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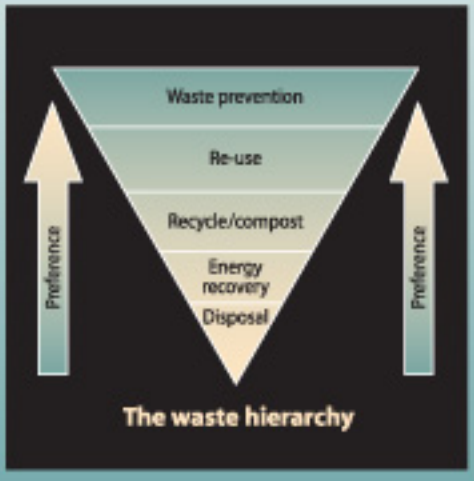

Handbook of waste management and co-product recovery in food processing

Volume 1

Edited by Keith Waldron

CRC

WP

Handbook of waste management and co-product recovery in food processing

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Keith Waldron

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Preface

The global intensification of agriculture and food production has led to the creation of vast quantities of food co-products and wastes, often in centralised locations as food processors seek to achieve economies of scale. Typically, the food industry produces considerable amounts of biodegradable wastes, including large volumes of effluent and residues with a high biological oxygen demand (BOD) and chemical oxygen demand (COD) content. Their uncontrolled spoilage and decomposition lead to the production of methane and other toxic moieties that are environmentally hazardous. In Europe alone, over 220 million tonnes of food-related waste are disposed of annually.

As a consequence of increased environmental awareness, the food industry is facing mounting pressures to reduce food processing and related wastes, for example in the form of legislation such as the EU Council Directive 1999/31/EC on the landfill of waste. Such pressures have contributed to an increase in costs of disposal and a reduction in landfill availability in many member states. Hence, methods to (a) reduce waste production, (b) valorize unused co-products, and (c) improve the management of unavoidable wastes, are becoming increasingly important to the food industry. Coincidentally, there is an increasing body of scientific literature relevant to exploiting food processing co-products. However, much of it is published in journals that do not focus specifically on this topic. This makes it more difficult for food technologists and industrialists to evaluate the 'state-of-the-art', and to exploit knowledge and expertise currently available.

It is in this context that the *Handbook of waste management and co-product recovery in food processing* has been conceived. The current volume

comprises contributions from an array of internationally recognized experts who have reviewed the latest developments in this area, with special reference to optimizing manufacturing processes to decrease waste, understanding how to reduce energy and water loss, and methods that may be used to valorize co-products.

The book provides expert opinion on topics relevant to the minimization of waste, and maximization of co-product recovery in food processing. There are five parts:

Part I Key drivers for waste management and co-product recovery in food processing

This section provides an introduction to the subject, with special reference to the principal drivers. These include legislative pressures (Chapter 1) and the interests of the consumer (Chapter 2).

Part II Optimizing manufacturing to minimize waste in food processing

This section focuses on the minimization of waste, both in terms of biowaste and efficient energy management. Chapter 3 highlights the importance of chain management and good housekeeping practices. Such management is important in reducing instances of irregular waste production that arise from managerial and technical problems. The waste is usually 'one-off' in nature, and hence is difficult to upgrade because of the absence of a regular co-product, thus negating any opportunities for economies of scale. This chapter also emphasizes chain management from an environmental and life-cycle analysis perspective. Chapter 4 explores the potential for minimization of energy use in food processing, highlighting the energy-intensive nature of the industry. For example, a typical energy requirement for the delivery of 1 J in the form of food consumes almost 10 J from natural resources. Chapter 5 completes Part II by evaluating opportunities to minimize water use and wastage. Such activities have the concomitant knock-on effect of reducing the effluent production, thereby reducing the requirement for additional treatment.

Part III Key issues and technologies for food waste separation and co-product recovery

This is a broad section bringing together expert opinion on research and technology associated with stabilization, fractionation, extraction and filtration of waste streams. Chapter 6 focuses on the microbiological stabilization of food processing waste which is fundamental to retaining food-grade quality characteristics and ensuring that maximum value can be obtained from subsequent processing activities. The chapter introduces the importance of HACCP development, not only before and during stabilization but during subsequent exploitation. Chapter 7 extends the concept of stabilization by considering the autolytic and natural deteriora-

tion of waste materials of biological origin once they have been damaged or taken out of their natural environment. Chapter 8 provides a general overview of combination processes that may be used in disassembling co-products – taking into account their structural, chemical and biochemical heterogeneity – whilst Chapter 9 looks at the use of biochemical routes for extraction and waste exploitation. This is followed by an evaluation of modern separation technologies. Chapter 10 explores supercritical CO_2 methods whilst Chapter 11 highlights the emerging and rapidly growing arena of membrane filtration technologies. This part is then completed by Chapter 12 on separation technologies used for food wastewater treatment, with special reference to the recovery of valuable products and water recycling.

Part IV Waste management in particular food industry sectors and recovery of specific co-products

The aim of this section is to provide specific examples of waste management in the main food processing sectors of meat-, fish- and plant-derived co-products. Chapter 13 provides a comprehensive account of waste management and co-product recovery in red and white meat processing, highlighting the difficulties encountered during the past decade due to legislation and food safety concerns. Chapter 14 focuses on dairy processing, an arena that has demonstrated huge improvements in efficiencies during the past 20 years; Chapter 15 gives a general overview of waste management and co-product recovery in fish processing. The remaining chapters in this section relate to the exploitation of plant-based wastes: Chapter 16 reviews the recovery and exploitation of trimmings and pulps from fruit and vegetable processing with emphasis on cell-wall polymer exploitation; Chapter 17 focuses on the extraction and exploitation of intracellular phytochemicals, revealing their potential for use in functional foods and pharmaceuticals. Chapter 18 takes this concept further by extending it into the cosmetics and pharmaceuticals arena. Chapter 19 explores the non-food exploitation of phytochemicals in relation to the production of natural dyes from food processing wastes, and Chapter 20 completes Part 4 by considering the problems of waste and co-product exploitation in the vegetable oil processing industry.

Part V Minimizing disposal: wastewater and solid waste management in the food industry

The final part of the book considers research and development and technologies relevant to the disposal of food processing wastes. Chapter 21 explores the treatment of wastewater generally with emphasis on general macro pollutants, COD, BOD, suspended solids, etc., and introduces anaerobic and aerobic treatments. Chapter 22 evaluates the technologies available for dewatering solid food processing waste streams, including

the use of modern electric field enhancement; Chapter 23 takes the anaerobic concept further by looking at the potential for generating a return on the waste through energy recovery in the form of biohydrogen production.

Keith Waldron

Part I

Key drivers for waste management and co-product recovery in food processing

1

Waste minimization, management and co-product recovery in food processing: an introduction

K. Waldron, Institute of Food Research, UK

1.1 Introduction: food processing waste – the scale of the problem

The global food and drink industry is one of the largest industry sectors and is essential to all economies. This reflects its role in contributing to the basic needs and requirements of every living person (Maslow, 1970). Consequently, the last 50 years has witnessed an immense increase in the demand for food due to the rapid growth in world population (Fig. 1.1) and the associated increase in wealth. The response to these drivers has been an intensification of agriculture, food production, transport and storage. Values for global food production are substantial. Annually, meat production is in the order of 200 million tonnes; dairy milk production is about 514 million tonnes, cereal production (including rice, wheat and coarse grains) is approximately 2 billion tonnes (OECD, 2004). In money-rich but time-poor 'developed countries', the increased consumption of food has been accompanied by the explosive growth in food processing, with particular emphasis on the development of energy-intensive, ready-to-heat/eat canned, frozen, dried and fresh meals. As globalization increases across all sectors, such processing is now being carried out throughout the world, and many final products are then transported to appropriate markets.

Food processing creates waste. Of approximately 3 billion tonnes of waste generated each year in Europe it has been estimated that the member states produce in the region of 222 million tonnes of food waste and by-products across the key sectors (AWARENET, 2004; Fig. 1.2). A

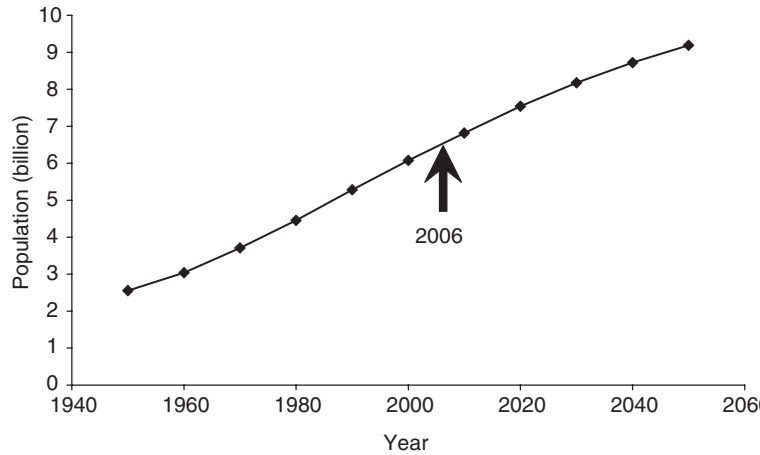

Fig. 1.1 Estimated previous and projected growth of the global human population (Source: Various).

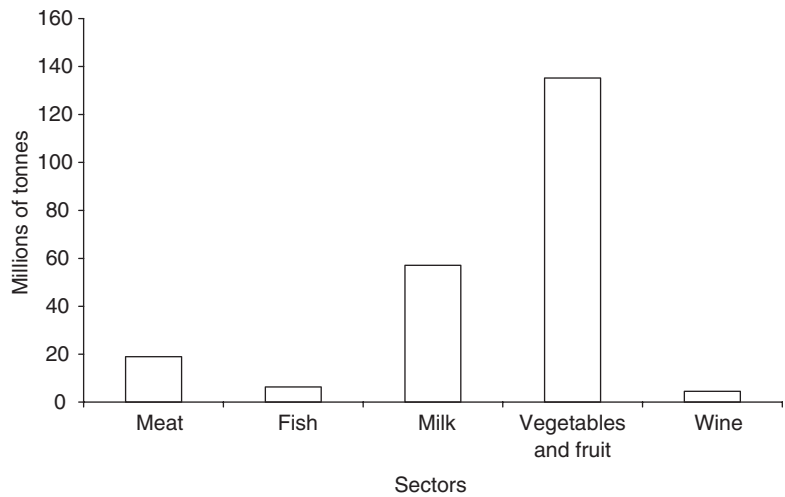

Fig. 1.2 European food waste across the different sectors (AWARENET, 2004).

significant proportion of this is exploited (valorized) to some extent, for example as a substrate for animal feed. However, large quantities of material are disposed of by landfill and other environmentally sensitive routes.

There are a number of reasons why so much food processing waste is produced, some economic and some technological. Traditional methods of food preparation result in relatively small amounts of locally produced domestic waste which, in the past, would have been disposed of as feed, by composting or through municipal waste disposal. However, industrial food processing, particularly that associated with the production of ready-to-eat meals, has created large, geographically localized waste streams which have generally increased over time as firms have sought to benefit from economies of scale. Furthermore, the majority of food processing systems were developed at least 20–30 (or more) years ago when waste disposal – particularly in the vegetable, cereal and fruit processing industries – was not the issue it is today. The amounts of waste, as a proportion of the raw material, are shown in Fig. 1.3. In the past, the value added by processing a portion of a raw food material to create a high-priced product outweighed the costs of disposal and, for many processes, there was little incentive to find alternative means to deal with the waste streams. Indeed, from a strategic viewpoint, such an approach would involve significant business risk (see Section 1.2 below). As a result, the development of technologies and approaches for exploiting waste streams was not such a priority; waste production remained integral to the development of food processing systems.

1.2 Diversification and risk

The difficulty of exploiting food processing waste may be considered from a business management perspective. Because of the specificity of a given raw material and its processing in relation to a particular product, surplus and waste food processing co-products are not readily utilized by the parent processors. Exploitation of the waste would necessitate a degree of diversification which would probably include the formulation of new products for current or new markets. This would create a high degree of risk (Ansoff, 1957; Fig. 1.4), which is not attractive to food industry players, especially since the industry is in a mature state, and the products are mostly commodities. It is not surprising that processors generally prefer their waste streams to be removed from their premises by third parties. The difficulties of dealing with wastes are compounded by the rapid deterioration of biological materials due to autolytic, chemical and microbial spoilage, resulting in a loss of food-grade potential and

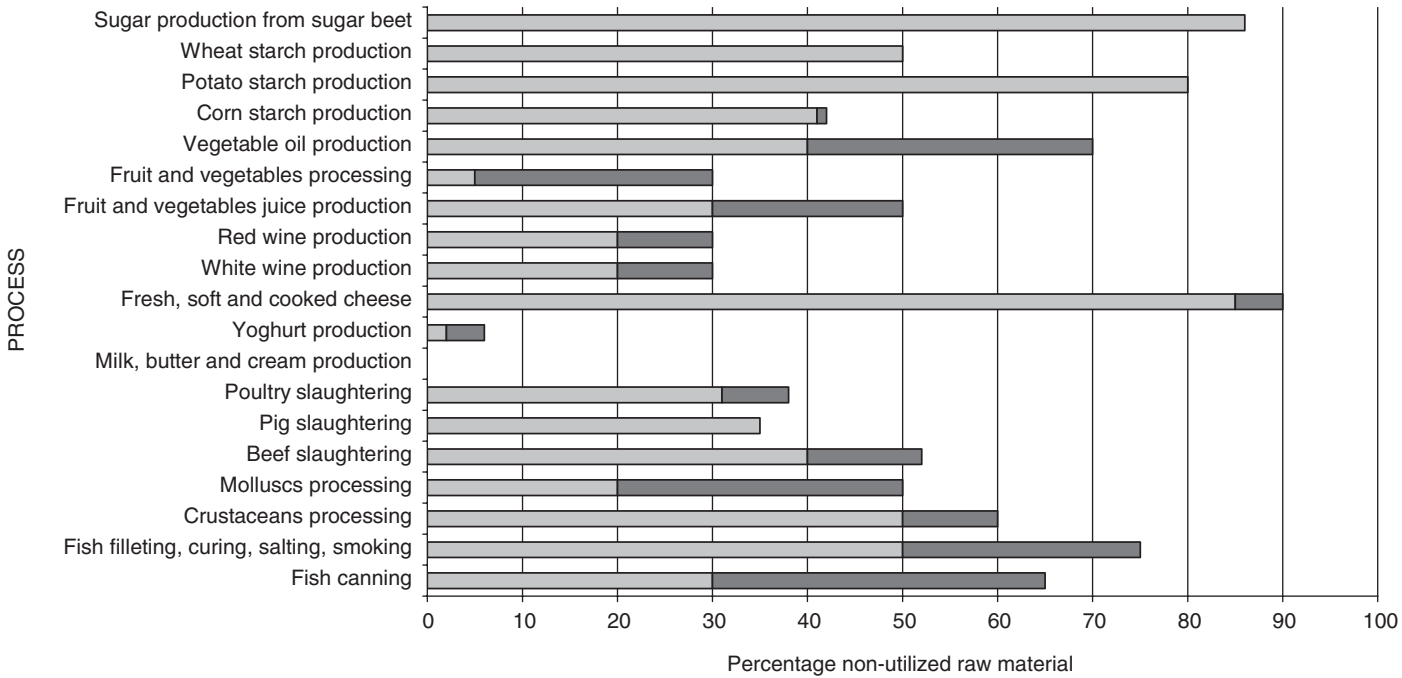

Fig. 1.3 Indication of the quantity of non-utilized raw material (light grey, minimum amount; dark grey, maximum amount) (AWARENET, 2004).

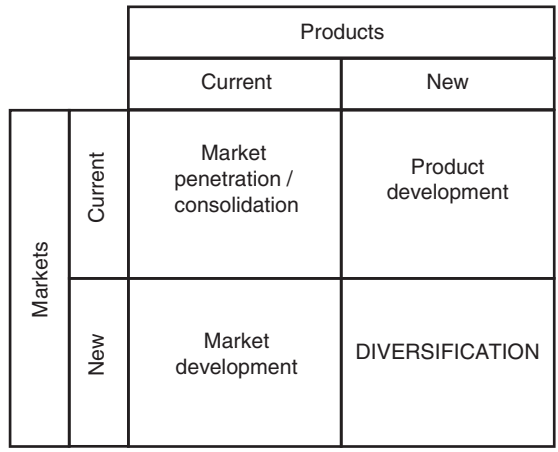

Fig. 1.4 Ansoff's matrix (after Ansoff, 1957).

associated value. Such materials would be fit only for non-food applications, or disposal.

1.3 Biological basis of biowastes

Food processing waste is derived from the processing of biological materials and is, in the main, biodegradable. Biowaste is defined in the landfill directive as 'waste capable of undergoing anaerobic or aerobic decomposition such as food and garden waste, and paper and cardboard'. The waste may be derived from plant, animal, fungal and bacterial sources, with the plant and animal origins predominating. A list of key production processes that create waste streams has been identified in the AWARENET handbook (2004; see also Fig. 1.3). A variety of waste streams will be created by the different stages of each process; these have also been described generically (AWARENET, 2004). Biological wastes are highly complex since they have been derived from highly intricate living organisms and can range from whole, unused (rejected) materials through to fractions and mixtures produced by physical, thermal, chemical and biochemical processing of the original raw material. Plant wastes include vegetable and fruit trimmings (comprising whole/partial organs and tissues) and cereal residues such as bran and extracted barley grain through to pulps and residues remaining after oil, starch, sugar and juice extraction. Animal-derived processing

wastes include blood, bone and neural tissues along with wastes from dairy processing. In addition, large quantities of wastewater are often produced. More complex mixed wastes are often produced in the production of ready meals which are composed of both animal and plant material. Hence, food processing wastes are heterogeneous in their composition due to the impact of the varied processing activities on the complex raw materials.

1.4 Legislation

This section provides a general and historical overview of some of the legislation relevant to the disposal or exploitation of food processing co-products. It is not definitive, and any formal advice and up-to-date information on legislative requirements should be sought from alternative and accredited sources.

1.4.1 Overview

Food wastes and effluents are rich in biodegradable components with high biological oxygen demand (BOD) and chemical oxygen demand (COD) content. If they are unmanaged and untreated, their uncontrolled decomposition is hazardous to the environment due to the production of methane and toxic materials (Waldron *et al.*, 2004). In order to reduce the impact of waste, a range of waste management strategies have been adopted at national and international levels. In the European Community (EC), a Community Strategy for Waste Management (see Sanders and Crosby, 2004) was published in 1989 and amended in 1996 (COM(96)0339) setting out key legal principles, including: (1) the prevention principle – to limit waste production; (2) the polluter pays principle – i.e. the polluter should pay for dealing with the waste; (3) the precautionary principle – waste problems should be predicted; (4) the proximity principle – as far as it is possible to do so, waste problems should be addressed near or at the place of production. These principles were created to form the framework of European waste policy. Sanders and Crosby (2004) have also highlighted three particular areas of importance within the strategy: (1) a waste management hierarchy (Fig. 1.5; AWARENET, 2004; DEFRA 1) in which priority should be given to the prevention and recovery of waste, and the optimization and minimization of disposal (often referred to as the '3 "R"s' 'reduce, reuse and recycle'); (2) producer responsibility whereby producers must take back end-of-life products; (3) control of waste shipments, with references to importing and exporting of waste within European Union (EU) countries.

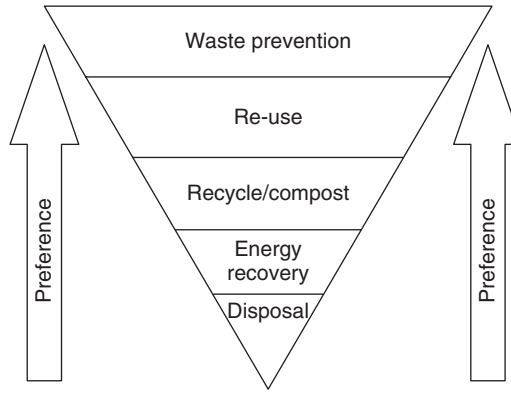

Fig. 1.5 The waste hierarchy.

In the EU, legislation comprises directives (to be implemented through the national legislation of member states) and regulations (directly applicable). In the United Kingdom (UK), information on implementation of EU directives via UK Government legislation may be found on the DEFRA website (DEFRA 2), and related links. In 1975, the EU introduced the Waste Framework Directive (WFD) (75/442/EEC) which was later modified by Directive 91/156/EEC to take into account the principles from the Community Strategy for Waste Management (1989 above). The overall regulatory and legislative arena concerning 'waste' covers a very broad range of materials and industrial sectors. Therefore it is appropriate to highlight the main legislation relevant to food industry waste as highlighted by AWARENET. These include:

1 Council Directive 96/61/EC on Integrated Pollution Prevention and Control – which seeks to prevent/reduce/eliminate pollution at source;
2 Council Directive 1999/31/EC on Landfill – which sets national targets for reduction of landfill of biodegradable municipal waste;
3 Regulation 1774/2002 on health rules regarding animal by-products not fit for human consumption;
4 Council Directive 2000/76/EC on incineration – to limit negative effects on the environment and human health.

At the time of the AWARENET report, a future directive on biowaste was foreseen prior to the end of 2004. However, this does not appear to have

materialized. Indications are that biowaste will be dealt with under the auspices of other legislative activites. Further information on EU legislation may be found in Table 1.1. Details of the legislation content may be found on the Europa websites (Europa, 2006). A more comprehensive overview of legislation (as of 2004) may be found in Sanders and Crosby (2004) and AWARENET (2004). A general history of waste legislation in the EU may be found on the EU Environmental Information and Legislation Database (see Section 1.9).

1.4.2 Definition of waste

The definition of waste has been surprisingly difficult to agree on. The Organisation for Economic Co-operation and Development (OECD), the Secretariat of the Basel Convention and the EU Commission each has formally its own definition of waste, and information on these can be found on the website of the European Topic Centre on Resource and Waste Management (2006). According to this source, the 'most

Table 1.1 Relevant EU legislation; see AWARENET (2004) and Sanders and Crosby (2004) for further details

Legislation on solid and liquid wastes
Council Directive 75/439/EEC on the disposal of waste oils
Council Directive 75/442/EEC on waste
Council Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community
Council Directive 80/68/EEC on measures and restrictions for the protection of groundwater against pollution caused by certain dangerous substances
Waste Framework Directive 91/156/EEC
Council Directive 91/271/EEC concerning urban wastewater treatment
Council Directive 91/689/EEC on hazardous waste
Council Regulation 259/93 on the supervision and control of shipments of waste
Council Directive 94/67/EEC on the incineration of hazardous waste
Commission Decision 96/350/EC for the adaptation of Annex IIA and IIB of Directive 75/442 EEC on waste
Council Directive 1999/31/EC on the landfill of waste
Commission Decision 2000/532/EC establishing a list of wastes
Water Framework Directive 2000/60/EC
Council Directive 2000/76/EC on the incineration of waste
Regulation 2150/2002/EC on waste statistics
Council Decision 2003/33/EC on criteria for acceptance of waste at landfills
Legislation concerning added-value products from food wastes
Regulation 1774/2002/EC on health rules concerning animal by-products not intended for human consumption
Directive 2003/30/EC on the promotion of the use of biofuels or other renewable fuels for transport

Table 1.1 cont'd

Council Directive 79/373/EEC on the marketing of compound feedingstuffs
Council Directive 90/667/EEC on veterinary rules for the disposal and processing of animal waste
Council Directive 95/53/EC concerning the organization of official inspections in the field of animal nutrition
Council Decision 1999/534/EC on measures on animal waste protecting against transmissible spongiform encephalopothies (TSE)
Council Decision 2000/7656/EC on protection measures regarding TSE and feeding of animal protein
Decision 2001/25/EC prohibiting the use of certain animal by-products in animal feed
Regulation 999/2001/EC for prevention, control and eradication of TSE
Regulation 811/2003/EC on intra-species recycling ban for fish, and burying and burial of animal by-products
Regulation 809/2003/EC on processing standards for category 3 material and manure used in composting plants
Regulation 810/2003/EC on processing standards for category 3 material and manure used in biogas plants

Novel foods
Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients
Commission Decision 2002/150 authorizing the marketing of coagulated potato proteins and hydrolysates as novel food ingredients

Additional specific legislation in specific agro-food sectors
Council Directive 90/496/EEC on nutrition labelling for foodstuffs
Council Regulation 91/493/EC laying down health conditions for production and the placing on the market of fishery products
Council Directive 94/435/EC on sweeteners for use in foodstuffs
Council Directive 94/435/EC on colours for use in foodstuffs
Council Directive 95/2/EC on food additivies other than colours and sweeteners
Council Regulation 2200/96/EC on the common organization of the market in fruit and vegetables
Council Regulation 1493/99/EC on the common organization of the market in wine
Commission Decision 1999/724/EC on specific health conditions for hygienic manufacture of gelatine intended for human consumption
Council Regulation 104/2000/EC on the common organization of the markets in fishery and aquaculture products
Commission Regulation (EC) No. 1623/2000 of 25 July 2000 laying down detailed rules for implementing Regulation (EC) No. 1493/1999 on the common organization of the market in wine with regard to market
Council Directive 2000/13/EC on the approximations of the laws in member states on labeling, presentation and advertising of foodstuffs
Commission Directive 2001/15/EC on substsances that may be added in foods for nutritional purposes
Commission Decision 2001/471/EC on regular checks on health conditions for production and marketing of fresh meat and fresh poultry meat
Council Regulation 178/2002/EC laying down general principles and requirements of food law

explicative' definition of waste may be found in the Joint Questionnaire OECD/Eurostat that is sent biennially to all EU countries and reads as follows:

> Waste refers here to materials that are not prime products (i.e. products produced for the market) for which the generator has no further use for their own purpose of production, transformation or consumption, and which he discards, or intends or is required to discard. Wastes may be generated during the extraction of raw materials, during the processing of raw materials to intermediate and final products, during the consumption of final products, and during any other human activity.
>
> Are excluded:
>
> – Residuals directly recycled or reused at the place of generation (i.e. establishment);
> – Waste materials that are directly discharged into ambient water or air.

The legal definition of waste is provided by the EU Commission in the Waste Framework Directive 75/442/EEC on waste as amended by Council Directive 91/156/EEC, Art.1(a), and is inseparably linked with the EU lists of waste categories and waste types:

> 'Waste' shall mean any substance or object in the categories set out in Annex I which the holder discards or intends or is required to discard.
>
> The Commission has drawn up a list of wastes belonging to the categories listed in Annex I.

Categories of waste from Annex 1 that are relevant to food processing are shown in Table 1.2.

In order to make the definition of waste more tangible and to reduce uncertainty, the European Commission has drawn up a list of wastes (Table 1.3; see 'Waste list' in reference section). This was established by Commission Decision 2000/532/EC.

Once a material is defined as a 'waste', it is then subject to a range of directives (e.g. the Waste Framework Directive and the Hazardous Waste Directive) and regulations (e.g. the Waste Shipment Directive) concerning the handling and treatment of waste streams. Therefore, the classification of co- or by-products that are currently disposed of as 'waste' is of importance if alternative exploitation routes are to be sought. Interestingly, there appears to be no legal definition of the term 'co- or by-product'.

A number of issues have arisen in the interpretation of the above waste definitions including the meaning of the term 'discard' (which lacks a common understanding) compared with 'dispose'. A number of disputes have required resolution by the European Court of Justice. The OECD Waste Management Policy Group has produced a guidance document with the aim of clarifying whether or not a material can be

Table 1.2 Categories of waste, Annex 1 of Directive 75/442/EEC

Categories of waste	
Directive 75/442/EEC, Annex I	
Q1	Production or consumption residues not otherwise specified below
Q2	Off-specification products
Q3	Products whose date for appropriate use has expired
Q4	Materials spilled, lost or having undergone other mishap, including any materials, equipment, etc. contaminated as a result of the mishap
Q5	Materials contaminated or soiled as a result of planned actions (e.g. residues from cleaning operations, packing materials, containers, etc.)
Q6	Unusable parts (e.g. reject batteries, exhausted catalysts, etc.)
Q7	Substances which no longer perform satisfactorily (e.g. contaminated acids, contaminated solvents, exhausted tempering salts, etc.)
Q8	Residues of industrial processes (e.g. slags, still bottoms, etc.)
Q9	Residues from pollution abatement processes (e.g. scrubber sludges, baghouse dusts, spent filters, etc.)
Q10	Machining/finishing residues (e.g. lathe turnings, mill scales, etc.)
Q11	Residues from raw materials extraction and processing (e.g. mining residues, oil field slops, etc.)
Q12	Adulterated materials (e.g. oils contaminated with PCBs, etc.)
Q13	Any materials, substances or products whose use has been banned by law
Q14	Products for which the holder has no further use (e.g. agricultural, household, office, commercial and shop discards, etc.)
Q15	Contaminated materials, substances or products resulting from remedial action with respect to land
Q16	Any materials, substances or products which are not contained in the above categories

regarded as waste. Nevertheless, the situation appears to be less clear at the European level as to the classification of materials that are to be recovered and used as raw materials in other processes (so-called 'secondary products') and is made more complicated by interpretations at the member state level. This seems to be an emergent situation, and will be influenced further by the development of technologies and approaches to exploit what would previously be regarded as waste material. A flow chart depicting the EC legislative relationships between waste, secondary raw material and product is shown in Fig. 1.6 (AWARENET, 2004).

1.4.3 Novel foods legislation

An important piece of legislation which should be considered in the exploitation of food co-products and wastes concerns the regulation on novel foods and novel food ingredients (Regulation (EC) No. 258/97).

Table 1.3 An extract from the EU list of wastes (see 'Waste list' in reference section)

02 02 wastes from the preparation and processing of meat, fish and other foods of animal origin	
02 02 01	sludges from washing and cleaning
02 02 02	animal-tissue waste
02 02 03	materials unsuitable for consumption or processing
02 02 04	sludges from on-site effluent treatment
02 02 99	wastes not otherwise specified
02 03 wastes from fruit, vegetables, cereals, edible oils, cocoa, coffee, tea and tobacco preparation and processing; conserve production; yeast and yeast extract production, molasses preparation and fermentation	
02 03 01	sludges from washing, cleaning, peeling, centrifuging and separation
02 03 02	wastes from preserving agents
02 03 03	wastes from solvent extraction
02 03 04	materials unsuitable for consumption processing
02 03 05	sludges from on-site effluent treatment
02 03 99	wastes not otherwise specified
02 04 wastes from sugar processing	
02 04 01	soil from cleaning and washing beet
02 04 02	off-specification calcium carbonate
02 04 03	sludges from on-site effluent treatment
02 04 99	wastes not otherwise specified
02 05 wastes from the dairy products industry	
02 05 01	materials unsuitable for consumption or processing
02 05 02	sludges from on-site effluent treatment
02 05 99	wastes not otherwise specified
02 06 wastes from the baking and confectionery industry	
02 06 01	materials unsuitable for consumption or processing
02 06 02	wastes from preserving agents
02 06 03	sludges from on-site effluent treatment
02 06 99	wastes not otherwise specified
02 07 wastes from the production of alcoholic and non-alcoholic beverages (except coffee, tea and cocoa)	
02 07 01	wastes from washing, cleaning and mechanical reduction of raw materials
02 07 02	wastes from spirits distillation
02 07 03	wastes from chemical treatment
02 07 04	materials unsuitable for consumption or processing
02 07 05	sludges from on-site effluent treatment

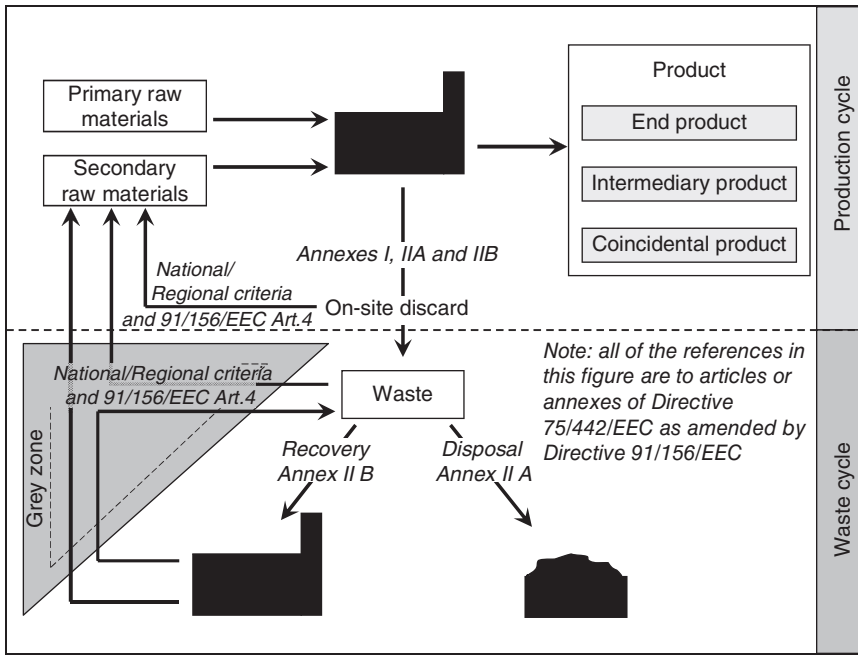

Fig. 1.6 Overview of the relationships between waste, secondary raw materials and products, with EC legislation (after AWARENET, 2004). The 'grey zone' relates to an area of dispute between member states.

Some of the components extracted and fractionated from co-products may require evaluation under this legislation. One example of how this might be relevant is given by the Commission Decision 2002/150 regarding the exploitation of potato proteins and hydrolysates as novel ingredients.

1.5 Implementation of the waste hierarchy concept in relation to food processing co-products and wastes

Figure 1.7 shows a simplified diagram of the production of waste materials from the food processing sector and highlights criteria consistent with the waste hierarchy (Fig. 1.5) that need to be addressed in order to minimize disposal requirements. Prevention of waste requires consideration of the

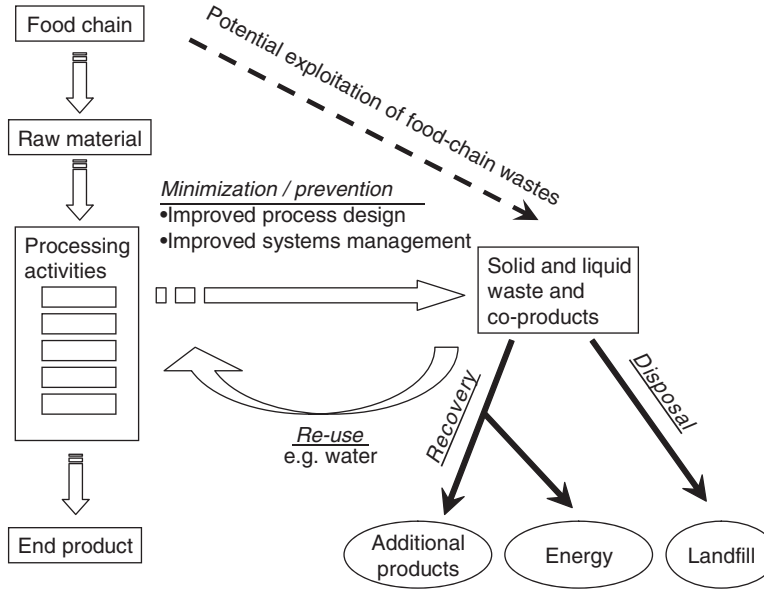

Fig. 1.7 Food processing waste in relation to the waste hierarchy.

reasons for its production. For example, waste that results from mistakes in process management – e.g. excess substrates, breakdowns, human error – might be addressed by improved systems management. In contrast, waste that is inherent to the process may have to be addressed by improving the design of the process and is likely to require investment in new plant. Some wastes, e.g. water, may be recycled if purified effectively, helping in the development of closed-loop factories. Those co-products that are not suitable for recycling may be exploited via the creation of novel products (see below). This approach may be unattractive since it is likely to involve a high-risk strategy on the part of the food processor (see Fig. 1.4). However, due to the increase in legislative pressures (see above) and consumer demand (see Chapter 2, this volume), this option is becoming increasingly relevant. Some very large co-product streams, e.g. whey from the dairy industry and starch from potato processing, have been successfully exploited for the production of ingredients for the food sector (Waldron *et al.*, 2004). However, this has taken between 10 and 20 years to occur, reflecting the time taken for development of new technologies and new markets.

1.6 High-value components and whole-waste exploitation

Many waste streams contain small levels of components that command an apparent and attractive market value. For example, phytochemicals from fruit and vegetable trimmings might be exploited for the production of nutraceuticals, cosmetics or even pharmaceuticals. There has been much interest and research dedicated to exploiting such components with a view to adding value to co-product streams (Waldron, 2004; Waldron *et al*., 2004). However, whilst it is often possible, and even relatively straightforward in a laboratory to develop a process to extract a 'high-value' component, it is frequently uneconomical to do so commercially. This may be because the bulk residues remaining after extraction are of lower value, and may actually cost more in disposal. Therefore, it is important to develop approaches that aim to exploit food co-products in their entirety, ensuring that all components so derived may be of marketable quality. This requires a research and development approach that links all the potential components in the waste stream to the potential markets available. A possible strategy for such an approach is shown in Fig. 1.8 and is based on that which has been proposed in the EU project REPRO (see website). This approach involves exploiting fully traceable, food-grade waste streams. Initially they should be stabilized against microbiological deterioration and autolysis to prevent them from losing their food-grade status. Subsequently they would be disassembled by physical and biochemical approaches (individually and in combination) involving modern enzyme and processing technologies. The aim is to provide a range of components, from high value to low value, all of which would contribute to achieving whole-waste exploitation. The process would require evaluation for acceptability, both in relation to safety (via Hazard Analysis Critical Control Point (HACCP) development), novel food legislation, consumer preference and marketing requirements. Such an approach requires close interaction with all stakeholders to maximize knowledge transfer and exploitation.

Finally, some co-products may be unsuitable for exploitation due, for example, to their complexity, uncontrolled spoilage or lack of traceability. In such cases, non-food exploitation as energy sources may be appropriate via fermentation and biogas production, and other microbially based disposal systems such as composting. New technologies may provide opportunities to convert such biomass into biofuels. Hence, it should be possible to reduce landfill considerably, and in the case of plant-based co-products, to avoid it altogether. Of course, the final arbiter will be the cost-effectiveness of this strategy, as measured by perceived return on investment, and this will have to take into account locally/

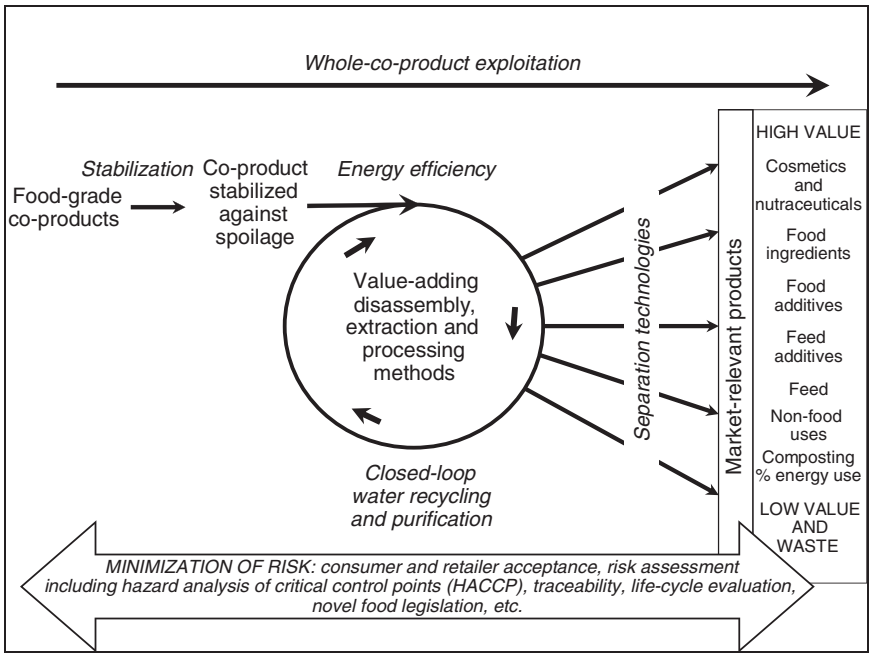

Fig. 1.8 A roadmap for whole-co-product exploitation.

sector-relevant factors including seasonality, market potential, and transport and storage costs.

1.7 Future trends

The world is changing rapidly. In particular, the large increases in fuel costs and the pressures to reduce carbon emissions are bound to have a significant impact on the food chain. It is likely that over the next 10 years the costs of food processing and transport will increase, as will demands to use more land for non-food crop production. This will be compounded by the costly energy requirements of modern intensive agriculture, including mechanized activities and the use of fertilizers. These drivers, in conjunction with the 'waste hierarchy' will add further pressure to ensure that the majority of food processing and food-chain co-products are fully exploited. In order to evaluate this complex arena from environmental and economic perspectives, the demand for user-friendly LCA is likely to increase.

1.8 Sources of further information and advice

AWARENET handbook for the prevention and minimization of waste and valorization of by-products in European agro-food industries: http://eea.eionet.europa.eu/Public/irc/envirowindows/awarenet/library?l=/awarenet_handbook&vm=detailed&sb=Title

EU Environmental Information and Legislation Database: http://www.ncte.ie/environ/welcome.htm and http://www.ncte.ie/environ/waste.htm

Europa legislation search facility: http://europa.eu.int/eur-lex/lex/RECH_legislation.do

Forum for the Future: http://www.forumforthefuture.org.uk/

Mass Balance UK Project Website: http://www.massbalance.org/

REPRO website: http://www.repro-food.net/

Resource Efficiency Network (UK): http://amf.globalwatchonline.com/epicentric_portal/site/AMF

Sustainable development commission: http://www.sd-commission.org.uk/

US Environmental Protection Agency: http://epa.gov/epahome/rules.html

Waste and Resources Action Programme (WRAP): http://www.wrap.org.uk/

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2

Consumer interests in food processing waste management and co-product recovery

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2.1 Introduction: consumer interests as a key driver to improve waste management in food processing

More than 10 million tonnes of food processing waste are produced within the European Community every year. The costs associated with handling the waste produced within the food industry constitute many tens of millions of euros, attributable to landfill costs and other waste disposal routes. However, this waste is also known to contain significant amounts of valuable components which remain unexploited as a result of current food-waste processing practices. There are many kinds of potentially valuable components in these wastes: nutrients and micronutrients (protein, dietary fibre, prebiotics, antioxidants and other bioactive polyphenolics), rheological agents (hydrocolloids, gelling agents, films and coatings), texturised residues, flavours and colourants (juices) (Cheunk *et al*., 2003). These could be utilised in pharmaceutical, cosmetic and nutraceutical high-value products, as well as in contributing to medium-value food and feed ingredients. In the following we will focus on possible food products that can be reprocessed from food waste.

In the past, most of the waste of the food processing industry has been used as landfill, or has been fed to farm animals. Recent changes in the legislative framework, which have been enacted as a response to various food safety incidences such as the bovine spongiform encephalopathy (BSE) crisis, have forced the food industry to reconsider recycling practices. However, at the present time, increasing attention is being paid to the concept of sustainability. As a consequence, this issue is becoming increasingly important to the food industry and other food producers. In addition,

the industry also recognises the need to respond to increased regulatory, end-user, non-governmental organisations (NGO) and (to some extent) consumer interest in the need to implement sustainable production systems. Of course, consumers are not just interested in sustainability but also in food safety, food quality and a number of other issues; for example, emotional responses to novel foods, which may arise as a consequence of food reprocessing. In this way consumer attitudes towards reprocessed food products have become a key driver to improve waste management in food processing.

In considering the issue of food-waste recycling as an integral part of the food chain, some key questions regarding consumer attitudes to resultant novel products need to be asked. These include, for example, questions about our understanding of how the public conceptualises sustainability, and whether this differs from expert views regarding sustainable food production practices. It is also important to understand how these beliefs and attitudes relate to the consumption of specific foods and food products. Individual and cross-cultural differences in the conceptualisation of, and attitudes towards, sustainable production processes must also be evaluated and incorporated into the assessment of the utility of emerging technologies and novel foods that may arise as a consequence of food-waste recycling. Research should identify the salient consumer beliefs regarding sustainable and, conversely, unsustainable products and product features, and the way in which people's values influence attitudes and opinions. At the present time, however, there has been little systematic empirical investigation of these various issues and questions. It may be possible to make some predictions of possible barriers to consumer acceptance, as well as reasons for consumer acceptance, regarding product commercialisation, by examination of the relevant literature on related topics.

In particular, due consideration of these issues should be made when considering recycling food waste into new food products developed for consumer purchase. Account should be taken of consumer acceptance of the different technological innovations applied during the production process, as well as consumer perceptions of the motives of producers in implementing these new technologies (for example, is there a dominant consumer attitude that industry is engaging in recycling practices in order to increase profitability, rather than to increase the sustainability of production processes?) and perceptions of risk. Previous risk events such as the BSE crisis may negatively predispose consumers towards recycling food wastes within the food chain, even if the recycled product is not destined for human consumption. During the BSE scare, consumer concern was underpinned by the recycling of animal waste into the human food chain in the form of animal feed. As a consequence there may be consumer concerns about the use of reprocessed waste in novel food production.

One might predict that effective commercialisation strategies will focus on regaining consumer confidence in food production in general, as well as

communicating effectively and involving consumers in the broader debate about sustainable food production, and how this might be achieved and implemented.

This chapter aims to discuss some of the consumer issues relevant to sustainable production in general, and recycling food waste into the food chain in particular (either as animal feed or products for human consumption). Due account will be taken of factors driving consumer attitudes towards novel food products (for example, risk perception, perceptions of benefit and consumer trust in food producers and regulators with responsibility for consumer protection). Finally, the need for greater consumer involvement early in the process of food innovation will be discussed.

2.2 Societal issues related to sustainability

2.2.1 Sustainability

The key underlying driver for upgrading food-chain waste is improved sustainability, linked to the consumer attitude of concern for the environment. Co-product exploitation and waste minimisation are vital to sustainable development. Literally, sustainability means 'the ability to sustain'. This implies maintaining something in the same state, or keeping it in the same state for a period of time, or providing support by giving help and encouragement or mobilising resources. In recent decades, the best-known and widely adopted definition of sustainable development was provided in the Brundtland report (1987), where sustainable development was defined as 'development that meets the needs of the present without compromising the ability of future generations to meet their own needs'. Despite widespread application of this particular definition, there are many different interpretations, modifications and reformulations of what sustainability actually is (Gremmen and Jacobs, 1997). A World Bank inventory (Jacobs, 2001) listed no less than 190 different attempts to provide definitions. Indeed, sustainability has become linked to a large variety of human activities, concepts and concerns, ranging from biodiversity preservation, through to corporate social responsibility. In addition, sustainability has become a priority for the world's policy makers. The concept is now at the centre of regulatory activities in many countries around the world and has, as a consequence, become a societal norm. Although there is no single definition of sustainability over all domains, for the remainder of this chapter we refer to the Brundtland definition as a working definition.

Generally, people will do what they believe is 'right', i.e. whatever is consistent with their personal beliefs and/or the norms of the society in which they live. In the view of many stakeholders, including consumers, the food production system has ceased to be sustainable. The development of increasingly intensive and specialised forms of agriculture has culminated

in what some refer to as 'industrial food production' or 'chemical farming systems' (Nap *et al.*, 2002). The farm is viewed as a factory with inputs and outputs, aiming at increased yields with reduced costs, often by exploiting the economies of scale. It is accompanied by use of and reliance on agrochemicals, large-scale mono-cropping, and mechanisation and energy consumption. The primary objective of such agricultural systems is to produce as much food and fibre as possible, for the least cost. According to Lyson (2002), 'current conventional agriculture is anchored to the scientific paradigm of reductionist experimental biology, in combination with the reductionism of neoclassical economics, driven by continued (desire for) industrialisation.'

Although successful in the past in terms of improving food security and food safety, virtually every aspect of conventional agricultural practice is viewed by many as problematic in terms of sustainability. The problems centre on both the environmental aspects and on the social and community aspects of food production. For example, current high-intensity practices in the agri-food sector not only have a negative impact on the supply and quality of water, these practices also cause erosion, unfertile soils, contamination by pesticides, excessive use of synthetic chemicals, and various levels of pollution of groundwater, soils and atmosphere. Arguably, existing methods of food production may contribute to negative changes within the ecosystem.

At the present time, there are increased efforts within the food industry to develop more sustainable food production practices, in part a response to consumer and regulatory demands. At the top of the sustainability agenda is conservation of resources related to energy and water use. Although sustainability in its broadest sense focuses on the conservation of natural resources, many of the goals of the food industry specifically reflect the need for increased sustainability in the sense of renewable resource use and a significantly reduced environmental burden of food production. International commitments towards improved sustainability are now underpinned by demanding legislative controls to minimise the impact of waste on the environment and human health. In the European Community (EC), the importance of reducing waste has been reflected in stringent legislation, particularly EC Council Directive 1999/31/EC (26 April, 1999) to reduce biodegradable waste disposed to landfill, 35% of 1995 levels by 2016, and the Hazardous Waste Directive (91/689/EEC) which includes cereal and vegetable food processing wastes. The Euro-legislation is implemented via appropriate national regulations (such as the UK Landfill Tax currently 20 euros per tonne). Across European Union (EU) member states, the amount of organic waste sent to landfill will be severely limited in the future. The impact of the legislation on food processors is also being increased by other regulations in the area of food safety. For example, there are now restrictions on feeding many co-products to animals, and animal husbandry across Europe has been reduced as a result of public concerns such as dietary well

being and safety issues such as BSE (Windhorst, 2001). As a consequence, despite their valuable constituents, agro-waste co-products currently represent a substantial negative cost within the food industry. A sustainable future for many food processors requires that these co-products are exploited in order to prevent them from becoming waste.

2.2.2 Consumer issues

There is a general concern across the food industry that consumers will not accept organic by-products as (ingredients in) food products because they will consider 'by-products' synonymous with 'waste'. Given public fears with respect to, for example, BSE and other issues related to recycling of waste products in the food chain, this would imply consumer rejection of the upgrading of organic by-products. Apart from the benefits to sustainability not being attained, consumer rejection of food products would run counter to the work of experts on sustainable food production. The 'by-product equals waste scenario' is far from hypothetical. In the literature, a commonly used terminology used is that of 'waste management in food processing'. The term 'waste management' may turn out to be one that does not facilitate consumer acceptance of food products. In the following we will use the term 'by-product' instead of 'waste', because it is a more neutral term.

As indicated before, there are a number of reasons to proceed carefully regarding the introduction of food-processing by-products in food products. Consumers should have an important role in this process of what, when and how novel products are introduced, and, therefore, the interaction between producers and (potential) consumers should be intensified. Hanssen *et al*. (2001) point to the examples of the societal debates on the application of biotechnology in the Netherlands, the UK and elsewhere in Europe, which illustrate the need for an increased involvement of users (consumers) in the process of product and technology development. Thus it behoves producers to attend not only to the issue of improved sustainability, but also to public attitudes and concerns relating to the technologies applied in order to attain improved sustainability.

One important reason for the opposition of consumers to novel agri-food technologies has been negative consumer responses to the 'technology push' by certain producers, most notably the manufacturers of genetically modified (GM) products. As a consequence, financial investment in public and private research and development in genetic engineering in the food and agriculture sector can be considered to be more or less wasted (Moors *et al*., 2002). There is a huge volume of literature on why European consumers rejected genetically modified crops. However, public attitudes towards agri-food technologies are not dependent on an analytical assessment of risks and benefits alone. Other factors, such as ethical and moral considerations, and other values such as concern about the integrity of nature and

trust in the regulatory system, also play a part in societal and consumer acceptance (Miles and Frewer, 2001; Jensen and Sandøe, 2002). Developing communication about substantial equivalence (i.e. that the content of GM foods was not substantially different to conventional counterparts) did not address consumer concerns, and was thus not relevant to consumer fears. Research also demonstrated that control over consumption of GM foods was enormously important to European consumers, necessitating the labelling of GM foods and implementation of effective traceability systems (Miles *et al*., 2005). The negative public reaction to GM foods was therefore less to do with risk, and more to do with consumer choice and the failure to deliver information about what was actually driving consumer concerns. Opaque risk analysis systems and decision-making practices were not helpful in reassuring the public. The absence of first-generation products with tangible and desirable consumer benefits did little to reassure consumers about the motives of the food industry in introducing these crops. Taken together, it seems unlikely that consumers will accept the introduction of new GM foods and ingredients in the human food chain.

It is important that, in the future, consumers are involved in innovation processes in the food processing industry. It is also important to recognise that consumers may represent a repository of ideas (linked, for example, to new products) which could be exploited far better during the innovation process than is currently the case (Moors *et al*., 2002). In this way the creative capacity of consumers can be used to shape technological development in all phases of the innovation process (Oudshoorn and Pinch, 2002; Smits and Kuhlmann, 2002). Of course, a potential hindrance to consumer involvement in novel product design may be the attitudes of stakeholders in the production process. Specifically, producers may believe '. . . that the consumer does not know what she/he wants, especially if we are talking about developments such as genomics that take at least 5–10 years before the first products come to the market. Only the research and development departments can tell us what is under development. As yet, the research is in a too early stage to inform the public/consumer, so it is better to wait until the first prototypes are there and then consult the consumer.' (Moors *et al*., 2002). However, this reaction may have limited validity, particularly as in the published literature innovation processes are considered to be dynamic, complex and interactive. The linear model of innovation (from science, to technology, to industry, to consumers) is replaced by an interactive network model. According to Rosenberg, innovation is very much a process of 'learning by using' and 'learning by doing' (Rosenberg, 1976). As a consequence, innovations emerge in close interaction with their (socio-economic) environment. Thus innovation processes may benefit from a constructive technology assessment (CTA) which proposes to broaden the design by bringing together all interested parties early on and throughout the design process, with the aim of shaping technology development processes in such a way that social aspects are symmetrically considered in the process itself

(Smits *et al*., 1995; Schot, 2001). Another method that may benefit the innovation process is strategic niche management (SNM). Through the development of certain protected spaces (without the constraints of the market), SNM brings different stakeholders together to experiment with promising technologies and to learn about the desirability of the new technology. In this way SNM enhances the further development and the rate of application of the new technology (Kemp *et al*., 1998).

Will the relationships between consumers and the food processing industry change when by-products are used in food? To answer this question it is necessary to initiate CTA processes early in the development of new food products containing by-products in order to increase consumer participation and influence in the innovation process itself.

The effective management of food by-products could lead to the use of improved food (e.g. better flavour, improved texture, larger fruits, advanced shelf life, increased nutritional value and cheaper products produced using sustainable processes). The consumer will also be able to purchase food supplemented with functional health-promoting ingredients derived from process co-products. The goal of these new food products is to assist consumers in improving their health and quality of life through better nutrition. This can be regarded as part of a more general shift in consumer trends in food consumption that Moors *et al*. (2002) have called 'eating for feeding to eating for health'. In other words, consumers are beginning to pay more attention to the healthy and nutritious aspects of food, and they are more aware that eating specific food products could contribute positively to their health status. However, in the case of new food made from by-products of the food processing industry, consumers may consider by-products unhealthy, although this question merits further empirical investigation. It cannot be expected *a priori* that consumers know about, or are indeed interested in, the management of by-products, and it may be expected that new food products will only be successful if consumers understand the newness (and benefits). The food industry must inform consumers about why and how products are developed and introduce an empirical analysis of consumer acceptance in an early stage of the development process. In addition, the producer should not be too far 'ahead' of the consumers regarding product development (Moors *et al*., 2002), otherwise effective communication with consumers about new products may be difficult.

An important success factor for the introduction of new functional foods (based on substances from by-products) is that the safety and health claims associated with these products can be trusted by consumers. The underlying factors that drive consumer trust in food safety have been well documented (Frewer *et al*., 1996), and may equally apply to sources of food-related information in general. Lundvall (1992) argues that without mutual trust, efficient and effective interactions will not be possible. One of the ways the food industry may build trust is to use scientific proof of safety or

health benefits. However, at the present time, there are few novel foods available that have been developed using by-products, and as a consequence consumers are not familiar with potential innovations in this area. This lack of knowledge may increase the distance between consumers and producers, and this could hinder effective interactions between them (Lundvall, 1992). It could lead to a lack of trust, because some consumers fear new food products based on new, unknown technological developments or even novel foods in general (Pliner and Hobden, 1992).

Much public negativity associated with the way food safety issues are managed and regulated has been the result of managers, assessors and other key actors in the process of risk analysis failing to take account of the actual concerns of the public when assessing, managing and communicating about food safety issues. One consequence has been increased public distrust in the motives of regulators, science and industry in taking decisions or actions in relation to risk assessment priorities, resource allocation and risk mitigation activities (Frewer, 1999). Jensen and Sandøe (2002) have observed that, despite the creation of new food safety institutions such as the European Food Safety Authority (EFSA), the decline in public confidence in food safety continues. This may, it is argued, be partly the result of communication about food safety issues being based on scientific risk assessments alone, and failing to incorporate public concerns, values and fears into a broader societal debate (Levidow and Marris, 2001). Communication that does not explicitly address public concerns is likely to have a limited role in reassuring the public.

One approach to gain the trust of consumers is to adopt the Fork-To-Farm approach. The consumer will be able to purchase food that – through waste avoidance, full traceability and Hazard Analysis for Critical Control Points (HACCP)-inclusion in co-product ingredient manufacture – has been produced in a healthy and environmentally friendly manner. The idea is that this will help to increase consumer confidence in the food supply, and that this will be augmented by the increase in efficiency of the food chain which will contribute towards a cheaper food supply. Direct consumer benefits will also include an improved quality of life through a reduction in noxious odours and microbiological hazards commonly associated with organic waste disposal. However, in order for the consumer to develop an enthusiastic response to the new food products, a system of information and labelling must be implemented in order to deliver the information to those that need it.

A second approach to developing trust has focused on greater public inclusion in the process of policy development. When people feel a lack of control over their exposure to potential hazards, risks are perceived as higher. This effect can be countered by reassuring conclusions from a trusted source. Therefore, in cases where there is a lack of control, trust in risk assessment and risk management is likely to be a particularly important determinant of public confidence in food safety. A case in point is that

of GM foods, where consumer concern did not focus primarily on risk *per se*, but rather on the lack of personal control on the part of the consumer over consumption (Miles and Frewer, 2001). It was reasoned by the policy community that more extensive public consultation and participation in risk management and other science and technology issues would restore public confidence in the institutions with responsibility for public and consumer protection (see, for example, Renn *et al*., 1995; Rowe *et al*., 2004). Indeed, this appeared to reflect institutional recognition that consumers' attitudes towards different hazards are not purely dependent on an analytical assessment of risk and benefit. Other factors, such as *ethical* and *moral* considerations, were recognised as being potentially influential in establishing the acceptability or otherwise of a particular hazard or establishing societal approval of the measures put into place to contain specific risks. Social inclusion is important if consumers are to build trust in both risk analysis and technology development and commercialisation, although consumer opinions should be seen to influence outcomes and technological developments otherwise the effect may be trust destroying rather than the converse.

One consequence of various food scares (for example, the controversies over GM foods and BSE in the 1990s), may be increased consumer cynicism regarding sustainability claims supporting 'risky' technology pushes. The questions that need to be asked are: under what circumstances will people accept potentially controversial technologies applied to production if there is a benefit to sustainability; and does improved sustainability in itself represent a substantial enough benefit to offset consumer negativity towards specific food production processes?

2.2.3 Risk management and transparency

Consumer risk perceptions differ from those held by other stakeholders involved in food production and risk analysis. It has been well established that people's risk perceptions determine how they react to different hazards. Some factors (for example, whether a hazard is voluntary in terms of exposure or technological in origin) predict people's responses *across* different hazard domains (i.e. the extent to which risk is perceived to be involuntary increases the threat perception for all kinds of hazards). Other factors are domain specific (for example, people may have concerns about the potential for negative effects on animal welfare in the case of BSE, concerns that will not apply to other types of potential hazard such as food irradiation).

Psychological factors are important in influencing people's responses to a particular hazard. The technical risk estimates traditionally provided by experts have little influence on people's behaviours and responses. In comparison, people's risk perceptions are a far more influential determinant of their responses to different risks. For example, a risk that people perceive to be involuntary in terms of their personal exposure is more threatening

than one that they choose to take, even if the probability of harm is the same, or possibly even less. For similar reasons, naturally occurring risks are less threatening than hazards that are technological in origin. Natural risks (for example, being struck by lightning) are perceived as less frightening than other equivalent risks that are technological in origin. People fear potentially catastrophic hazards more than those that affect a similar number of people, but at different times and places (Slovic, 1993; Katsuya 2001). Other concerns are very specific to particular hazard domains, and this is very much the case in relation to food (for example, see Miles and Frewer, 2001).

Public risk perceptions have been shown to be particularly important determinants of public responses to activities in the agri-food area. These include food safety (Fife-Schaw and Rowe, 2000; Verbeke, 2001), the biosciences, (Frewer *et al*., 1997), and the possible unintended negative environmental and health impacts of technology (Levidow and Marris, 2001). All of these examples reflect the observation that public risk perception has been driven by the failure to provide information relevant to the actual concerns of consumers, and instead providing information that focused on the technical risk estimates derived from expert knowledge.

It should also be remembered that food choice is as much a cultural, social and emotional process as it is a rational choice, and the purchase of products perceived as recycled waste may be problematic in terms of perceived quality reduction. Consumers also demand the enforcement of effective traceability systems and, as a consequence, are likely to demand the introduction of utilitarian labelling strategies focusing on both sustainability and food production. Consumer perceptions that food-waste recycling is occurring in a non-transparent manner may compromise acceptability of the resultant products, particularly in the food sector. Potentially negative emotional responses regarding the consumption of food waste may also be problematic. Finally, consumers are not homogeneous with respect to their attitudes, perceptions, and food choices, and what is acceptable or desirable to some consumers may be viewed negatively by others. It is important, therefore, to ensure that consumers are able to choose whether or not to eat foods produced using particular technologies or addressing some other agenda (for example, sustainable production).

It is useful to consider the example of the BSE scare in the context of by-product recycling, as it has had a potential impact on consumer acceptance of other attempts to recycle food waste. Miles and Frewer (2001) have noted that public risk perceptions associated with the BSE crisis were driven by the failure of government and the industry to provide information relevant to the actual concerns of consumers. BSE-related risk communication was based on technical risk assessments, ignoring key issues of concern to the public. These included worry about animal welfare and effective communication regarding risk uncertainty. The latter was particularly salient given that consumers perceived that uncertainty regarding the risks

of BSE was being hidden by the authorities prior to 1996 in order to protect their own interests, and those of industry. Other consumer concerns focused on the use of technology in food production *per se* (for example, technology applied to animal husbandry), and the potential that unintended effects associated with the application of these processes could occur. Frewer and Salter (2002) observe that, as a consequence of the BSE scare, the decline in the public's trust in science has passed a 'threshold point' where the legitimacy of scientific judgement is questioned. The rise of 'consumer citizens' (who express informed choice via purchase behaviours), combined with the diminished role of the 'expert' as a consequence of wide availability of specialist information through the Internet, means that simply explaining that a product is safe and sustainable will not result in successful commercialisation. In addition, research must be conducted to prevent product developers misunderstanding consumer preferences regarding novel product development.

2.3 Implications for food processors

Food processing is a costly enterprise. Research and development costs are very high, but profit margins are low compared with other sectors. The level of regulatory scrutiny currently imposed on new food products is high and unprecedented, increasing the cost of developing novel products. The development and costs of such regulatory requirements may have significant negative impacts on by-product management in food processing. This implies that only a few companies may decide on the management of by-products. The corporate control of food processing in general is likely to generate considerable social concern. Despite all the promises, agenda setting in the food industry still seems focused on short-term goals. These relate to conventional, high-yield industries aiming at profit. Consumer concerns about corporate control are immediately related to issues of ownership and food-supply-chain control. Management of by-products is seen as economic investment that requires return. This will raise concerns about the accessibility, monitoring and desirability of the use of by-products in food products.

The use of by-products could be perceived by consumers as a technological solution for improved sustainability which will cause other problems. Some consumers may perceive that innovations in this area will contribute to a further industrialisation, economisation and mechanisation of agricultural production that is seen as undesirable. Thus the development of effective commercialisation strategies regarding novel, sustainably produced food products must focus on regaining consumer confidence in food production and food technology, as well as communicating effectively and involving consumers in the broader debate on sustainable food production, and how this might be achieved. The emphasis should be on understanding

what *consumers* understand by sustainability, and how this might be introduced into product design. Consumers must not be regarded as passive recipients in the process of the introduction of new food products, but should be taken into partnerships as the potential sources of inspiration regarding new innovations. Several studies have demonstrated that there is a positive effect associated with consumer involvement in product development. This explains why producers of innovative food products who work closely with consumers have a larger innovation success rate (e.g. Von Hippel, 1976, 1988, 2001; Lundvall, 1988, 1992; Coombs *et al*., 2001; Hoogma and Schot, 2001). Adopting such a strategy may overcome consumer negativity linked to the application of food technologies to food production. Such consumer negativity may be problematic in the context of food-waste recycling, particularly if the resulting novel products are destined for human or animal consumption. Depending on its research agenda and achievements, the food industry may change its perspective on sustainability in society. Successful by-product management in the future is contingent on societal recognition of a clear relationship between the food industry and sustainable practices. Initially, this could be attempted on a case-by-case basis, the results of different cases being used to generate generic strategies for commercialisation of food by-products in the future. Unless consumers can agree that the benefits of by-products management are equivalent to sustainable, desirable and acceptable food production practices, consumers are unlikely to recognise and realise many of the potential benefits of by-products management.

It is, of course, important that the food industry develops a 'code of conduct' regarding the socio-economic impact of sustainable production processes. Such a code may not just serve humanitarian, ethical, environmental and other 'non-competitive' goals, but may *also* serve an economic purpose. Quality and environmental policy are, for example, integrated parts of the ISO 14000 certification. Codes of conduct are likely to be particularly relevant when producers face a lack of trust from their stakeholders, when laws and regulations of the government are not specific regarding the issue at hand and when people from different cultures meet and interact. In the absence of an international government or shared norms and values, codes of conduct may offer a solution that is clear to all those involved in the issue at hand. Ingenbleek and Mol (2003) have noted that such codes are of particular interest as part of strategies where firms adopt codes of conduct on food safety and/or sustainability. If customers perceive these attributes as not being beneficial to themselves, but as beneficial to society more generally, they may not be willing to pay for the development and implementation of the strategy because of the resultant increased prices in the retail sector. Thus codes of conduct regarding sustainable production may reflect *societal* value rather than *customer* value, but should not be associated with increased prices relative to similar products not produced in a sustainable way.

2.4 Future trends

Overall, there seems to be a place and a need for substantial research into the mechanisms of agenda setting in the food processing industry, the extent of corporate control and the diversity of society's evaluation of such issues. Policy makers and researchers seem aware of the need to include society and the need to deserve a 'license to produce' and a 'license to sell', rather than to exclude society and go on regardless. How this awareness can be incorporated into day-to-day management decisions is as yet unclear and requires more analyses and possibly new approaches. However, it is clear that consumer acceptance of novel foods produced using food by-products is contingent on understanding consumer concerns and preferences relating to both food products themselves, and the processes by which they have been produced.

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Part II

Optimising manufacturing to minimise waste in food processing

3

Chain management issues and good housekeeping procedures to minimise food processing waste

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3.1 Introduction

The environmental movement of the 1990s prompted many business managers to think about the impact of their activities on the environment. Before then, most managers seemed to think that generating waste was an unavoidable function of conducting business. Currently, managers are increasingly focusing on minimising waste and adding value to waste by-products.

Minimising the volume of pre- and post-production organic and non-organic waste is vital to companies in the food processing supply chain. Essential business objectives for minimising solid waste throughout the supply chain are to minimise inputs and reduce the potential for waste, and to contemplate re-engineering or creating co-products to add value.

Waste minimisation is an efficient and up-front solution to waste management that can significantly alter the way a business thinks about and treats waste (May & Flannery 1995). Although recycling is the solution that is usually embraced by those concerned about waste, waste minimisation is a superior approach because it aims to eliminate or reduce the quantity or toxicity of manufacturing wastes in addition to waste generated by products at the end of their useful life. Waste minimisation approaches range from good housekeeping practices to product redesign, and result in less waste being created, stored, treated, or discarded by a company.

Not only is it an environmentally superior alternative, waste minimisation is a competitively sound tactic for companies. It allows firms to reduce escalating disposal costs, adhere to governmental regulations, be viewed as a 'green' (environmentally sound) company, and increase their profits.

The main themes of this chapter are the key reasons to minimise waste, chain management to minimise waste, good housekeeping procedures to minimise raw material waste, the effective implementation of measures to minimise waste, and future trends. A case study is presented to demonstrate that a company can realise significant waste reduction and associated cost savings by forming a supply-chain partnership programme. At the end of the chapter, sources of further information and advice are presented.

3.2 Key reasons to minimise waste

The amount of food waste and by-products generated in the European Union (EU) is approximately 222 million tonnes annually. The food industry produces an immense amount of biodegradable waste that causes problems in landfills and wastewater treatment systems. When buried in landfills, biodegradable waste generates methane (which contributes to the greenhouse effect) and causes the release of toxic materials that can contaminate groundwater. Wastewater treatment systems can be overloaded with biological and chemical oxygen demand (BOD and COD) from the sizeable quantities of effluents and residues discarded by food processing facilities. Solid waste generated by food processing facilities includes excessive packaging, waste oils, off-spec products, food waste, outdated inventory, empty raw materials containers, damaged pallets, spent and dirty filters, and sludge. All of these solid wastes represent a loss of resources and money.

The most effective way to minimise the losses associated with waste is to avoid producing the waste in the first place. Reducing the amount of solid waste generated will lower operating costs, reduce waste disposal costs, enhance workplace safety and health, decrease long-term liability, help sustain environmental quality, and project a positive public image.

Excessive packaging has contributed to an overabundance of solid waste. Rising solid waste disposal costs, decreasing availability of landfill space, and consumer pressure are causing food processing manufacturers to re-evaluate their use of packaging. Legislation is also influencing packaging changes.

Disposing of waste in landfills has been declared unsustainable by the European Union. Disposal to landfill is the last option in the solid waste hierarchy. There are a number of reasons why landfilling is considered unsustainable. Disposal wastes potentially valuable resources and increases the depletion of resources. Landfills take up precious land space and cause water, air, and soil pollution. They discharge toxic chemicals into the soil and groundwater, and carbon dioxide and methane into the atmosphere.

3.2.1 Legislation

A Community Strategy for Waste Management was published in 1989 to address the problem of increasing amounts of waste being disposed. The

Waste Strategy, as amended in 1996 (COM(96)0339), lays down legal principles that will form the framework of the European Waste Policy. These legal principles include:

- The prevention principle: waste generation should be minimised as much as possible.
- The polluter pays principle: whoever produced the waste should pay for handling it.
- The precautionary principle: one should anticipate problems related to waste.
- The proximity principle: waste should be handled as close as possible to where it was generated.

There were three areas of significance distinguished within the Waste Strategy:

1 Waste management hierarchy: waste prevention, recovery, optimisation and minimisation of disposal must be given priority.
2 Producer responsibility: producers must take back end-of-life products.
3 Control of waste shipments: imports and exports of waste within EU countries are regulated.

The United Kingdom (UK) adopted Waste Strategy 2000 for England and Wales, which sets challenging targets for business and industry. For example, it requires the reduction of industrial waste landfilled to 85% of 1998 levels.

The Integrated Pollution Prevention and Control Directive 96/61/EC lays out procedures to eliminate, prevent, and reduce pollution generated at industrial facilities. The polluter pays principle is emphasised in this directive. Certain large food processing facilities must obtain operating permits under this directive.

The EC Packaging and Packaging Waste Directive 94/62/EC (1994, amended 2004) promotes waste minimisation, reuse, and recycling. It set recycling rates for businesses of 70% recovery of packaging waste by 2008. It also set material-specific recovery targets by weight for glass (60%), paper and paperboard (60%), metals (50%), plastics (22.5%), and wood (15%).

Packaging (Essential Requirements) Regulations 2003 set requirements that all packaging items must meet before entering the UK market. Packaging volume and weight must be the minimum amount needed to meet safety and hygiene requirements, and packaging must be recoverable. The UK's system sets recovery and recycling targets for businesses that are 'producers'. Businesses required to follow the regulations are those with an annual turnover in excess of £2 million and handling more than 50 tonnes of packaging; these businesses are required to recover and recycle specified tonnages of packaging waste each year, based on the packaging that goes through their business.

The Landfill Directive (1999/31/EC) is dramatically changing the way the UK manages waste. The directive took effect in the UK on 15 June 2002 as Landfill (England and Wales) Regulations 2002. The regulations reclassified types of landfills, banned liquids and certain materials (including biodegradable wastes), and encouraged recovery and reuse of waste. This directive has tremendous implications for the food industry since they produce so much biodegradable waste. The directive will stimulate the development of technology for alternative uses of food waste. A Biowaste Directive is being developed and its final adoption is anticipated in 2006.

3.3 Chain management to minimise waste

The food supply chain is a network of companies that handle food, from the farmer to the retailer. Supply-chain management is a relatively new concept that first appeared in the 1980s in the Japanese motor industry. The Japanese recognised that a business working in a vacuum would find it increasingly challenging to improve its success in increasingly competitive markets. In order to meet consumer needs more efficiently and effectively, a business must work cooperatively with its suppliers and distributors. Although the food industry has been slow to replicate the motor industry's success in optimising chain management, it appears that it is gradually becoming a priority throughout the global food industry.

A fundamental shift in the operation of the food supply chain took place as a result of the Efficient Consumer Response initiative, which originated in the United States (US) grocery industry in 1993 and later spread throughout Europe. Cooperation and coordination replaced adversarial trading relationships, and companies began exchanging strategic and operational information. Food manufacturers that have adopted chain management strategies are reducing production lead times by days, inventory levels by weeks, and new product development cycles by months, resulting in substantially lower costs.

Economic pressures are driving the evolution of the global agri-food chain and promoting greater vertical and horizontal coordination to develop collaborative marketing ventures. By establishing closer partnerships between suppliers at all levels in the chain, it is enhancing the total performance of the entire supply chain and increasing its efficiency and responsiveness.

A crucial factor in supply-chain waste reduction programmes is partnership based on trust between each of the participating members. This differs from the competitive manner in which business is presently conducted and the negotiation of the 'best price' for goods and services provided throughout the supply chain. For that reason, it is vital to obtain

a solid union among members of the supply chain and establish a cooperative culture.

Environmental supply-chain management emphasises activities that reduce, reuse, recycle, compost, vermicompost, or substitute materials. To minimise waste, the principles of integration and partnership should be utilised together. Companies in the food processing supply chain must work together using an integrated approach to waste management. A life-cycle approach ought to be applied to the environmental impacts of each product.

The best way to minimise, or eliminate, food waste is to not create it. This may be accomplished by streamlining processes and using resources more efficiently.

Some of the world's top companies are using the following approaches to enhance environmental supply-chain management:

1 A supply-chain integration approach to environmental management links reduction in energy consumption to waste generation.
2 Top management commitment to environmental issues is critical.
3 Employee suggestions must be elicited and rewarded to solve environmental problems.
4 A synergy between a company's environmental image and environmentally sensitive products is imperative.
5 Using life-cycle management and environmental audits improves environmental and operating performance.
6 Setting quantitative targets for different environmental performance measures is valuable.
7 Identifying vendors that can collect, clean, and reship process waste back to the company.

The distribution of cost savings between supply-chain members who undertake waste minimisation practices is difficult to determine as improved efficiencies lead to less product/raw material being purchased. In some ways the inefficiencies of supply chains are promoted by the businesses concerned because it increases their sales volumes.

Despite the barriers to supply-chain cooperation on waste reduction issues, it will probably continue to move forward as the corporate community acknowledges its joint responsibility for the environmental impacts of its products.

3.4 Good housekeeping procedures to minimise raw material waste

Housekeeping is a general term used to describe how a facility's daily activities are carried out. Housekeeping measures include storage, inventory control, cleaning, maintenance, and record keeping. Good housekeeping is

Table 3.1 A hierarchy of waste minimization approaches (from May & Flannery 1995)

I	**Waste prevention and reduction** • Good housekeeping: improve maintenance procedures, monitor leaky valves and fittings, maintain proper material handling and transfer, segregate waste streams, and tighten inventory controls. • Material or product substitution: replace hazardous materials and products with less toxic or less hazardous ones. Use water-based paints and cleaners instead of oil-based equivalents. • Process modification: alter manufacturing processes themselves. Use 'environmental re-engineering' to change thinking about the environmental impact of such processes.
II	**Recycle and reuse** • Collect and treat waste streams such as used oils and solvents. Use on-site or off-site recycling processes. • Design products for disassembly and reuse.
III	**Waste treatment** • Prevent hazardous waste from entering the environment. Treat waste with biological, chemical, or physical processes to break down waste components into harmless compounds. • Develop waste treatment services and offer services to customers and other businesses.
IV	**Waste disposal** • Ensure that landfills used for waste disposal are designed to protect the environment, particularly underground water supplies.

crucial for ensuring that a company is run efficiently. It is also the first and most essential step to minimising waste and saving resources. Good housekeeping is usually at the top of the list of waste minimisation strategies because it is the simplest, most cost-effective approach (see Table 3.1). The objective of good housekeeping is to minimise material losses and prevent unnecessary waste generation through the practice of routine procedures. Good housekeeping practices in commercial settings are similar to those in a home. They are things that can be done to maintain cleanliness and prevent spills or accidents. Good housekeeping approaches include improving maintenance procedures, monitoring leaky valves and fittings, maintaining proper material handling and transfer, segregating waste streams, and tightening inventory controls.

Good housekeeping procedures can be applied to all activities in food processing facilities, including purchasing, materials accounting, inventory control, receiving, delivery, storage, proper labelling, optimising operations and processes, preventive maintenance, and recovery/reuse/recycling.

3.4.1 Good housekeeping measures for specific activities

The following are specific good housekeeping and waste minimisation measures that can be taken in each area of business activity.

Purchasing

- Conduct material purchases through a central person or department to make sure that waste reduction purchasing guidelines are followed and to eliminate unnecessary acquisitions.
- Draw up purchase agreements that spell out terms and conditions for receiving supplies. Include provisions that allow the inspection of materials before accepting them. Specify terms of responsibility for each party in case of a material release. Ensure that specified procedures are followed by documenting agreements with suppliers and making sure that shipping personnel have a written copy.
- Assess the materials used and buy those that are the least toxic and least costly to handle.
- Before purchasing new equipment, make sure it can be easily maintained, as preventive maintenance can save three to four times the equipment cost by reducing breakdowns.
- Purchase supplies and packaging with recycled content.
- Work with farmers to grade produce more effectively so that poor-quality produce remains on-farm (this reduces storage and disposal).
- Buy pre-cleaned raw products to reduce cleaning required in-process.
- Work with suppliers to develop innovative options for product delivery.
- Containers: take into account the intended use of various materials and standard operating practices, and buy them in the size and type of container that will minimize material losses and costs. If materials are usually transferred from large to smaller containers before use, purchase smaller containers to reduce potential spillage, material evaporation, contamination from returning unused material to the original container, and expiration of unused materials. Reduce handling losses by purchasing pre-weighed packages. Buy in bulk to reduce the number of containers requiring disposal. Purchase containers that are wider than they are tall so that less material clings to the sides; more material gets used so there is less container residue. Purchase containers that minimise disposal challenges (e.g. substitute single-use aerosol spray cans with fillable pressurised spray cans).

Materials accounting

- Integrate materials purchasing, handling, inventory, and sales procedures to facilitate accurate materials accounting.
- Devise real-time materials tracking and monitoring systems that report on waste generation and discrepancies from efficiency standards.

Inventory control

- Minimise inventory by purchasing and storing only what you need as the disposal of excess or out-of-date supplies may outweigh the costs of waiting for resupply shipments.

- Use a 'first in, first out' inventory policy for raw materials, and rotate perishable stock at every delivery.
- Maintain proper temperature, humidity, etc. to reduce material degradation.
- Computerise your tracking system.
- Negotiate with vendors to accept back excess, off-spec, or expired materials.
- Look for substitutes with longer shelf lives.
- Reduce the number of similar types of products.
- Use waste exchanges for overstock or off-spec materials.

Receiving

- Reduce unnecessary waste generation, spills, and returns, and prevent acceptance of shipments that are improperly packaged, off-spec or incorrect by using the following procedures.
  - Receive incoming materials in a designated area and design the area to prevent and control spillage of materials.
  - Prevent waste disposal, property losses, and injuries by training receiving employees how to properly handle incoming shipments.
  - Before accepting incoming goods, check for damaged, opened, or leaking containers, test for off-spec materials, and verify expiration dates.
  - Choose suppliers that are reliable and have high-quality service.
  - Ask suppliers to offer products with minimal and reusable packaging.

Delivery

- Ask customers to receive shipments in a designated area to prevent and control accidental material releases.
- Draw up delivery agreements that require customers to inspect deliveries and document that the materials were received in an acceptable condition.

Storage

- Maintain proper temperature and humidity to reduce material degradation.
- Keep storage areas well lit and clean, and allow space to easily access all containers.
- Keep aisles free of obstructions.
- Place new materials behind older stock.
- Use high-quality containers that can be resealed to prevent spills, evaporative losses, and contamination.
- Stack containers in a way that will not allow them to tip, puncture, tear, or break.
- Do not lean or stack equipment against containers.
- Segregate hazardous from non-hazardous wastes.

- Use dry clean-up methods such as brooms, vacuums, and absorbent materials.
- Plug floor drains in material storage areas.
- Remove water supplies from storage areas.
- Store dry materials off the floor and away from liquids by placing catch pans beneath the material.
- Build kerbs around storage areas to keep spills in and water out.
- Prevent corrosion from concrete sweating by storing drums off the floor.

Labelling

- As materials are received, ensure they have labels indicating product name, weight, concentration, lot number, date, hazard class, and intended use.
- Label all portable bottles.
- Label containers and tanks that contain hazardous materials or wastes.

Operations

- Scrutinise layout of plant and equipment to identify opportunities to improve cleaning and maintenance efficiency.
- Improve process start-up and shut-down, and changeover frequency.
- Test the incoming water supply for minerals or chemicals that can spoil the process and cause waste.
- Prevent spills by providing suitable training, equipment, tools, and work areas for operators. Dispense bulk liquids with gravity spigots or pumps. Use a spout and funnel to transfer liquids to different containers.
- Provide recipes and measuring equipment for each chemical treatment operation.
- Reduce the use of salt and dye by optimising the chemistry and temperature of the process.
- Label valves and settings clearly to reduce the chance of inexperienced staff changing things incorrectly or turning them in the wrong direction.
- Use small-volume equipment for small production runs.
- Reduce cleaning requirements by scheduling sequentially the products that use the same production line or equipment.
- Dedicate mixing and production lines to particular products to reduce changeover cleanups.
- Schedule jobs in batches and maximise batch size.
- Avoid shifting production schedules for rush orders.
- Routinely calibrate and adjust automatic process control devices to prevent loss and increase productivity.
- Use high-quality valve and piping material that minimises corrosion and leaks.
- Install spring-loaded nozzles or timers on water supplies to turn them off when not in use.

- Keep records of when and why larger spills occurred to help determine how to prevent future spills.
- Use mechanical or manual wall wipers on tanks.
- Check and service equipment on a regular basis to avoid breakdowns and waste creation.
- Conduct periodic waste audits to identify opportunities for improvement.
- Redesign products to make them less wasteful for the end user.
- Remove soil and other solid wastes without using water.
- Separate useful products from the waste stream early in the process to prevent contamination and maximise the potential for recovery and reuse.

Preventive maintenance

- Inspect storage areas for expired materials and proper labelling.
- Routinely look for potential sources of leaks and spills by checking: pipes, valves, and hoses; process and storage tanks, and equipment attached to them; storage areas for materials, wastes, and empty containers.
- Prevent leaks by using welded pipes instead of flanged pipe joints.
- Install and routinely inspect spill and leak control equipment such as leak detection systems, overflow control devices and alarms, relief valves, interlock devices to stop flow to leaking sections, splash guards and drip boards, and spill basins on dikes.
- Use pumps with double mechanical seals or canned (seal-less) pumps.
- Use flange guards, double seals, and bellow-sealed valves.

Recovery/reuse/recycling

- Keep waste streams separate to maximise reuse and recycling. Provide separate bins to enable proper segregation.
- Use local waste exchange programs to share wastes with other companies.
- Collect solids and reincorporate into the product.
- Make food waste a food source for animals or convert to a by-product for human consumption.
- Recycle lubrication oils.
- Sell to recycling companies, composters, and worm farms.
- Set up an area for unwanted materials and equipment that employees can take home.
- Donate unwanted equipment and materials to schools, charities, and other non-profit-making organisations.
- Reuse, recycle, or compost excess, off-spec materials, and samples taken for quality control testing. Off-spec material can be added to an already existing waste stream that is used for animal feed. If some organic waste products are not suitable for use as animal feed, then compost them.

There are several ways that packaging waste can be reduced. Packaging waste from shipping and receiving activities includes cardboard boxes, pallets, plastic-lined cartons, plastic bags, plastic containers, plastic film, metal cans, paper, and aluminium foil. Choose to purchase goods in bulk, with less packaging. Buy larger sizes for products that are used a lot. Discover ways to reuse some of the packaging internally. Arrange with vendors and customers to use returnable packaging (such as reusable plastic pallets), reduce packaging, use packaging that may be easily recycled, and use packaging with recycled content. Return empty containers to suppliers for refilling. Buy heavy-gauge steel drums that can be reconditioned, then recondition them on-site or contract with a drum conditioner.

3.4.2 Good housekeeping recommendations for specific industries to reduce waste

Fruit and vegetables

The principal processing steps include: (i) general cleaning and dirt removal; (ii) removal of leaves, skin, and seeds; (iii) blanching; (iv) washing and cooling; (v) packaging; and (vi) clean-up (US-AEP 1997). Waste includes peelings, stems, seeds, shells, etc. and products that are off-spec, damaged, out-of-date, or returned. Reduce waste by using air flotation units to remove debris from raw fruits and vegetables. In addition, try to wash, grade, and trim crops in the field so that the waste can biodegrade in nature rather than becoming a solid waste problem in buildings. This type of waste can be reused as animal feed or converted to compost, mulch, or soil conditioners.

Bakery

This waste is caused by overproduction, product deterioration, damaged goods, spills, or operator errors. Improving process control will have the greatest impact on reducing this waste stream, since most of the waste is due to cutting errors, incorrect weight, misforming, and contamination. Waste can also be decreased by completely draining mixers, troughs, and tanks before cleaning and removing solids from equipment or the floor before wetting those surfaces. Bakery waste can be reused by feeding to animals or composting.

Prepared foods

This waste includes raw products, sauces, grease, spices, additives, oils, drippings, off-spec product, spoiled materials, and damaged finished products. Waste can be decreased by appropriately storing raw vegetables in reusable containers to prevent dehydration and spoilage, and recycling used grease, cooking oil, and meat fat. Prepared food waste can be reused or recycled by donating unspoiled perishable or non-perishable foods, processing it into animal feed, composting it, or rendering it.

Meat and poultry

The principal steps in processing livestock include: (1) rendering and bleeding; (2) scalding and/or skin removal; (3) internal organ evisceration; (4) washing, chilling, and cooling; (5) packaging; and (6) clean-up (US-AEP 1997). Waste includes carcasses, hides, hoofs, heads, feathers, manure, offal, viscera, bones, fat and meat trimmings, blood and other fluids, and off-spec animals and meat. There are several options for reducing this waste. Install strainers along evisceration lines to keep by-products off the floor. Attach strainers to drains in the de-hairing process area. Send blood to a blood collection facility. Remove fat from conveyor belts by scraping rather than spraying. Before washing areas, collect solids with squeegees, brooms, or shovels. Waste reuse and recycling options include animal and pet food, composting or vermicomposting of paunch manure, fertilizers, cosmetics, blood meal, gelatin from heads, and glue from hides.

Dairy products

The dairy sector consists of two segments: fluid milk and processed milk products (which originate from fluid milk). The principal processing steps are: (1) clarification or filtration; (2) blending and mixing; (3) pasteurisation and homogenisation; (4) process manufacturing; (5) packaging; and (6) clean-up (US-AEP 1997). This waste consists of off-spec goods, damaged or out-of-date products, cheese solids, curd, whey, and milk sludge from the separation process. Reduce solid waste by dedicating lines to particular products, avoiding product spillage when disconnecting hoses and pipes, provide a foolproof whey collection system to avoid leaks from valves and fittings, provide whey storage tanks of twice the maximum daily volume to avoid tank overflow, and prevent sludge from entering the wastewater stream. Reuse or recycle waste generated by collecting spilled solids for reprocessing or animal feed, developing markets for whey solids, creating processes to extract and produce proteins and carbohydrates from whey, and using anaerobic digestion to create methane or to ferment for alcohol production.

Seafood

Waste includes: off-spec, by-catch, and rubbish from fishing operations; skins, bones, cuttings, viscera, oils, and blood; brines, sauces, spoiled products; damaged, out-of-date, or returned goods. To reduce waste: improve the quality of fish delivered to the plant; use a vacuum to remove skin, fat, and flesh from the skinner drum; install trays and chutes around filleting machines to catch solids; vacuum offal for oily fish processing; and remove offal from the filleting line by dry methods instead of using water. Waste can be reused or recycled into pet food, protein hydrolysate, fish meal and oil, bait, or compost.

3.5 Effective implementation of measures to minimise waste

In addition to improving good housekeeping practices, source reduction methods that may easily be employed at food processing facilities include making process modifications, substituting more environmentally friendly raw materials, and segregating waste streams for reuse or recycling.

After taking the first step of improving housekeeping, companies can move on to technology improvements and material substitutions. Such changes can require some capital outlay, however savings in waste disposals, and energy and water use, can result in relatively short payback periods.

A company's commitment to waste reduction should begin at the top. If managers fully support waste minimisation, employees will feel motivated to participate. Companies should consider writing a formal environmental policy that includes waste minimisation. They should also publicise their commitment to waste reduction, as customers feel good about doing business with an environmentally responsible company.

Waste minimisation training should be provided to all employees so that they are aware of the sources of waste generation and proper waste handling methods. Training ought to be repeated regularly for reinforcement and to allow for employee turnover. Employees should be invited to help design and implement waste reduction measures. A reward system will encourage employees to adopt waste minimisation techniques and to suggest changes in design or operating procedures that would reduce more waste. Companies have drastically cut their wastes and saved millions of pounds by following employee suggestions submitted through reward programmes.

Before designing a waste minimisation programme, it is important for companies to know what materials are being discarded. By examining their waste stream, companies can determine the types and amount of waste items being disposed of and decide which of the items can be eliminated, reused, recycled, or composted. A waste audit will help companies better understand their current purchasing, waste generation, and waste disposal practices. A waste audit also provides a baseline from which to measure the success of a waste minimisation programme. While collecting information about the costs associated with solid waste management, company managers who view waste disposal as a fixed overhead cost may be surprised to see the various costs involved. Checking purchasing records may also be an enlightening activity that encourages companies to change their waste management and purchasing policies. When conducting a waste audit of a facility, list all wastes generated, ascertain the composition and source of each waste material, and identify options for reducing each waste. Companies should also periodically reassess their operations and waste handling practices to ensure that they have not slipped back into wasteful routines and to identify additional opportunities to reduce waste.

Companies can use environmental management accounting (EMA) to get an accurate and comprehensive view of materials use and the costs of wasted raw materials. EMA provides information on quantities, flows, and disposal of materials and energy. This allows businesses to track and manage raw materials and wastes more accurately. EMA also improves supply-chain management, purchasing, inventory and production planning, and performance evaluation and benchmarking.

A number of processing options are available to vegetable, fruit, and meat processors. Solids can be de-watered to a moisture content of less than 10% and used for animal feed. Fermentation can add value to solid waste by creating fermented food or compost. Food wastes abounding with carbohydrates can be converted to sugars by a process called enzymatic hydrolysis. Solid wastes can also be directly converted to biodiesel fuel or converted to methane by anaerobic digestion.

The next priority after surplus food elimination or reduction is to reduce and reuse material within the production unit. This can be accomplished partially through more efficient transformations, better matching of supply to demand, and then reuse. By separating surplus food for human consumption, this enables the food to be recycled outside the production unit as either finished goods fit for human consumption or edible raw materials that can be processed into meals. A secondary option to food for humans is animal feed. Next on the hierarchy is the beneficial treatment of surplus food through composting or anaerobic digestion. Incineration or landfilling is the last resort (see Table 3.2).

If edible surplus food is produced, the food supply chain should make a commitment to recycle it for human consumption. Organisations such as Second Harvest and FareShare collect and distribute surplus food at all stages in the food chain. In the United States, Second Harvest collects and distributes 500 000 tonnes annually to the hungry. Several major companies such as Kellogs, Nestlé, Coca-Cola, Unilever, and McCain donate their surplus to this charity. In the UK, FareShare distributes 1500 tonnes of food per year from 100 companies.

Excessive packaging is being reduced and recyclable products such as aluminium, high-density polyethylene (HDPE), polylethylene terephthalate (PET), and glass are being used whenever possible. Some of the

Table 3.2 The food recovery hierarchy (from Johnstona & Green 2004)

- Eliminate surplus
- Reduce surplus and reuse within processing unit
- Recover and recycle surplus outside processing unity (edible finished goods and raw materials)
- People first, animal second
- Beneficial treatment
- Disposal/destruction

packaging changes taking place at food manufacturing facilities are the use of plastic liners in corrugated cartons used within a plant, use of HDPE plastic bags, and substituting foam food packaging containers with biodegradable ones. In the US, Tyson Foods created an incentive programme for employee feedback on how to reduce packaging. One suggestion to redesign an entrée dinner dish saved over a million pounds (in weight) of packaging per year. Food manufacturers that advertise their packaging as more environmentally friendly rapidly gain an advantage over their competition and improve their public image.

Automated technology is increasingly being used by food processing facilities to improve efficiency, control raw material inputs, and reduce the amount of waste generated. Analytical sensors can control contamination levels and flow rates. Although automation has been used for years by the dairy and beverage industries, until recently it has not been used to a vast degree in the vegetable, fruit, and meat processing sectors. Computers can now be used for evaluating conditions that, in the past, only workers could assess. The use of automation helps to reduce the chance of human error in the food processing industry.

3.6 Case study

In 2001, without spending money on new technology, Heinz Australia realised savings of AUS$60 000 per year by reducing waste in the production and retailing of tomato ketchup. This was accomplished by forming a supply-chain partnership programme to develop environmental management systems (EMS) for supply chains in the tomato ketchup industry. Infotech Research headed up the project, with funding and support from The Commonweath Department of Environment and Heritage. Infotech worked with the following organisations involved with the production and retailing of ketchup: Heinz-Watties Australasia, Australian Processing Tomato Research Council, ACI Plastics, Safeway grocery stores, and Visy Industries (a glass bottle manufacturer). The team examined the environmental performance of a bottle of ketchup throughout the entire supply chain and focused on discovering opportunities to reduce the amount of raw material wasted and packaging waste created, in addition to decreasing water and energy use. They discovered that an astounding 56.6% of the tomatoes grown for processing into ketchup are wasted. Most of the waste takes place with growers and at the early stages of processing. Packaging wastes throughout the supply chain are also high, at 0.57 kilograms per litre of ketchup.

This supply chain consists of the following steps and participants. Tomato growers supply their products to the nearby Heinz-Watties tomato paste manufacturing plant in Girgarre, Victoria. At the plant, tomatoes are cleaned, cooked, and converted to paste; the paste is then packed into 1000

litre bladders stored in wooden boxes. Ketchup is then packaged in PET and glass bottles provided by ACI Plastics and Visy Industries, respectively, and stored at Girgarre; it is then shipped to the Heinz-Watties Dandenong warehouse before being distributed to retailers, including Safeway supermarkets.

Of the 56.6% loss of tomato product, 49% is lost by local growers with unharvested fruit or tomatoes in inferior condition or with inadequate colour that fail to meet the quality standards for the paste. There are no other markets for the tomatoes, as they are grown specifically for paste production. Another 10% of the tomato paste is discarded because it does not meet quality criteria at the paste production stage. This is an ongoing loss that costs money in addition to requiring biological treatment before being disposed of in landfills. Subsequent to paste manufacture, tomato-based losses of 2% continue at each stage of the supply chain.

The majority of ketchup packaging waste is generated at the supply chain's formulation, retail, and consumption stages. Half of the ketchup is bottled in PET plastic and the other half is put into glass containers in a range of sizes. In the processing plant, the bottles are placed in cardboard cartons on pallets then wrapped in shrink-wrap before shipping to the warehouse. Half of all of the packaging wastes are recycled. Most of the recycling takes place at the supply chain's warehouse and retail stages, particularly with cardboard waste. Significant amounts of bottling wastes are generated at the formulation stage when newly bottled ketchup does not meet Heinz-Watties quality standards. However, recycling of the bottled ketchup is low because the packaging prevents it from being composted and the bottles must be cleaned before being recycled. So the poor-quality bottled waste is usually sent to landfill. Some of the bottled waste is caused by breakage during storage and transfer, with twice as many glass bottles damaged than PET plastic bottles. Finally, waste generated at the growing stage is comparatively minor and mainly consists of polypropylene drip tape and agricultural chemical containers. The containers are challenging to dispose of as they cannot be directly tipped to landfill.

Based on their findings, the supply-chain members devised goals and plans for reducing waste. Beginning with the growing stages, the growers and Heinz-Watties set a goal of reducing harvest losses by 20% per year, saving AUS$20 000 in the initial year. Their plan was to improve coordination of tomato harvesting and delivery to the facility for processing to reduce tomato losses and poor-quality tomato paste. Growers set the goal of reducing polypropylene drip tape waste landfilled by recycling 80% and saving approximately AUS$500. Another AUS$500 would be saved by recycling chemical containers through the Australian DrumMuster Program. Three packaging waste reduction goals were set. Change bottling from half PET: half glass to 60:40 PET:glass to reduce breakages and transport fuel costs, for anticipated savings of AUS$38 000 per year. Another AUS$2000 per year is expected to be saved by identifying and quantifying causes of

damaged packaging and reducing landfill disposal by 10% per annum. The second goal was to increase recycling of damaged PET bottles and those containing off-spec ketchup. The last goal was to investigate options for adding value to organic wastes by reusing them as refined extracts in tomato sauces or as food additives.

A total of AUS$60 000 per year in savings is anticipated by implementing these initiatives. No new equipment or technology is required; instead, the key to waste reduction is communication between supply-chain partners and organisation of the improvement schemes.

3.7 Future trends

Companies will continue to examine ways to minimise waste, use less packaging, utilise reusable and recyclable packaging, and use more biodegradable packing products. Waste-reducing technology will continue to be created; for example, improved sensors and process control technology will be developed to manage specific portions of the manufacturing process to reduce wastes and increase productivity.

There is a drive to take organic waste out of landfill, which means that food processing operations will have to shift to using more sustainable alternatives. The emerging trend is for source separation systems to aim to minimise the biodegradable fraction of the waste stream. In addition, organic wastes are finding ever-increasing markets for resale. Most food wastes can be processed into valuable by-products and then resold as fertilizer, animal feed, and by-products for human consumption.

A growing trend is the principle of zero emissions, which relies on a network of companies utilizing one company's waste streams as another company's raw materials. Another trend is the development of new methods to add value to food by-products. For example, a consortium of food research organisations from the European Union and select other countries that have experience in adding value to food processing co-products has formed a group called REPRO. With funding from the European Commission, they are working to transform vegetable trimmings and cereal co-products (such as brewer's spent grain) into high- and medium-added-value food, feed and related ingredients such as biopolymers, phytochemicals, nutrients, and micronutrients. Their work will have significant impact, as approximately 1 million tonnes of vegetable trimmings from the vegetable processing industry and 3.4 million tonnes of spent grain from the brewing industry are generated in the European Union annually. The team is developing innovative hybrid systems (such as bioprocesses plus advanced separation/extraction technologies) to deconstruct co-products into marketable product streams; precision enzyme-based bioprocesses to deconstruct and tailor co-products components; and integrated procedures for ensuring microbiological safety,

stability, and traceability of co-products. The project also involves developing strategies to minimise the market risk of the new processes. They will ensure acceptance by consumers and retailers by conducting risk assessments of the technological feasibility, economic viability, environmental safety, and compliance with legislation.

Finally, current and upcoming legislation will continue to drive the food processing industry closer to sustainable practices of waste reduction and recycling.

3.8 Sources of further information and advice

Waste minimisation technical assistance may be obtained from governmental agencies, consulting engineers, professional organisations, non-profit-making organisations, and the internet. The following information details some of the services available in the UK.

In March 2005, a new €284 million initiative was launched to help businesses reduce waste and manage resources more efficiently. The Business Resource Efficiency and Waste (BREW) programme specifically targets waste minimisation. Free services help businesses cut waste at every stage of the business process including research and development, market development, product design, operations, market solutions to waste, and compliance with legislation.

The Envirowise waste minimisation programme helps companies in the United Kingdom reduce the use of raw materials and the production of waste while at the same time saving money. The programme promotes the formation of regional and local waste minimisation clubs. From 1999 to March 2007, the UK government will spend €95.9 million on the programme in an effort to save ten times that amount by reducing waste at the source. Improved management is the primary method of waste minimisation being introduced by the programme. Envirowise helps to change management attitudes by showing that the true cost of waste to an organisation is much greater than they estimate and that significant savings can be realised by reducing waste. Envirowise provides education to companies through publications, seminars, and a telephone hotline. Companies with fewer than 250 employees may receive free visits from consultants. By 2002, the Envirowise programme had helped reduce the material use by over 240 000 tonnes per year, reduced waste disposal by over 1 million tonnes annually, and lowered costs by €200 million per year.

International standards developed by the International Organization for Standardization (ISO) provide a global environmental management system. Called ISO 14000, these voluntary standards help companies manage and evaluate the environmental facets of their operations without being prescriptive. More information about ISO 14000 may be found on the ISO website (http://www.iso-14001.org.uk/).

In April 2004, the first international conference on reducing food processing waste, 'Total Food 2004', was hosted by the Institute of Food Research. Nearly 100 people attended from a wide range of interest groups including food processors, research scientists, consumer scientists, animal feed industries, and non-governmental agencies. Lectures addressed drivers to reduce waste, associated legislation, and consumer concerns. Some promising solutions to waste management concerns were presented, including fractionation and extraction of co-products to obtain potentially valuable components for use as ingredients and nutriceuticals, extraction of functional foods, and whole co-product exploitation. An example was given of how the whey industry has turned a conventional waste stream into a valuable source of nutrients (in protein bars, drinks, and other products) and pharmaceuticals. Plans are being made for a biennial series of conferences to be held internationally, the next conference will therefore be Total Food 2007.

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4

Process optimisation to minimise energy use in food processing

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4.1 Introduction: energy use in food processing

The production of food, which sustains the human energy balance, requires a considerable and continuous supply of energy delivered from natural resources, principally in the form of fossil fuels, such as coal, oil and natural gas. For example, a typical energy requirement for the delivery of 1 J in the form of food consumes almost 10 J from natural resources. In the production of food for human consumption, the processing of food and drink requires a considerable part of this energy (see for example, Hufendiek and Klemeš, 1997; Klemeš *et al*., 1999a,b). The steady increase in the human population of the planet and its growing nutritional demands has produced an annual increase in the energy consumption of the food and drink industry of up to 40% in the last decade.

The accelerating development of many countries with large populations, such as China and India, has resulted in a large increase in energy demands and a steady increase in energy cost. The growing demand for energy from the increase in world population has also resulted in unpredictable environmental conditions in many areas because of increased emissions of CO_2, NO_x, SO_x, dust, black carbon and combustion processes waste (Klemeš *et al*., 2005a). As the developing world increases its food production, at the same time it is becoming increasingly important to ensure that the production/processing industry takes advantage of recent developments in energy efficiency and minimises the amount of waste that is produced.

The food and drink industry has many processes that consume energy. A comprehensive guide to these energy consumers is given in a recently published Best Available Techniques Reference Document (BREF) in the

food, drink, and milk industries (Institute for Prospective Technological Studies, 2006a), and includes the consumers: shown in Table 4.1.

Another related published BREF deals with slaughterhouses and animal by-products (Institute for Prospective Technological Studies, 2006b).

The energy and related environmental cost, and imposed emission and effluent limits, charges and taxation, contribute substantially to the cost of production. A potential solution to the problem is the optimisation of energy consumption, increasing the efficiency of processing and decreasing the emissions and effluents (Klemeš *et al*., 2005a).

However, there are some specific features in food processing that make optimisation for energy efficiency and total cost reduction more difficult when compared with other processing industries; for example in the oil refining industry, there is a continuous mass production concentrated in a few locations which offers an obvious potential for large energy savings (Al-Riyami *et al*., 2001). In the main, food processing is distributed over very large areas and is often producing during specific and limited time periods, for example in the case of campaigns in the sugar industry. In addition, the industry is frequently extremely diverse and relies heavily on small producers and processors.

These particular features of the food production/processing industry have resulted in less intense activity with regard to energy optimisation than has been the case in other comparably sized industries. This has also been the case in targeting for energy savings where the main purpose of such analysis has always been centred around economic performance. If the

Table 4.1 Energy consumers in the food and drink industry

- Material handling and storage
- Sorting/screening
- Peeling
- Washing and thawing
- Cutting/slicing/chopping
- Mixing/blending
- Grinding/milling/crushing
- Forming/moulding/extruding
- Extraction
- Centrifugation sedimentation
- Filtration
- Membrane separation
- Crystallisation
- Removal of fatty acids
- Bleaching
- Deodorisation
- Decolourisation
- Distillation
- Dissolving
- Solubilisation/alkalising
- Fermentation
- Coagulation
- Germination
- Smoking
- Hardening
- Carbonation
- Melting
- Blanching
- Cooking and boiling
- Baking
- Roasting
- Frying
- Tempering
- Pasteurisation
- Evaporation
- Drying
- Dehydration
- Cooling and chilling
- Freezing
- Freeze-drying
- Packing and filling
- Cleaning and disinfection
- Refrigeration
- Compressed air generation

production process is not concentrated, large scale and continuously running, it is more difficult to achieve an attractive payback period, i.e. a short time when invested capital is returned by improved economic performance – by lower energy costs and lower related environmental costs.

However, because of the pressure of ever increasing energy costs and concerns about environmental degradation, even previously economically less attractive and energy-consuming food processing plants – such as those producing sugar, ethanol, glucose, dry milk, tomato paste, vegetable oil, fruit juice, etc. – have become strong candidates for retrofits to reduce energy costs and environmental impact.

In addition, the food processing industry has the potential for integrating the use of renewable energy sources in order to reduce pollution and waste generation, and so reduce overall costs. A typical example is the use of bagasse as a biofuel for generating the energy needed for processing in a cane sugar plant and exporting any surplus electricity into the distribution network.

There are a number of well established methodologies available to optimise the use of energy, and consequently reduce operating costs. Many of these methods only require good management practice: good housekeeping, objective analysis based on optimum measurement policy and planning, and optimum supply chain management based on workflow optimisation. There is also an increasing role in waste management and co-product recovery for life-cycle assessment (LCA), not only in the production chain, but within the complete life span of production, processing, consumption and waste disposal (Koroneos *et al*., 2005; Lundie and Peters, 2005).

An advanced methodology for the improvement of energy efficiency – which has been widely applied in the chemical, power generating and oil refining industry – is process integration (Linnhoff *et al*., 1982, 1994; Shenoy, 1995). This methodology has also been referred to as 'pinch technology' (Linnhoff and Vredeveld, 1984), and the area of the technology mainly associated with heating reduction costs is often referred to as 'heat integration'. This methodology has a large potential in the food processing industry. This chapter is concerned, therefore, with presenting basic information and references, supported by case studies, to demonstrate the energy saving potential of these advanced methodologies when used within the food industry.

4.2 Energy saving and minimisation: process integration/ pinch technology, combined heat and power minimisation and combined energy and water minimisation

A novel methodology to reduce energy demand and emissions on a site comprising of individual processing units and an integrated utility system, and at the same time maximising the production of cogeneration shaft

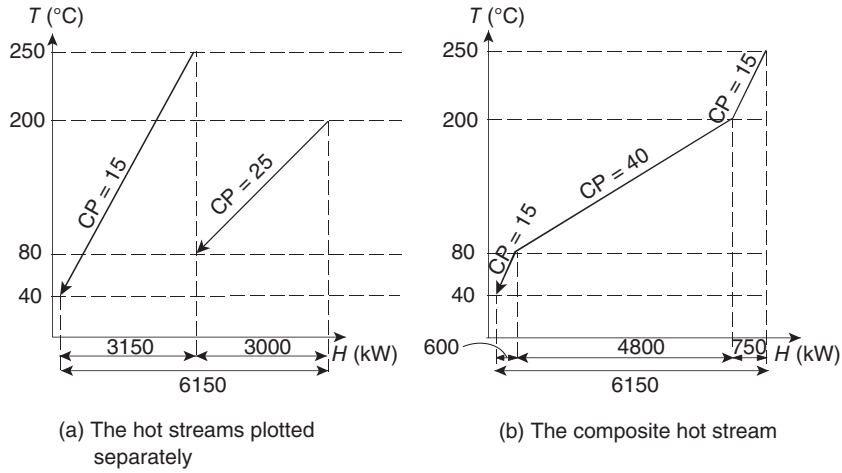
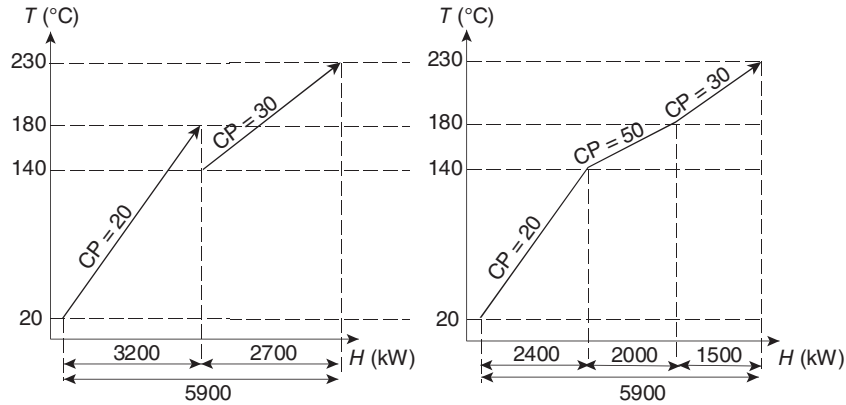


Fig. 4.1 Amalgamating hot streams to create a hot composite curve. CP = $C_p m$ (heat capacity flowrate = specific heat × mass flow) (after CPI 2004a and 2005a).

power, was developed and pioneered by the Department of Process Integration, University of Manchester Institute of Science and Technology (UMIST) (now the Centre for Process Integration at the School of Chemical Engineering and Analytical Science (CEAS), The University of Manchester) in the late 1980s and 1990s (Linnhoff and Hindmarsh 1982; Linnhoff *et al.*, 1982, 1994; Linnhoff and Vredeveld, 1984; Smith, 2005). This methodology is based on the analysis and understanding of the heat exchange between process streams through the use of a temperature–enthalpy diagram. The specific steps for drawing the curves in this diagram are presented in Figs 4.1 to 4.3. The methodology first identifies sources of heat (termed 'hot streams') and sinks of heat (termed 'cold streams') in the process flowsheet. Table 4.2 presents a simple example. Sources of heat can be combined together to construct the composite hot stream (Fig. 4.1) and sinks of heat can likewise be combined together to construct the composite cold stream (Fig. 4.2). The relative location of these curves on the temperature–enthalpy diagram is dependent on the allowable temperature difference for heat exchange. The next step is therefore to select a minimum permissible temperature approach between the hot and cold streams, ΔT_{min}. The selection of the most appropriate or optimum ΔT_{min} is a result of an economical assessment and trade-off between the capital and operating costs (which are mainly costs for energy usage) of the process being analysed. A large ΔT_{min} implies higher energy use and costs and lower capital costs. Consequently for increasing energy cost (for example the price of gas) the optimum ΔT_{min} is reduced, meaning the heat exchanger system is allowed to recover more energy, but at the expense of more capital to pay for the greater heat transfer area. This issue has been discussed in greater detail elsewhere (Taal *et al.*, 2003; Donnelly *et al.*, 2005; Smith, 2005).



(a) The cold streams plotted separately

(b) The composite cold stream

Fig. 4.2 Amalgamating cold streams to create a cold composite curve (after CPI 2004a and 2005a).

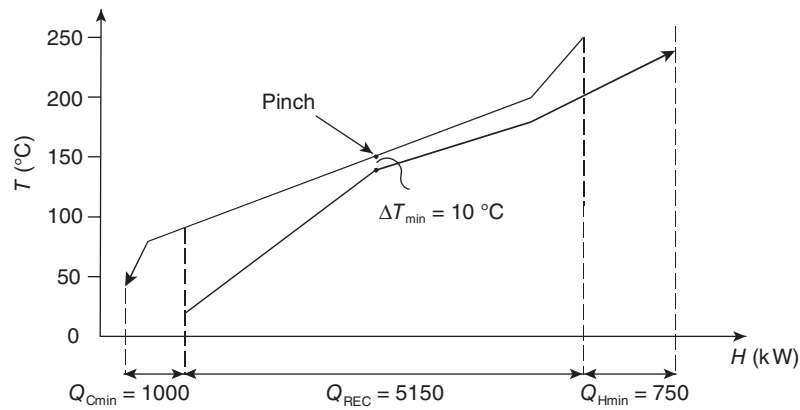


Fig. 4.3 Plotting the hot and cold composite curves. T_{min}, minimum temperature difference; Q_{Cmin}, minimum cooling requirement (cooling duty); Q_{Hmin}, minimum heating requirement (heating duty); Q_{REC}, heat recovery (after CPI 2004a and 2005a).

Table 4.2 Hot and cold streams

Stream	Type	Supply temp. T_S (°C)	Target temp. T_T (°C)	ΔH (kW)	Heat capacity flowrate, CP (kW/°C)
Fresh water	Cold	20	180	3200	20
Hot product 1	Hot	250	40	–3150	15
Juice circulation	Cold	140	230	2700	30
Hot product 2	Hot	200	80	–3000	25

CP = $C_P m$ (heat capacity flowrate = specific heat × mass flow).

In this example, a ΔT_{min} of 10 °C was selected for simplicity. Plotting the composite curves in the same graphical space (Fig. 4.3) allows values to be derived for maximum heat recovery, and minimum hot and cold utilities. These are known as targets. In this particular case of $\Delta T_{min} = 10$ °C, the minimum hot utility requirement is 750 kW and the minimum cold utility requirement is 1000 kW.

In Fig. 4.3 we can also determine the position of the pinch. The pinch represents the position where the hot composite and cold composite curves are at their closest (for a ΔT_{min} of greater than zero). The pinch has provided the name for the heat integration methodology ('pinch technology') and has various important features that make a substantial contribution to the design of maximum energy recovery systems and also to the design of the most economically efficient heat exchanger network.

Various design methods have been developed that allow these targets to be achieved in practice for both grass-roots designs (Linnhoff *et al.*, 1982, 1994), and more importantly, for the retrofit of existing plants (Asante and Zhu, 1997; Urbaniec *et al.*, 2000; Al-Riyami *et al.*, 2001). These methodologies are supported by process integration software that provides both design and retrofit support, and also automated design (SPRINT, 2006; STAR, 2006).

However, in most cases, we have more than one hot and one cold utility available for providing heating and cooling requirements after energy recovery in food processing plants. In these situations our task is to find and evaluate the cheapest and most desirable combination of utilities available (Fig. 4.4). To assist with this choice and to further enhance the information derived from the hot and cold composite curves, an additional graphical construction has been developed. This is known as the grand composite curve (Fig. 4.5) and provides clear guidelines for the optimum placement and scaling of hot and cold utilities. The grand composite curve, together with the balanced composite curves (the composite curves with the selected utilities added) provides a convenient tool for the optimum placement and selection of hot and cold utilities. An example of the selection of utilities and their placement is shown in Fig. 4.6.

The grand composite curve is also a useful tool for targeting the cooling requirements in sub-ambient food processes that require some form of chilling or compression refrigeration. An example of a single refrigeration level providing low-temperature cooling to a process is shown in Fig. 4.7. In this case the grand composite curve provides a target for the heat that has to be removed by the refrigeration process, and shows the temperature at which the refrigeration is needed. However, the overall process/utility system can be improved (Fig. 4.8) by using the heat rejected by the refrigeration system to provide low-level heating to the process above ambient, thereby saving heat supplied by another utility source (such as hot water). Further improvements to the system can also be contemplated, as shown in Fig. 4.9 by using a two-level refrigeration system. This system, compared

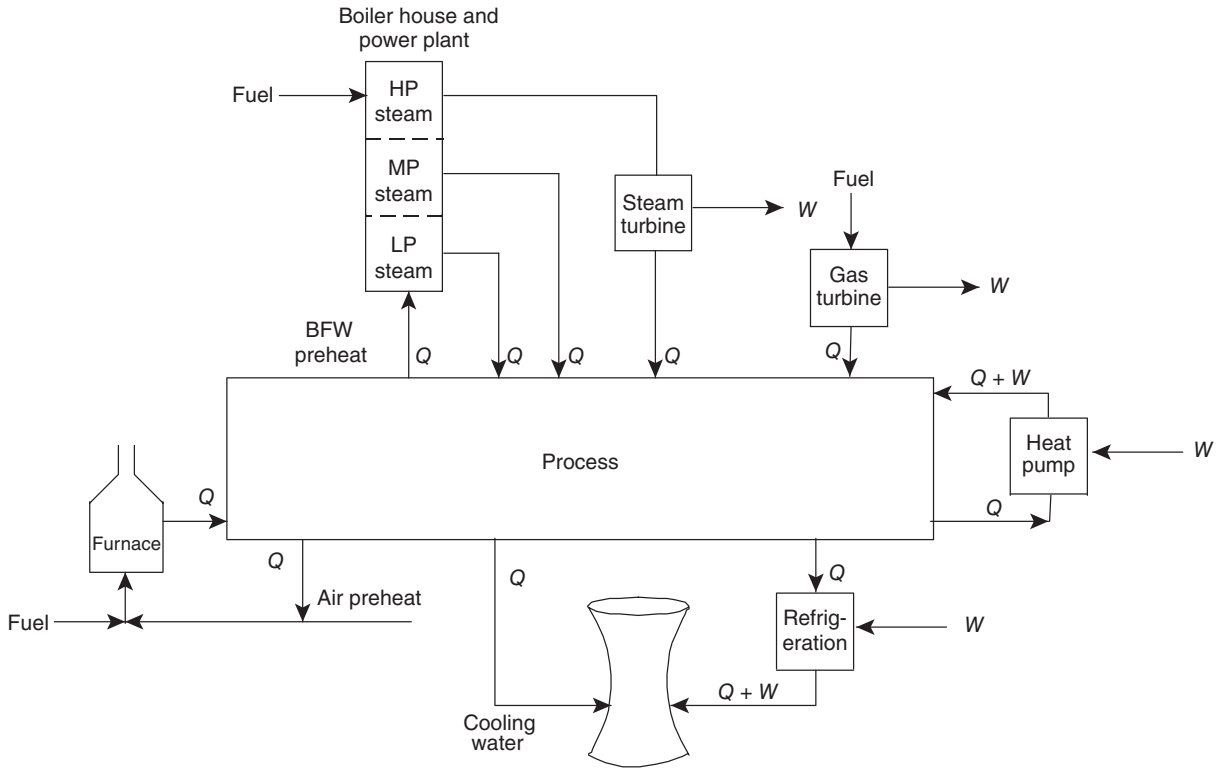


Fig. 4.4 Potential for the choice of hot and cold utilities. HP, MP and LP are high, medium and low pressure, respectively (after CPI 2004a and 2005a).

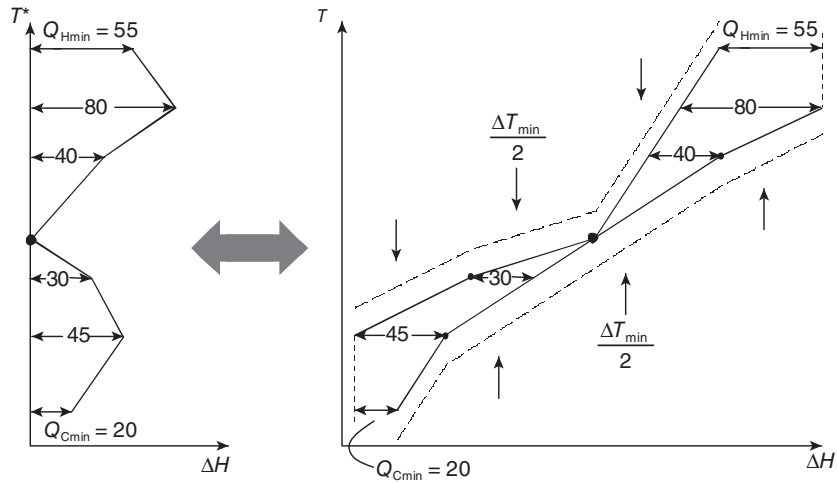


Fig. 4.5 Construction of the grand composite curve from composite curves. *T**, shifted temperature (after CPI 2004a and 2005a).

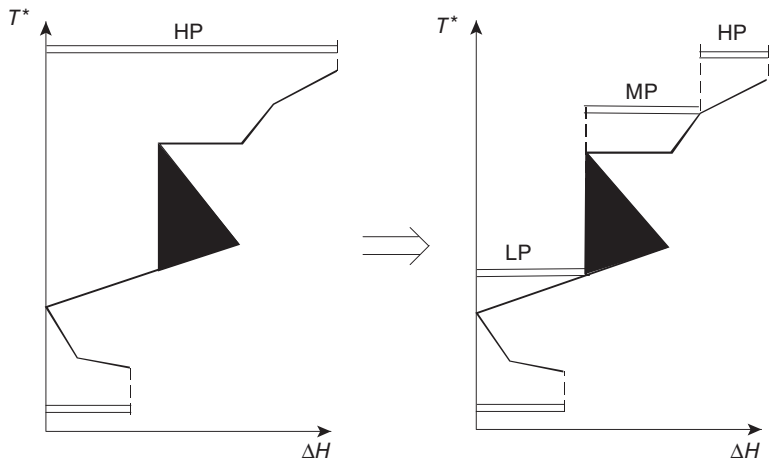


Fig. 4.6 Placement of utilities with the help of the grand composite curve (after CPI 2004a and 2005a).

with the one-level system, reduces the load on the coldest refrigeration cycle, and in most circumstances would reduce utility cost. The location and number of cooling/refrigeration levels required by a food process to achieve maximum energy efficiency and minimal cost is an overall optimisation problem (CPI 2004, 2005; Smith, 2005).

Traditional pinch analysis assesses the minimum practical energy needs for a process through a systematic design procedure involving five steps:

1 Collection of plant data.
2 Setting targets for minimum practical energy requirements.
3 Examination of process changes that contribute to meeting the target.

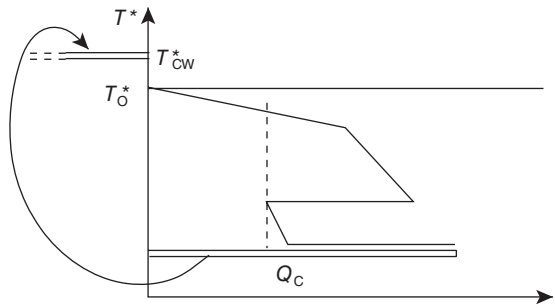


Fig. 4.7 The grand composite curve with cooling provided by a single refrigeration level and heat rejected to ambient. T^*_{CW} and T^*_O, shifted temperature of cooling water and ambient shifted temperature, respectively; Q_C, cooling duty (after CPI 2004b and 2005b).

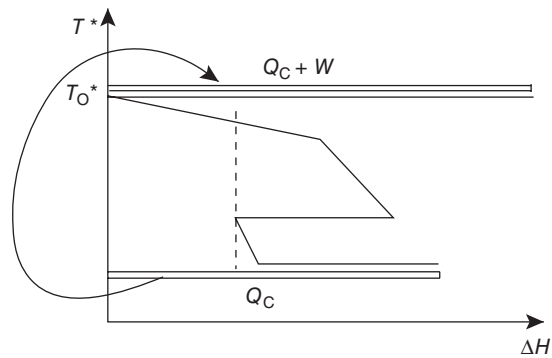


Fig. 4.8 The grand composite curve with cooling provided by a single refrigeration level and heat rejected to the process (after CPI 2004b and 2005b).

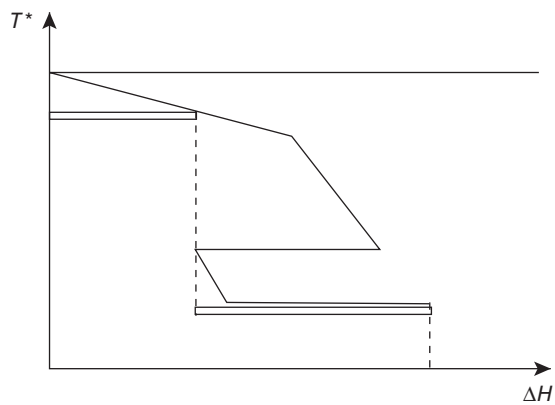


Fig. 4.9 The grand composite curve with cooling provided by two refrigeration levels (after 2004b and 2005b).

4 Obtaining the minimum energy design that achieves the target.
5 Optimisation, which allows a trade-off between energy costs and capital costs.

Heat integration methodology has been further extended to include total sites, which are defined as a combination of processing plants integrated with the utility supply system (Klemeš *et al.*, 1997). Total site analysis has extended the scope for energy savings in many industries, including the food processing industry. A typical example is a site that includes a sugar refinery with additional production of ethanol. Both processes are served by a power plant, which additionally, mainly in winter, acts as a heat supply for a nearby town (Fig. 4.10).

Total site methodology (Dhole and Linnhoff, 1993) produces integrated process designs coupled to logical investment strategies that can result in major savings. Savings of up to 20% in fuel use were found on the total sites studied after accounting for cogeneration. These savings have been achieved by simultaneous optimisation of the production processes and site-wide utility systems. The total site analyses have also resulted in the reduction of global CO_2 levels and other emissions by at least 50% when compared with the reductions achieved for individual process improvements. The specific features of semi-continuous and batch operations, as well as multi-objective optimisation, were taken into consideration in the design strategy for the total sites. The environmental cost and possible regulatory actions were also incorporated. Software tools supporting the methodology were developed. Total site projects have led to the concept of a 'road map' for investments in processes and in the site utility system.

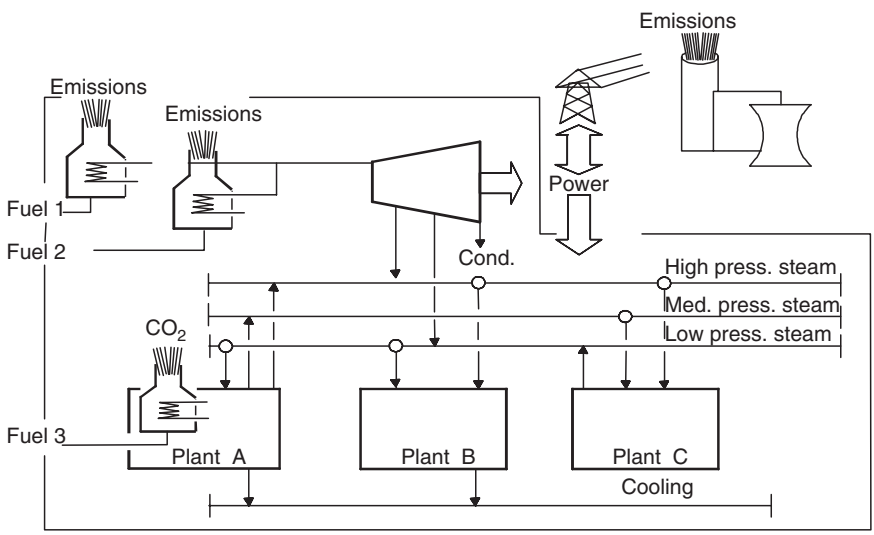


Fig. 4.10 Total site utilities arrangements (after Klemeš *et al.*, 1997).

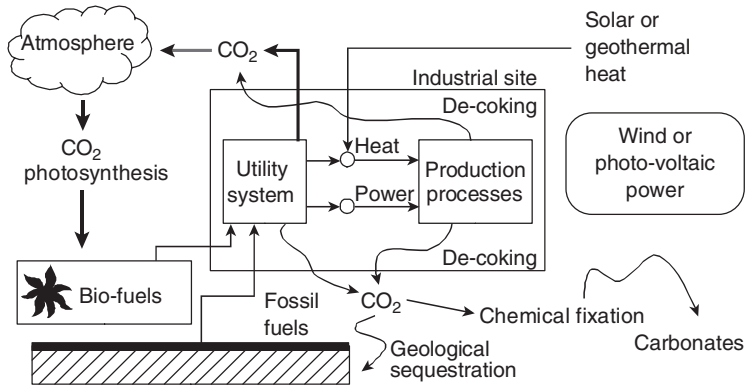


Fig. 4.11 A utility system and its interaction with the ambient tracing the CO_2 circulation (after Varbanov *et al.*, 2005).

The methodology has been further developed and extended to include the optimal synthesis of utility systems (Varbanov *et al.*, 2005) that feature low greenhouse gas emissions. A simplified site configuration is shown in Fig. 4.11.

In the most typical of total site systems, the utility system supplies steam for heating to the site processes. Alternatively, steam can be generated from high-temperature process cooling which is then passed to the steam system. The cooling demands are met by using cooling water, air cooling or refrigeration. In addition, the utility system is required to satisfy the power demands of the site. There are three important groups of interactions relating to the operation of utility systems. Firstly, it is most unlikely that the total site will be in power balance. Often they are required to import or export power. Secondly, economic conditions such as the market prices of fuels and electricity vary with time. There are also variations in product demands, feedstock compositions, ambient conditions, etc. Thirdly, environmental impact of a utility system needs to be integrated into a synthesis model. This is dictated by the need for significant reduction of emissions. The integration should be carried out accounting for the economics, since the decisions in industry are driven by profitability. The most recent developments in the field have been summarised by Klemeš and Stehlík (2003) and by Klemeš and Friedler (2005).

4.3 Renewables in the food industry

There are a number of slightly different definitions of renewable energy. The US Energy Information Administration (EIA, 2005) defines renewable energy as 'Energy obtained from sources that are essentially inexhaustible (unlike, for example, fossil fuels, of which there is a finite supply). Renewable

sources of energy include wood, waste, geothermal, wind, photovoltaic, and solar thermal energy'. The definition presented by the Texas Renewable Energy Industries Association (TREIA, 2005) carries a more detailed description: 'Any energy resource that is naturally regenerated over a short time scale and derived directly from the sun (such as thermal, photochemical, and photoelectric), indirectly from the sun (such as wind, hydropower, and photosynthetic energy stored in biomass), or from other natural movements and mechanisms of the environment (such as geothermal and tidal energy). Renewable energy does not include energy resources derived from fossil fuels, waste products from fossil sources, or waste products from inorganic sources.'

There are no reasons why the food processing industry could not use electricity generated by any renewable energy source. However, a very specific potential offers a co-product recovery from food processing that can use photosynthetic energy stored in biomass. In addition to the previously mentioned bagasse from sugar cane, the use of straw, grass and similar organic by-products from food processing is increasing.

The UK Department of Trade and Industry (DTI, 2005) classifies the biomass-dependent resources as those that include the by-products and waste generated by agricultural, industrial and commercial processes. This includes forest products, waste wood, straw, slurry, chicken litter, and industrial and municipal wastes (e.g. from food processing). For example, for every tonne of wheat harvested, a certain amount of 'waste' straw is created. Similarly, for every tonne of processed food, a certain amount of (mostly biological) waste is created. These by-products can be used as biomass fuels. In 2004, biomass accounted for 84% of renewable energy sources in the UK and this figure includes biomass used for both heat and electricity generation. Most of the energy was generated from landfill gas (33%) and waste combustion (14%) (DTI, 2005).

4.4 Overview of selected case studies

The principles of heat integration have been used in food and drink processing for many years. These techniques have included, for example, vapours bleeding from multiple-stage evaporators in sugar production, and heat recovery during sterilisation and pasteurisation of milk and other food solutions. However, these principles and techniques have not been used as frequently in other branches of food processing industry and there is scope for further research in this area.

The application of pinch technology and heat integration in different sectors of food and drink processing depends on the specific character of the particular production process. As a whole, food processing is characterised by relatively low temperatures of process streams (rarely above 120–140 °C), a low number of hot streams (some with non-fixed final

temperatures, for example secondary condensate of multiple-stage evaporation systems), low boiling point elevation of food solutions, intensive deposition of scale in evaporator and recovery systems, and seasonal performance. The development of heat integration is obstructed by some specific technological and design requirements, for example direct steam heating, difficulties in cleaning heat exchanger surfaces and high utility temperatures.

Nevertheless, higher efficiency, optimised heat systems and low energy consumption could be obtained and therefore reduce the quantity of environmentally hazardous emissions. Moreover, heat integration also results in technological improvements such as reduced deposition of scale due to reduced utility temperatures, self-regulation of heat process, etc.

An example of the benefits resulting from the analysis of existing heat systems is provided by the production of refined sunflower oil (Klemeš *et al*., 1998). These production systems operate with a minimal temperature difference of 65 °C at the process pinch and use two types of hot utilities (dautherm steam and water steam) and two cold utilities (cooling water and ice water). As a result of increased heat integration and optimisation the minimum temperature difference was reduced to 8–14 °C, the heat transfer area was increased, but the hot utility and cold utility consumption was reduced considerably. An additional benefit is that there was no necessity to use water steam and cooling water as utilities, considerably simplifying the design.

Similarly, good opportunities for heat integration also exist in sites extracting raw sunflower oil (Klemeš *et al*., 1998). A problem cited frequently with this process was the inappropriate placement of a one-stage evaporation system for separating the solvent, benzine. The problem could not be solved by changes in the pressure because of benzine's flammability and the sharp increase in the boiling point elevation. This disadvantage could be partially compensated for by an appropriately placed indirect heat pump, pumping heat from a condensation temperature level to the utility temperature level in the evaporation system.

The advantages of heat integration can also be illustrated by the case of crystalline glucose production (Klemeš *et al*., 1998). Operating plants in this process widely use vapours bleeding from a multiple-stage evaporation system for concentrating the water–glucose solution. Pinch analysis of these processing systems showed that using vapour bleeding results in the unnecessary over-expenditure of utilities due to the fact that the multiple-stage evaporation system was inappropriately placed across the process pinch. The adjustment of the evaporation system was difficult because of restrictions in maximum boiling temperatures.

A further example of the use of improved heat integration is observed in fruit squash production. During sterilisation, heat recovery between the cold feed and the hot product is difficult because of intensive deposition of scale and the fact that it is impossible to clean the shell and tube-side heat

exchange surfaces. This problem can be solved by employing single or multiple throttling of the solution using steam-ejecting compressors for vapours, intermediate utilities, etc.

In tomato concentrate production the main heat consumer is a multiple-stage evaporation system. Appropriate placement of the system is difficult because of the considerable disproportion between the heat consumption of the evaporation and recovery systems. Additionally, the available temperature difference in the evaporator is insufficient for a greater number of stages. Despite this, considerable utility savings can be obtained by increasing the two- and three-stage evaporators to four stages and by using steam-ejecting compressors.

The heat systems involved in alcohol production from grain and potato raw materials are characterised by considerable complexity, for example the availability of a large number of hot and cold streams, several (usually four) fractional distillation columns and a multiple-stage evaporation system. Applying heat integration to these heat systems confirms the efficiency of new three-stage fractional distillation systems. However, their appropriate placement requires heat pumps.

A further example of the use of heat integration and the appropriate choice of utilities is given by a case study of a whisky distillery (Smith and Linnhoff, 1988). In this case it was recognised that steam was being used incorrectly for process heating. The steam use was related to the use of a heat pump and by reducing the size of the heat pump, the steam used below the process pinch was eliminated. However, steam now had to be used for process heating above the process pinch, but overall energy costs were reduced due to the reduction in compressor duty.

4.5 A case study: sugar processing

Sugar manufacturing is a large industrial sector that has an important economic impact in more than 30 European countries. The total sugar output of Europe is about 30 million tonnes per year. Nearly two-thirds of this amount originates from EU countries, among which Belgium, the Czech Republic, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain and the UK are the largest producers. The remaining one-third of the European sugar output is predominantly supplied by the New Independent States (NIS): Belarus, Ukraine and Russia.

The total energy consumption of the European sugar industries is of the order of 300 000 TJ/year. The energy efficiency differs widely throughout the region. When compared with the 'old' fifteen EU countries, specific energy consumption (per unit mass of sugar produced) in the new EC countries, and especially in the NIS, is more than twice as high.

Beet-sugar production is one of the oldest and most intensively explored branches in the food processing industry with high energy consumption. The heat systems are characterised by a high degree of efficient heat

recovery. However, despite this, there is often the opportunity for further improvements (Vaccari *et al*., 2002, 2004). For example, sugar plants are designed to operate with a minimum temperature difference (ΔT_{min}) of 8–15 °C in the utility pinches due to vapour bleeding from multiple-stage evaporation. Contemporary economic conditions state that the optimal ΔT_{min} should be in the region of 4–6 °C. This reduction of minimum temperature difference can therefore lead to an 8–10% reduction in the hot utility consumption and with under an economically justified increase in heat exchange surfaces. The main problem remains the inappropriate placement of the evaporation system as a whole, and particularly the inappropriate placement of vacuum pans due to the low temperatures of the vapours contained within them.

The plant undergoing analysis was producing white refined sugar and confectioneries. The raw material was sugar beet and the plant had medium production levels. The diffusion system was a Decline double-screw extractor DC, equipped with hot juice purification, quadruple evaporation effect and a double-products crystallisation scheme. The plant production flowsheet is given in Fig. 4.12.

The hot utility was dry saturated steam at 136 °C, supplied by a public utility power plant. The designed hot utility consumption was 42.2 kg per 100 kg beet with an actual hot utility consumption of 48–52 kg per 100 kg beet. The required cold utility was provided by an internal spring. The cooling water, however, had a range of temperature, fluctuating within the range of 15–40 °C. Heat transfer was secured by a five-level evaporator in quadruple effect and 15 heat exchangers. The existing heat exchangers are shown in Fig. 4.13, along with the network and the overall process layout. The stream data extracted from the process flowsheet and used for the construction of the hot and cold composite curves are given in Table 4.3.

The composite curves were generated using the SPRINT software and are shown in Fig. 4.14. The related grand composite curves, also generated by SPRINT, are shown in Fig. 4.15.

The initial analysis of the data, using these heat integration techniques, has shown a number of areas for potential improvement. Firstly, there is a considerable excess of hot utility consumption, up to 35%, above the designed value, due to the fact that the plant is operating at a capacity lower than the designed one. Secondly, there is unsteady loading of the vacuum pans, with a low degree of process control resulting in 33 t/h of actual steam consumption compared with 26.4 t/h of designed steam consumption. There is also a considerable surplus of heat exchange area and a low level of heat exchange loading; 5601 m^2 are available but only 4317 m^2 are employed.

The potential for improvement in energy use in the process was centred on the following: increasing the number of evaporation system effects; increasing the use of available heat exchange through the reduction in the temperature driving forces; better heat integration and overall shifting of the vapours bleeding system towards the last evaporation effects. In

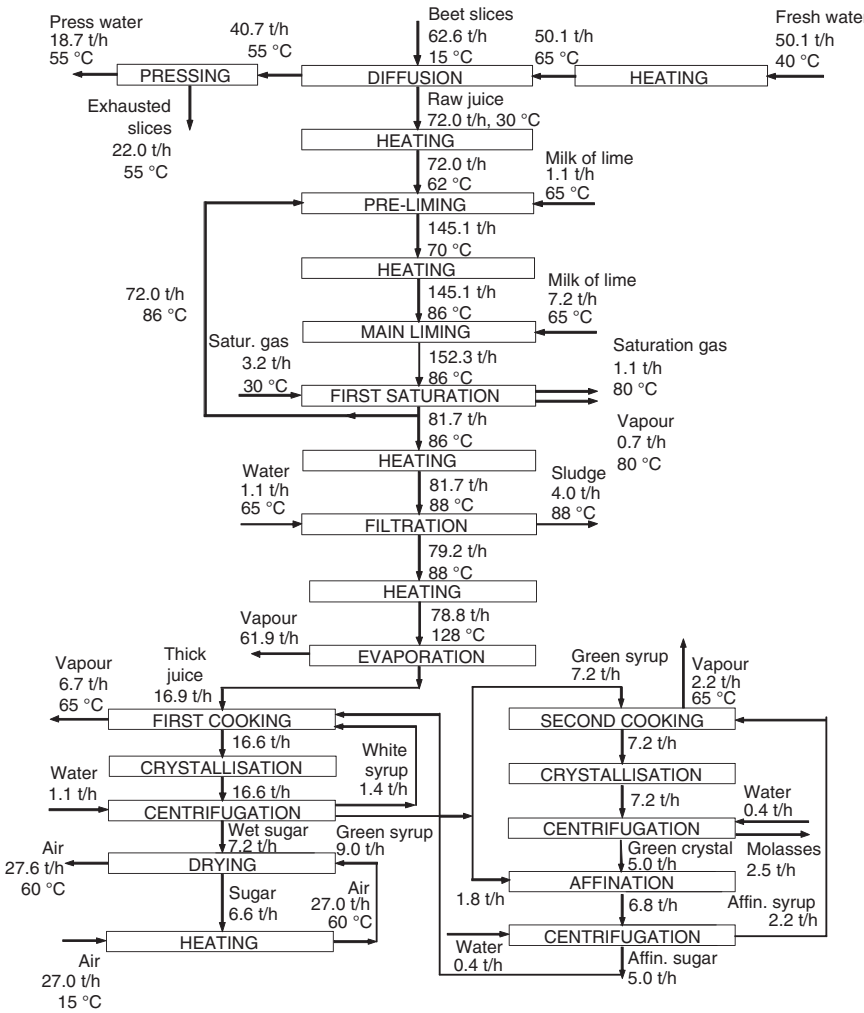


Fig. 4.12 Sugar plant production flowsheet.

addition, there was the possibility of improving the utilisation of condensate heat and modifying the condensate gathering system.

The suggested retrofit modifications included the transformation of the quadruple-effect evaporation system into a quintuple-effect system through the inclusion of the existing reserve evaporation body. Also included were internal changes in the network structure comprising the rearrangement of the existing heat exchangers (Fig. 4.16). Further, it was also suggested that the condensate gathering system should be changed to a pressure sequenced system. The modified heat exchanger network is shown in Fig. 4.17, and the simplified flowsheet diagram representing the heat exchanger network is

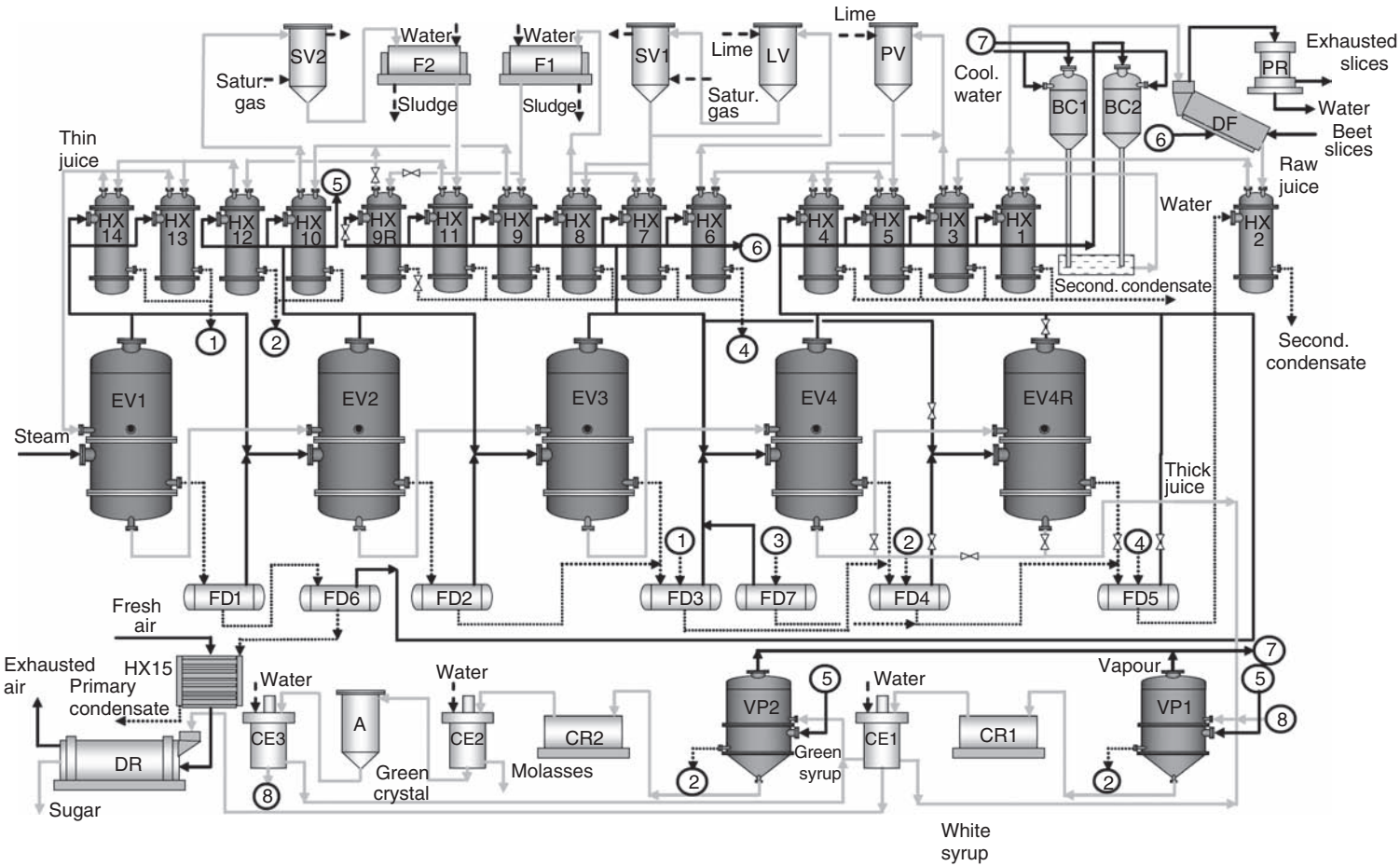


Fig. 4.13 Existing heat exchanger network and overall process.

Table 4.3 The stream data extracted for construction of hot and cold composite curves

Streams	Heat capacity flowrate (kW/°C)	Inlet temperature (°C)	Outlet temperature (°C)	Heat duty (kW)	Heat exchangers
Cold					
1. Fresh water	58.2	40	65	1455	HX1
2. Raw juice	75.9	30	43	1150	HX2
2. Raw juice	75.9	43	62	1271	HX3
3. Juice liming	153.1	70	82	1562	HX4, HX5
3. Juice liming	153.1	82	86	870	HX6
4. Juice filtrate	86.4	86	88	173	HX7, HX8
5. Juice satur.	83.8	88	91	251	HX9
5. Juice satur.	83.8	91	94	251	HX10
6. Thin juice	83.1	93	100	582	HX11
6. Thin juice	83.1	100	108	665	HX12
6. Thin juice	83.1	108	123	1247	HX13, HX14
7. Diffusion	–	–	–	1362	DF
8. Cooking	LH	75	75	7716	VP
9. Air	9.0	15	60	405	HX15
10. Boil. I eff.	LH	128	128	16747	EV1
11. Boil. II eff.	LH	119	119	15024	EV2
12. Boil. III eff.	LH	107	107	7047	EV3
13. Boil. I eff.	LH	95	95	4734	EV4
Hot					
1. Primary cond.	27.4	90	75	405	HX15
2. Secondary cond.	65.3	90	72	1150	HX2
3. Vap. cook.	LH	65	65	7017	CO
4. Vap. I eff.	LH	126	126	15915	EV
5. Vap. II eff.	LH	117	117	15323	EV2
6. Vap. III eff.	LH	103	103	7526	EV3
7. Vap. IV eff.	LH	88	88	4228	EV4

Abbreviations are: satur., saturation; eff., effect; cond., condensate; boil., boiler; vap., vaporation; cook., cooking.

presented in Fig. 4.18. The updated composite and grand composite curves, obtained using SPRINT, are given in Figs 4.19 and 4.20.

The main achievement of the heat integration retrofit methodology was the reduction of designed steam consumption from 26.4 t/h to 24.0 t/h (9%) without any additional capital costs (beside the cost of re-piping). This was achieved by making use of some low-usage heat transfer area and the re-allocation of some heat exchanger area to more appropriate usage. Moreover, the solution advocated was especially beneficial for a sugar plant that is run for less than five months per annum. The retrofit potential modifications and the benefits of these suggestions are given in Table 4.4.

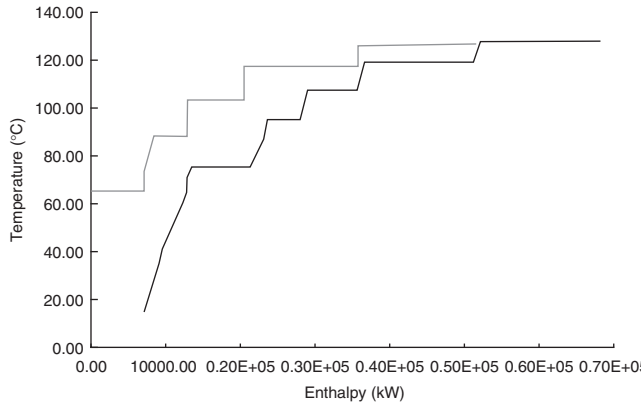


Fig. 4.14 Sugar plant composite curves.

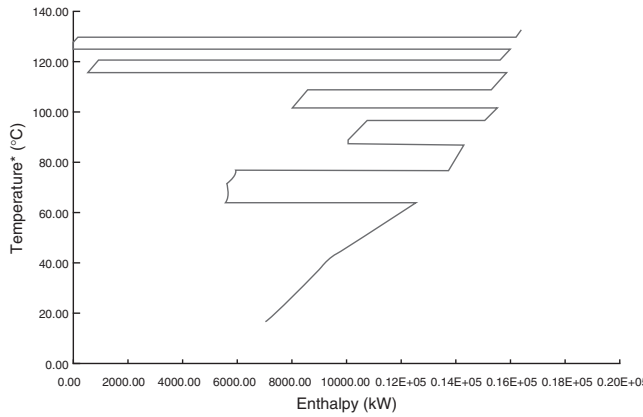
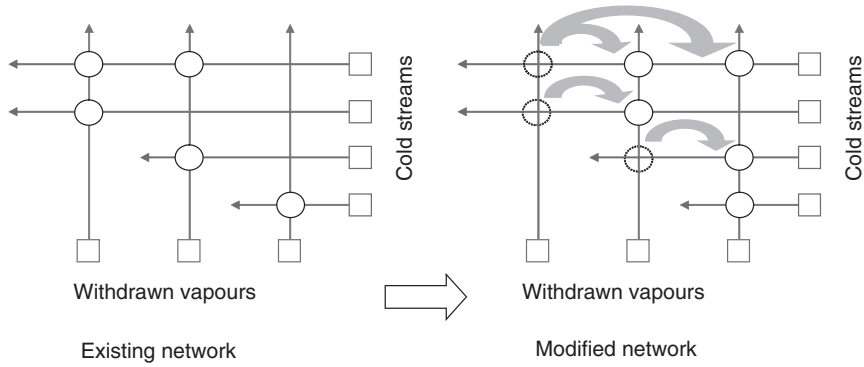


Fig. 4.15 Sugar plant grand composite curves.

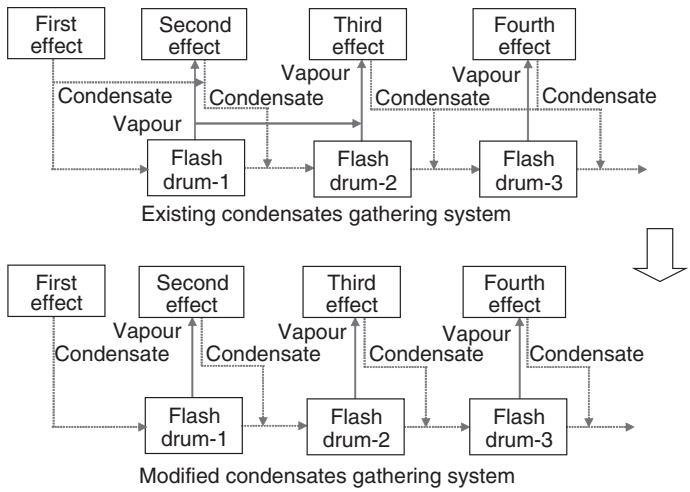
In addition to the retrofit suggestions already considered for the sugar processing plant, further improvements in steam consumption could be achieved through the application of a heat exchanger retrofit analysis and improved process control. If these modifications were adopted they would provide a further 9.0 t/h reduction in the steam consumption (27% compared with the actual consumption). This measure would require a more detailed economic analysis (for some guidelines see Donnelly *et al.*, 2005).

4.6 Further studies

Another case study analysing a sugar plant by heat integration methodology in a developing country has been recently published by Raghu Ram and Banerjee (2003). In this particular case pinch analysis was used to determine the hot utility requirement, which was found to be 9% less than the amount that was actually being consumed. Modifications to the



(a) Internal changes comprising rearrangement of the existing heat exchangers



(b) Tuning of condensates gathering system into a pressure sequenced system

Fig. 4.16 Retrofit modifications.

evaporator system were proposed that would reduce the steam consumption by 9 t/h, in line with the results provided by the pinch study. Additionally, the analysis was extended to include elements of total site targeting and analysis. The findings lead to proposals for a cogeneration system producing 26.8 MW of power.

Further case studies related to energy efficiency in the sugar industry have been presented; for example, Klemeš *et al.* (1999c) and Grabowski *et al.* (2001, 2002a,b). Heat integration analysis of a brewery with resultant considerable energy savings were presented by Hufendiek and Klemeš (1997). Klemeš *et al.* (1998) presented a comprehensive study covering a sugar plant, a raw sunflower oil plant and a corn crystal glucose plant.

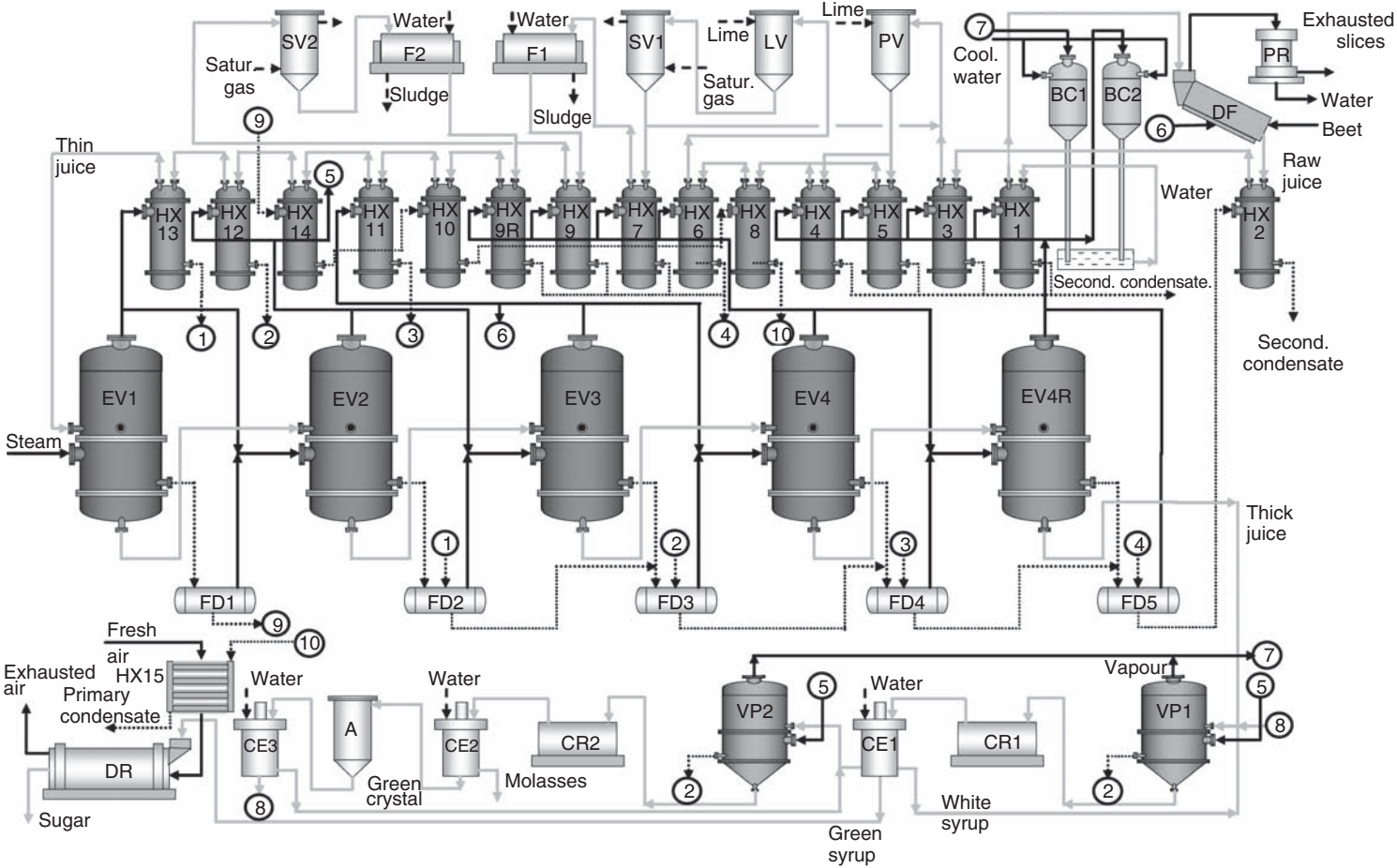


Fig. 4.17 Modified heat exchanger network and overall process.

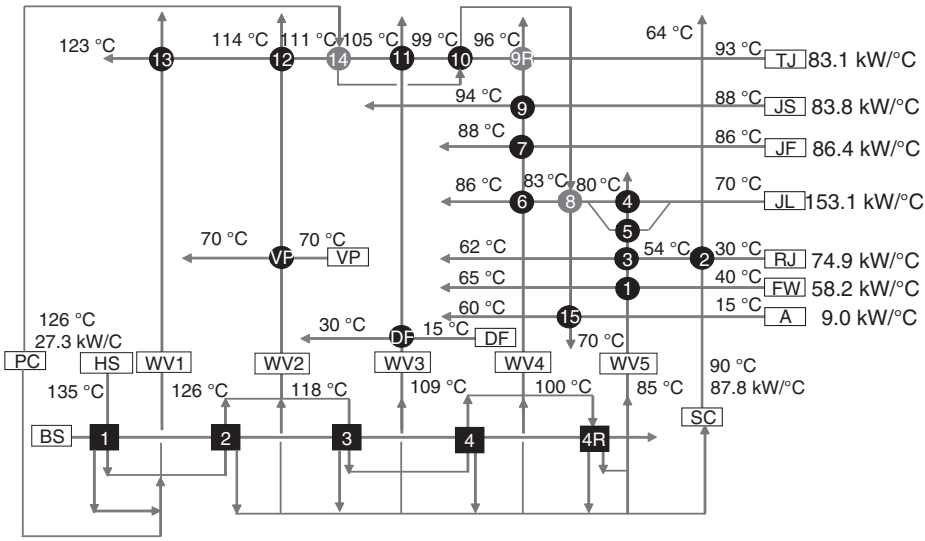


Fig. 4.18 Modified heat exchanger network.

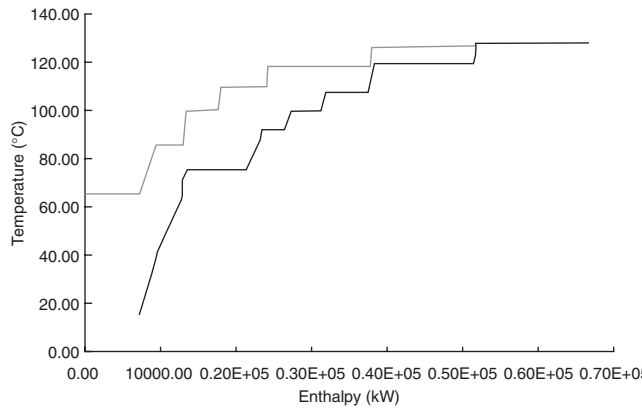


Fig. 4.19 Composite curves for the modified network. Hot utility, 14 850 kW; cold utility 7015 kW.

Several case studies have been documented by the Department of the Environment, Transport and the Regions (DETR, 1996, 1997). They developed a waste heat recovery potential for the United Kingdom alone of 8.3 PJ/year which at that time represented around £14 million, more than £20 million in today's energy prices. They concluded that, in the dairy industry, the pasteurisation process is already highly efficient in terms of heat recovery (up to 95%), but that this was not the case for sterilisation, which is more energy intensive with bottle sterilisation consuming 300–500 MJ/t. They mentioned several energy saving measures implemented by Associated Dairies.

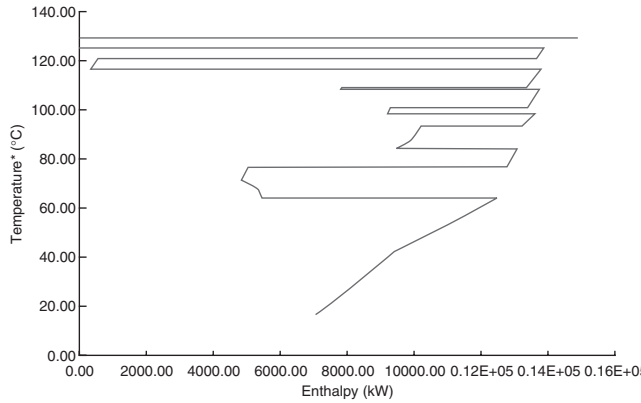


Fig. 4.20 Grand composite curve for modified network.

Table 4.4 Overview of the sugar plant retrofit results

Network	Steam consumption		Cooling water consumption	Total area (m^2)		Energy cost (£/campaign)
	(t/h)	(kg/100 kg beet)	(m^3/h)	Available	Used	
Existing	26.4	42.2	250	5.601	4.317	647 670
Retrofitted	24.0	38.3	250	5.601	5.091	591 540
Change (absolute)	–2.4	–3.9	0	0	774	56 129
Change (%)	–9.0	–9.0	0	0	+18	–9

The other food and drink processes reviewed by DETR (1996, 1997) were bakeries, breweries, drying in the production of flavourings and ingredients, and a developed example of a breadcrumb dryer plant where an energy saving potential of about 30% was identified.

A number of case studies have been completed by Linnhoff March–KBC Advanced Technologies (see Section 4.7.2). The details are mostly confidential, but publicity information can be obtained from the company (Table 4.5). This table demonstrates the variety of potential applications of heat integration and energy efficiency improvement in food processing industries.

4.7 Sources of further information and advice

4.7.1 Literature and conferences (papers, books, relevant conferences and journals, websites to study with a short evaluation)

There are two main groups of conferences dealing with minimising energy use. The first deals with energy minimisation in general and includes the following conferences:

Table 4.5 Case studies completed by Linnhoff March

AFFCO	Meat processing works
Alimentos Heinz	Tomato paste plant: CHP design
Allied Breweries	Energy survey and Pinch Study, M & T programme
American Crystal Sugar	Energy saving
American Fructose	Corn wet milling (two projects)
American Maize	Process studies and utility model (three projects)
Anheuser-Busch	Energy audit/scoping study
Archer Daniels Midlands (ADM)	Energy audit and detailed study/utility modelling at two sites, Pinch and ProSteam training courses, Total Site, Pinch Study
Avebe	Starch/protein
Bass	Scoping studies at two large breweries, implementation of water systems improvements, refrigeration review and investment strategy development
Batchelors (Unilever)	Food processing
Birds Eye Walls (Unilever)	Vegetable processing: water minimisation study
Borculo Domo Ingredients	Energy projects package for a dairy, feasibility study for a new refrigeration system
British Gas/Bass/Canada EMR	Generic brewery design
British Sugar	Confidential projects
Cache Valley Cheese	Cheese plant energy study
Cadbury Schweppes	Milk drying/agglomeration energy study
Campbell Soup	Energy study and project ideas definition
Cargill, USA	13 projects at several sites (Dayton, Blair, Wichita, Lafayette, Kansas City, Chesapeake, Gainesville, Raleigh, Cedar Rapids and Memphis), Process studies and Total Site analyses. Training and technology transfer. Pinch support
Cargill, UK	Edible oils (3 projects at 2 sites)
Cargill, Germany	New design audit for Salzgitte, operational analysis for Salzgitter
Cargill, Australia	West Footscray study
Cargill, Malaysia	Pinch training and case studies
Carlton & United Breweries	Study of refrigeration and compressed air systems, specific energy benchmarking study
Cereol	Site appraisal, edible oil refinery
Cerestar, Europe	Total Site studies of corn processing facilities (Holland, France and Germany), utility modelling (France, Germany, Holland)
Chivas Brothers (Seagrams)	Malt whisky distilleries, by-products plant
Combination of Rothes Distillers	Distillery by-products energy scoping study
Courage	Water systems study

Table 4.5 cont'd

CPC (UK)	Corn wet milling
CPC (USA)	Corn processing
Distillers	Maltings energy and electricity appraisal
Dominion Breweries	Pinch studies of two breweries
Domino Sugar Corporation	Total Site energy study
Douwe Egberts	Coffee
East Midlands Electricity (UK)	Food processing
El Turia, Spain	Energy audit and Pinch Study
EOI (Unilever)	Edible oil refinery
ETSU (UK DOE)	Edible oils
Eutech	Brewery energy benchmarking
Express Foods	Dairy products (two sites) energy studies
Fleischman's Yeast	Yeast
FMC (Ireland)	Analysis of steam and condensate systems
FMC (UK)	Site energy assessment
Fonterra Co-operative Group	Pinch analysis and process/utility modelling at two sites in New Zealand, Pinch Study, Steam Study
Gist Brocades	Yeast
Glanbia Foods	Dairy products
Golden Wonder	Potato products
Grain Processing Corporation	Corn processing/starch
Greenall Whitley	Pinch Study and detailed engineering of projects M & T programme
Guinness	Energy survey and Pinch Study of stout and lager breweries. Definition of future refrigeration supply strategy
Heinz Australia	Cannery energy scoping study
H J Heinz of Canada	Food processing energy scoping study
Highland Distilleries	Malt whisky distilleries and malting pinch studies. CHP appraisal and project ideas definition
Hubinger	Corn wet milling
Ind Coope, Burton	Definition of refrigeration duties and detailed design of new plant systems
Invergordon Distillers	Grain whisky distillery energy scoping study
John Dewer & Sons Ltd	Energy survey (2 sites)
Kraft Foods	Baking processes energy study
Labatt's	Pinch Study of major brewery
Lantic Sugar	Cane sugar
Loders Croklaan (Unilever)	Edible oil (3 projects)
Long John International (Whitbread)	Grain whisky distillery energy/ debottlenecking study and project ideas definition
J Lyons & Co (Allied Lyons)	Energy studies for coffee, ice cream and cereal production
Master Foods (Mars, Belgium)	Rice processing
Meadow Lea Foods, Australia	Edible oils
Meggle Milch Industrie	Dairy products
Midwest Grain	Corn processing
Miller Brewing	Energy survey and Pinch Study of large brewery

Table 4.5 cont'd

Moosehead	M & T programme, Pinch Study
Murray Goulburn Co-op	Dairy energy scoping study
National Starch	Corn processing
NZ Co-op Dairy Company	Dairy energy study. Total Site
Ocean Spray	Food processing
Procter & Gamble	Citrus products energy study
Pura Foods	Vegetable oil processing
Quest International	Energy audit executed under ETSU/CIA agreement
Redpath Sugar	Sugar refinery and utility debottlenecking study
Rich Products	Non-dairy creamers
Richmond	Meat processing works
Rowntree Mackintosh (Nestlé)	Confectionery, waste management energy study and project ideas definition
Sorrento	Dairy
Staley	Corn wet milling (3 projects at Decatur and Sagamore)
Stevensons	Pig meat rendering
Suiker Unie	Sugar refinery energy study
Svenska Nestlé AB	Food processing
Tate & Lyle	Energy study and project ideas definition (2 refineries) BFW heating optimisation study
Tetley Walker (Allied Breweries)	Pinch Study and detailed engineering of projects, energy audit of brewery
Tunnel Refineries, UK	Corn wet milling (2 projects)
United Distillers (Guinness)	Maltings, heat recovery and heat pump evaluation
Van den Berghs & Jurgens (Unilever)	Edible oil refineries (4 projects at 2 sites)
Van Grieken Melk	Milk products energy study and project ideas definition
Waikato Dairy Co-op (New Zealand)	Dairy
Weddel Tomoana	Meat processing plant
Wendt's Dairy	Fluid milk plant CHP project appraisal
Whitbread	Scoping study of 3 large breweries, electrical survey of 2 breweries, formulation of electrical strategy for all breweries

Abbreviations: M & T, Measurement & Testing programme; ETSU, Energy Technology Support Unit; CIA, The Chemical Industries Association; BFW, boiler feed water.

- PRES: Process Integration, Modelling and Optimisation for Energy Saving and Pollution Reduction. This conference has been organised annually from 1999 (www.conferencepres.com).
- CIWEM: Chartered Institution of Water and Environmental Management National Conferences (www.ciwem.org/events/3rdNational ConferenceBrochure.pdf).

- World Renewable Energy Congress: Innovation in Europe (web.taed.unifi.it/abitaweb/wrec.htm).
- World Bioenergy (www.elmia.se/worldbioenergy/).
- World Sustainable Energy Days (www.energiesparverband.com/esv/).
- Europe Energy Efficiency Conference (www.esv.or.at/esv/index.php?id=1484&L=1).

The second group are conferences dealing with the specific issue of food processing and, in particular, energy efficiency.

- Action Energy (2004) Steam distributions costs. *Energy Consumption Guide*, ECG 092, Carbon Trust, London.
- Action Energy (2004) Energy efficient operation of boilers. *Good Practice Guide*, GPG 369, Carbon Trust, London.
- Amla B L and Potty V H (1985) Development of energy-saving technologies for the food processing industry. The United Nations University Press, *Food and Nutrition Bulletin* **7** (2).
- Energetics, Incorporated for US Department of Energy (2004) *Technology Roadmap – Energy Loss Reduction and Recovery in Industrial Energy Systems*.
- US Department of Energy, Energy Efficiency and Renewable Energy (2003) *How to Calculate The True Cost of Steam.* Industrial technologies Program, A Best Practice Steam Technical Brief.

4.7.2 Sources of practical information (service providers, professional bodies, EC projects and networks)

Service and advice providers

DEFRA (Department for Environment, Food and Rural Affairs, UK) (www.defra.gov.uk/ and especially www.defra.gov.uk/environment/energy/index.htm).

SEPA (Scottish Environment Protection Agency) (www.sepa.org.uk).

European Integrated Pollution Prevention and Control Bureau, Institute for Prospective Technological Studies producing BREFs (Best Available Techniques (BAT) Reference Documents) (eippcb.jrc.es/pages/FActivities.htm).

ADAS, Insight and Solutions, Woodthorne, Wergs Road, Wolverhampton, WV6 8TQ, UK, Tel. +44 (0)845 7660085 (www.adas.co.uk/contact/index.html).

Centre for Process Integration, CEAS, The University of Manchester, PO Box 88, Manchester, M60 1QD, UK, Tel. +44 (0)161 306 4380, Fax +44 (0)161 236 7439 (www.ceas.manchester.ac.uk/research/researchcentres/centreforprocessintegration/).

Linnhoff March–KBC Advanced Technologies, Targeting House, Gadbrook Park, Northwich, Cheshire, CW9 7UZ, UK, Tel. +44(0)1606 815100, Fax +44(0)1606 815151 (www.linnhoffmarch.com/contact/uk.html and www.kbcat.com).

COWI A/S, Parallelvej 2, DK-2800, Kongens Lyngby, Denmark. Contact: John Jørgensen, Communication Manager (DJ), Tel. +45 4597 1494, Mobile +45 2030 6494 (jhj@cowi.dk, www.cowi.com/).

Some EC-supported projects

- AWARENET (Agro-food Wastes Minimisation and Reduction Network): provides valuable and comprehensive information on various aspects of waste minimisation, including energy minimisation. EC GROWTH, GRD1-CT2000-28033 (de las Fuentes *et al*., 2002a,b) (www.cordis.lu/data/PROJ_FP5/ACTIONeqDndSESSIONeq112242005919ndDOCeq154ndTBLeqEN_PROJ.htm; accessed 10 December 2005).
- Project SUCLEAN: research on minimisation of energy and water use in sugar production by cooling crystallisation of concentrated raw juice. IC15960734 (Klemeš *et al*., 1999b) (cordis.europa.eu.int/inco/fp4/projects/ACTIONeqDndSESSIONeq6118200595ndDOCeq42ndTBLeqEN_PROJ.htm; accessed 3 January 2006).
- AVICENNE: integrated concept for the fermentation of sewage sludge and organic waste as a source of renewable energy and for use of the fermented products as a hygienic fertiliser and soil improver. AVI*94005, Universität Stuttgart, Germany, 1997.
- ANDI-POWER-CIFRU: an anaerobic digestion power plant for citrus fruit residues. FP5 EESD NNE5/364/2000, Envisec S A, Greece, 2004 (www.cordis.lu/data/PROJ_EESD/ACTIONeqDndSESSIONeq7826200595ndDOCeq1ndTBLeqEN_PROJ.htm; accessed 14 March 2006).
- BIOGAS BY BIOAUGMENT: optimised biogas production and resource recovery through bio-augmentation in a joint plant treating poultry and pig waste. NNE5/46/1999, Centro para a Conservaçao de Energia, Estrada de Alfragide, Praceta 1, 2720-537 Amadora, Portugal (www.cordis.lu/data/PROJ_EESD/ACTIONeqDndSESSIONeq7826200595ndDOCeq4ndTBLeqEN_PROJ.htm; accessed 14 March 2006).
- DEP-PROJECT: power plant based on fluidised bed fired with poultry litter: FP5 NNE5/75/1999, 2003, Energy Systems BV, De Vest 51, Postbus 218, 5555 XP Valkenswaard, Netherlands (www.cordis.lu/data/PROJ_EESD/ACTIONeqDndSESSIONeq7826200595ndDOCeq13ndTBLeqEN_PROJ.htm; accessed 14 March 2006).
- Development of a CHP process using grease and oil waste from plants and animals. FP5 EESD ENK5 CT-2000-35008, Sud recuperation Sarl, France, 2001 (www.cordis.lu/data/PROJ_EESD/ACTIONeqDndSESSIONeq26119200595ndDOCeq216ndTBLeqEN_PROJ.htm; accessed 14 March 2006).
- WREED: development of an energy efficient to reduce the cost of drying food and feed waste. FP5 EESD ENK6-CT2001-30002, Ceramic Drying Systems Ltd, UK, 2004 (www.cordis.lu/data/PROJ_EESD/ACTIONeqDndSESSIONeq3155200595ndDOCeq440ndTBLeqEN_PROJ.htm; accessed 14 March 2006).

- Efficient drying of by-products in starch production from wheat. ENG-THERMIE 1/IN/01013/91, Cerestar Deutschland GmbH, Germany, 1994.
- Development of a new process for energy efficient protein extraction from cornstarch production effluents. FP6 LIFE QUALITY/QLK1-CT-2001-42103, Umwelt- und Wassertechnik GmbH, Nienhagen, Germany, 2002 (www.cordis.lu/data/PROJ_FP5/ACTIONeqDndSESSIONeq112482005919ndDOCeq634ndTBLeqEN_PROJ.htm; accessed 14 March 2006).

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AWARENET (2004), *Handbook for the Prevention and Minimisation of Waste and Valorisation of By-products in European Agro-food Industries*, BI-223-04. Fundacion Gaiker, Zamudio, Spain.

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DHOLE, V. R. and LINNHOFF, B. (1993), Total site targets for fuel, co-generation, emissions, and cooling. *Computers and Chemical Engineering* **17**, Suppl. S101–S109.

DONNELLY, N., KLEMEŠ, J. and PERRY, S. (2005), Impact of economic criteria and cost uncertainty on heat exchanger network design and retrofit, *Proceedings of 8th Conference on Process Integration, Modelling and Optimisation for Energy Saving and Pollution Reduction – PRES'05*, Ed. J Klemeš, May 2005, Giardini Naxos, Italy, pp.127–132.

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5

Process optimisation to minimise water use in food processing

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5.1 Introduction: water use and wastage in food processing

5.1.1 Key reasons (economic, environmental, legislative, etc.) to optimise processes to minimise water use and wastage

Water is used in most process industries for a wide range of applications. Industrial processes and systems using water are today being subjected to increasingly stringent environmental regulations relating to the discharge of effluents and there is a growing demand for fresh water. The changes taking place and the pace of these changes have increased the need for improved water management and wastewater minimisation. The adoption of water minimisation techniques can effectively reduce overall fresh water demand in water-using processes and subsequently reduce the amount of effluent generated, with the result of reducing both the costs incurred in the acquisition of fresh water and the costs involved in the treatment of effluent streams.

Important methodologies that have been applied to the minimisation of water use and effluent generation have included:

- The minimisation of water consumption by the efficient management and control of process operations.
- The optimisation of material and energy balances of processes by the application of advanced optimisation strategies aiming at waste reduction.
- The integration of optimisation and production planning techniques in conjunction with real-time plant measurements and control for product quality and minimisation of losses.

- The growing use of enhanced intelligent support to operators by the application of knowledge-based decision-making procedures to select those options that best protect the environment.
- The use of process integration techniques based on pinch analysis.

In the food processing industry, with its own unique and specific features, it is considered advisable to progress from the simplest measures – such as good housekeeping based on efficient management, control and maintenance – to more advanced methodologies.

Food processing is not, in many cases, a continuous process running 7 days a week for the whole year, but one that is intermittent and highly dependent on the availability of the feed stock. Typical campaign productions occur in sugar, fruit juice or cereal processing plants. On the other hand, breweries are in operation on a continuous basis, but are batch processed. These features influence the investment in processing plant and technologies adopted, including those involving water and waste minimisation and processing.

5.1.2 European legislation

Water use and wastewater discharge are covered by both national and international legislations. For European Union (EU) member countries the legislation follows ECC directives, the most relevant are listed below; see the Europa website for further details (Europa, 2005).

- The new European Water Policy: river basin management.
- The Water Framework Directive (2000/60/EC).
- Towards a European action programme on flood risk management.
- Strategies against chemical pollution of surface water under the Water Framework Directive.
- Priority substances under Article 16 of the Water Framework Directive.
- Discharges of Dangerous Substances Directive (76/464/EEC).
- Water pollution coming from urban waste water and certain industrial sectors.
- Urban Waste Water Treatment Directive (91/271/EEC).
- Water pollution caused by nitrates from agricultural sources.
- Nitrates Directive (91/676/EEC).
- Bathing Water Quality of rivers, lakes and coastal waters.
- Bathing Water Quality Directive (Council Directive 76/160/EEC concerning the quality of bathing water) and its proposed revision.
- Drinking Water Quality.
- Drinking Water Directive (98/83/EC).

Many of the above have either direct or indirect relation to water used and waste water discharged by food processing industries.

5.1.3 Best available techniques

The Integrated Pollution Prevention and Control (IPPC) Directive (96/61/EC) (IPPC, 1996) has introduced a framework requiring EU member states to issue operating permits for industrial installations performing activities. These permits must contain conditions that are based on best available techniques (BAT), and aim to offer a high level of protection to the environment as a whole.

A key feature of the IPPC Directive is to stimulate an intensive exchange of information on BAT between the European member states and the industries considered. The European IPPC Bureau organises this exchange of information and produces BAT reference documents (BREFs). Member states are required to take these BREFs into account when determining permit conditions. The Bureau carries out its work through Technical Working Groups (TWGs) comprising nominated experts from EU member states, European Free Trade (EFTA) countries, industry and environmental non-governmental organisations (NGOs). The European IPPC Bureau is located in Sevilla, Spain. For this reason, we refer to the activities carried out within the framework of the IPPC Directive as 'the Sevilla process'.

BAT correspond to the techniques with the best overall environmental performance that can be introduced at a reasonable cost. There have been several studies made in food processing industries. A good example comes from The Flemish Centre for Best Available Techniques (BAT centre) (2005). This document contains an overview of available information on the 'fruit and vegetable processing industry'. Using BAT as guidance, the study proposes:

- to the Flemish authorities, permit conditions and techniques for which investment support may be offered because they are favourable to the environment;
- to the Flemish companies, guidelines for the implementation of the concept of BAT.

The 'fruit and vegetable processing industry', as described in the study, contains the sectors of 'frozen fruits and vegetables', 'canned fruits and vegetables', 'processed potatoes', 'peeled potatoes' and 'fruit juices'. The most important environmental problems are the use of large volumes of ground water and the production of waste water polluted with organic carbon, nitrogen and phosphorus. Information on candidate BAT was mainly obtained from expertise present in Belgium and the neighbouring countries. Over one hundred different BAT were selected. The technical and economical feasibility of these BAT were discussed. BAT on wastewater treatment are, for example:

- primary and aerobic wastewater treatment for small, potato peeling enterprises;
- primary, anaerobic and aerobic wastewater treatment, including nitrification/denitrification/dephosphatation for larger companies.

Based on the BAT it was concluded that the Flemish wastewater discharge limits on surface water are technologically and economically feasible. Additional discharge limits for total phosphorus (25–50 mg/l) were suggested. Annual wastewater treatment costs for an average enterprise were estimated to be €2.5–3.5 M. For small, potato peeling companies, wastewater discharge into the sewers was found to be more appropriate. Water-saving measures and reuse of water may cause a 25–30% decrease in the use of ground water.

A good source for BAT practices is Envirowise – Practical Environmental Advice for Business, a government programme managed by Momenta (an operating division of AEA Technology Plc) and TTI (an operating division of Serco Ltd). Their website, (Envirowise, 2006), provides a wide range of information from news to best practice examples.

5.2 How to minimise water usage and wastewater treatment – present state-of-the-art and future trends

The task of minimisation of water usage and minimising wastewater discharge has received considerable attention over the last few years as water has become more and more costly and an environmentally strategic concern. Most of these trends are general and are not limited to food processing.

Smith (2005) summed up the main water usage and water and wastewater minimisation measures as follows.

1 *Process changes.* These include increasing the number of stages in an extraction processes that use water, changing from wet cooling towers to air coolers, improving energy efficiency to reduce steam demand, increasing condensate return from steam systems, etc. Under 'process changes' we can also add good housekeeping, which includes analysing and measuring water use and wastage, reducing water wastage, cleaning operations, maintenance of equipment and daily work practices. It is also possible to decrease water consumption or replace some of the water-consuming operations, such as hazardous cleaning agents, chemicals and additives. Additionally, 'process changes' also include major process changes driven either by inspection findings or by the process optimisation results of some modern technologies, e.g. process integration/pinch technology. It is possible to adopt new technologies or replace equipment in order to reduce the generation of wastes and increase the efficiency of the process.
2 *Reuse*. This can occur when waste water is used directly in other operations, but only if the existing pollutants do not disturb the process. Maximum water reuse methodologies and methods to recycle water are discussed in more detail in Chapter 21 of this book, together with the opportunities to use recycled water in food processing.

3 *Regeneration reuse*. This is defined as the process of purifying waste water and reusing it in another operation or process.
4 *Regeneration recycling*. This occurs when contaminants from waste water are partly eliminated and the waste water is then returned to the same process afterwards.

A good review of wastewater minimisation has been published by Blomquist and Brown (2004). They looked at a large number of pre-assessment and assessment techniques developed during the last few years for, respectively, identifying waste minimisation focus areas (opportunities) or options (solutions) during a waste minimisation audit. They critically reviewed these techniques and assessed their relative merits. The pre-assessment techniques are analysed in terms of their ease and speed of implementation, while the usefulness and applications of the available general assessment techniques are considered.

5.2.1 Water contamination and wastewater treatment

The food processing industry generates large volumes of waste water and large amounts of solid waste that can produce environmental problems and whose management increases the cost of processes. It is normally necessary to adopt some measures to decrease the environmental impacts of the processes and reduce the costs that are incurred in the treatment and the disposal of the wastes generated. These measures should be focused on the reduction of the generation of wastes rather than their treatment. It is always more cost-effective to reduce the amount of wastes generated rather than treating them before discharge (minimisation at source is better then end-of-pipe treatment).

Water contamination by food processing

There are numerous methods of wastewater treatment and new ones are continuously being developed. There are many handbooks, for example Baruth published by AWWA (2004) and monographs (Kawamura, 2000; Crittenden and Montgomery, 2005) available, where it is possible to study the existing methodologies in detail.

As this field is developing rapidly, potential users will probably be most interested in practical applications from sources of continuously updated information or specialised company websites. For example a Dutch company Lenntech Water- & Luchtbeh. Holding b.v. (2005) offers:

- physical–chemical treatment systems;
- sludge treatment;
- anaerobic/aerobic treatment;
- drum and disc filtration;
- membrane filtration;
- advanced oxidation systems;

- equalisation tanks;
- biological excess sludge reduction.

As food processing has some specific contaminants, mostly biological, then appropriate specific wastewater treatment methodologies should be selected.

The final documentation of the EU AWARENET project (de las Fuentes *et al*., 2002a,b; AWARENET, 2006) provides the following overview of the main technologies for food processing:

- mechanical treatment (sedimentation, screening, degreasing, flotation, membrane separation);
- biological treatment (land application, activated sludge treatment, trickling filter and submerged contact aerator, constructed wetlands, anaerobic treatment);
- physical–chemical treatment (neutralisation, precipitation, flocculation, flotation, oxidation/reduction and disinfection, ion exchange, adsorption, incineration, NH_3 stripping, evaporation, membrane separation).

Wastewater treatment

Methodologies for wastewater handling can also be subdivided into different levels of treatment as follows.

1 *Pre-treatment*: mechanical separation of coarse particles (e.g. sticks, plastics, etc.).
2 *Primary treatment*: removal of suspended solids by physical or physical–chemical treatment. This can consist of natural sedimentation or assisted sedimentation via coagulants and/or flocculants addition, or via centrifugation. This step also includes neutralisation, stripping (elimination of NH_3) and the removal of oils and grease by flotation.
3 *Secondary treatment*: this is used to eliminate colloids and similar matters from the waste water. With this treatment the organic load of the waste water is removed to a large extent. It can include chemical and biological processes. The most common processes are activated sludge treatment and anaerobic digestion which lead to important removals of chemical oxygen demand (COD), biological oxygen demand (BOD), phosphate and ammonia.
4 *Tertiary treatment*: this comprises physical and chemical processes to eliminate defined pollutants such as phosphate, ammonia, minerals, heavy metals, organic compounds, etc. These types of treatments are considered as a 'polishing phase' and are usually more expensive than conventional ones. The need of the application of this type of treatment is dictated mainly by two potential factors:
   - The requirement to meet discharge conditions based on Environmental Quality Standards (ESQs) (Environmental Quality Standards, 2006), which may be stricter than the requirements of BAT. Relevant

substances include ammonia, List I and List II substances and suspended solids.
- Recycling of the waste water for its use in the factory, either as process water or washing water.

These approaches can be also applied in sensitive areas where the effluent has to have a very low charge of N and P.

5.2.2 Water and wastewater minimisation: process integration/pinch technology and other optimisation techniques

Smith *et al*. (1994) presented a comprehensive overview of how to minimise both water and waste water. Water pinch analysis embedded in wastewater minimisation techniques offers simple methods and beneficial results when applied to water-using industries. Traditional approaches to water minimisation such as changing washing operations, when complemented with water pinch analysis methodology have been shown to achieve 30–60% fresh water savings in industrial applications, as stated by Smith and Petela (1991–92, 1994).

Smith (2005) has comprehensively described the technique that has been developed to minimise the amount of water that is used in water-using processes. He lists the main water-using operations as:

- reaction medium (vapour or liquid);
- extraction processes;
- steam stripping;
- steam ejectors for production of vacuum;
- equipment washing;
- hosing operations.

These are grouped together as they have the common feature of bringing water into contact with contaminants. The transfer of contaminant mass into the water increases the overall concentration of contaminant as shown in Fig. 5.1. The flowrate of water in the operation determines the concentration of contaminant exiting from the operation. If the flowrate of water is decreased, and the same mass transfer of contaminant is required, then the concentration of contaminant exiting from the operation is increased (Fig. 5.2). Consequently, if a water flowrate reduction is required, then the consequence will be an increase in contamination concentration at the outlet. This may not be permissible for the following reasons:

- maximum solubility;
- corrosion limitations;
- fouling limitations;
- minimum of mass transfer driving force;
- minimum flowrate requirements;
- maximum inlet concentration for downstream treatment.

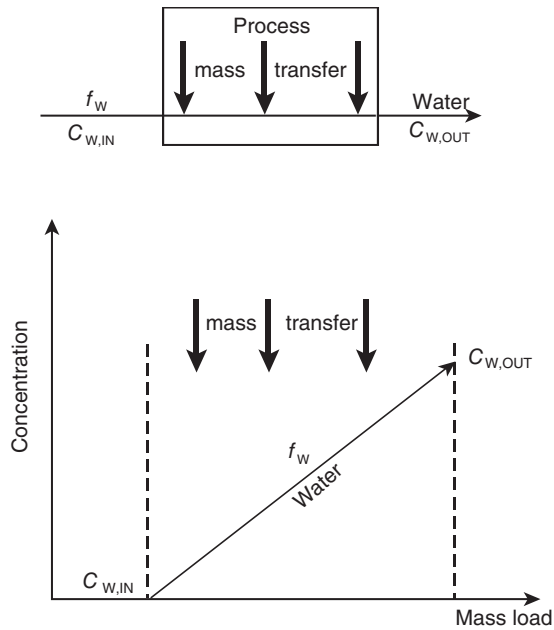

Fig. 5.1 Contaminant mass transfer in water. f_W, water flowrate; $C_{W,IN}$, contaminant mass flow in; $C_{W,OUT}$, contaminant mass flow out (after CPI 2004 and 2005).

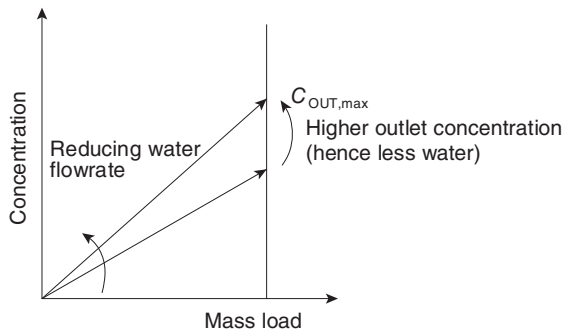

Fig. 5.2 Concentration of contaminant related to water flowrate (after CPI 2004 and 2005).

Assuming that all process operations use clean water, minimisation of water use can be achieved by reducing the flowrate to its minimum. However, as Smith (2005) emphasises, this loses the opportunity to reuse water, which can considerably reduce the overall amount of clean or fresh water used by the process operations.

In order to reuse water between operations, thereby further reducing the amount of clean water required by the water-using operations, some level of inlet concentration of contaminant has to be set. This is illustrated in Fig. 5.3. This figure shows a water-using profile where the contaminant

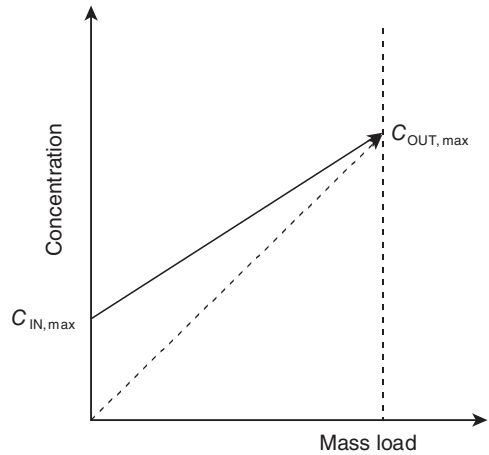

Fig. 5.3 Setting targets for contaminant concentration (after CPI 2004 and 2005).

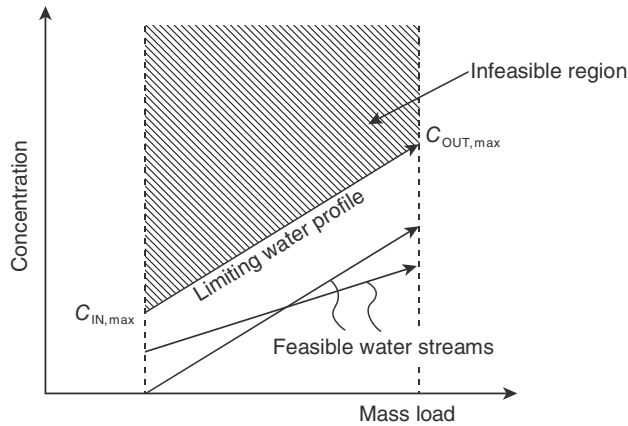

Fig. 5.4 Limiting contaminant water profile (after CPI 2004 and 2005).

concentrations at the inlet and outlet have been set to their maximum values. This setting can be used to define the limiting water profile, which provides a boundary between feasible and infeasible concentrations. If the contamination concentration of a water profile is below that of the limiting water profile, then the concentration is feasible (Fig. 5.4). This feature can be used to recognise reuse opportunities and according to Smith (2005) has a number of advantages:

- Water-using operations that have different characteristics can be easily compared using a common basis for comparison.
- Calculation of the mass transfer of contaminant does not require a model of the operation.
- The flow pattern of the operation is not required for analysis.

Table 5.1 Problem data for four water-using operations (after Wang and Smith, 1994)

Operation number	Contaminant mass (kg/h)	C_{IN} (ppm)	C_{OUT} (ppm)	f_L (t/h)
1	2	0	100	20
2	5	50	100	100
3	30	50	800	40
4	4	400	800	10

- The methodology is applicable across the entire range of water-using operations.

Wang and Smith (1994) have used these principles to determine the amount of water required by a series of operations, employing water reuse, compared with these operations using fresh water. They use a simple example, making use of the limiting composite curve, for four water-using operations (Table 5.1). The table gives the maximum inlet and outlet concentrations (C_{IN} and C_{OUT}, respectively) for a single contaminant for four operations; it also gives the limiting water flowrate (f_L), which is the flowrate required by the operation if the mass of contaminant is taken up by the water between the inlet and outlet concentrations. It should be noted however, that if fresh water is available, and an operation has an inlet concentration greater than zero, then using uncontaminated fresh water would allow a lower flowrate than the limiting water flowrate for that operation. A simple analysis of the given problem, reveals that the total fresh water required by operations (assuming zero concentration of contaminant in the fresh water) is 112.5 t/h, with the four operations requiring 20, 50, 37.5 and 5 t/h, respectively. However, if reuse of water is allowed, then analysis, making use of the limiting composite curve, gives a targeted minimum flowrate of 90 t/h. The limiting composite curve of the four water-using operations is given in Fig. 5.5. The water supply line, satisfying the water-using operations represented by the limiting composite curve, has its origin at zero concentration, and lies below the composite. The slope of the line is such that it touches the composite at one point, known as the water pinch. Other water supply lines with the same origin could be drawn, but these would not touch the composite, and would indicate flowrates larger than the minimum. If the water supply line was drawn with a steeper slope, indicating a smaller flowrate, then this line would cross the limiting composite curve, and would not be feasible.

Smith and Wang (1994), as well as other researchers (Kuo and Smith, 1998; Savulescu *et al.*, 2005a,b) extended the methodology for calculating the minimum flowrate of water, including reuse, required to remove contaminants from water-using operations, also provided a methodology for the design of the water system. For the problem above, the final design of

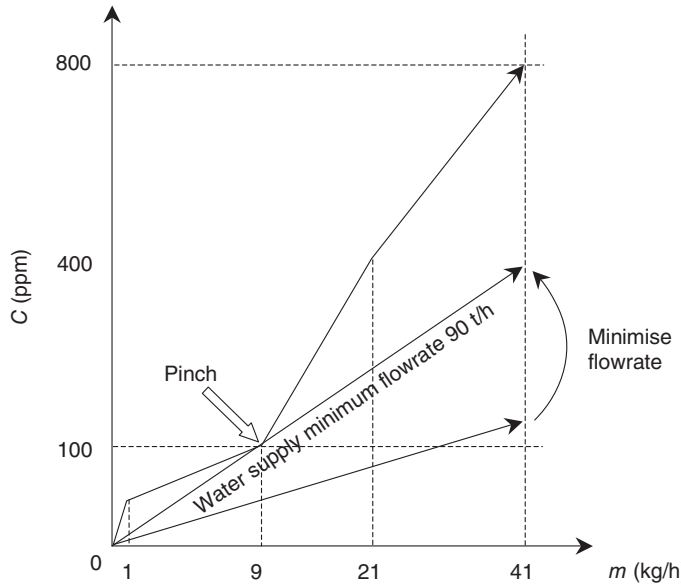

Fig. 5.5 Limiting composite curve and water pinch (after CPI 2004 and 2005).

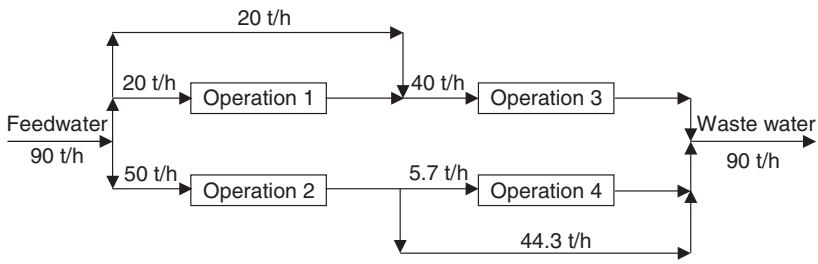

Fig. 5.6 Final design of water treatment using water pinch methodology (after CPI 2004 and 2005).

the water operation is given in Fig. 5.6. This shows that of the original targeted amount of fresh water of 90 t/h, 20 t/h is fed to operation 1 and 50 t/h is fed to operation 2. The remaining 20 t/h is fed to operation 3, along with 20 t/h from operation 1. Of the original 50 t/h fed to operation 2, 5.7 t/h is fed to operation 4, while the remaining 44.3 t/h goes directly to waste water. However, the authors state that this design could be further evolved to produce alternative networks.

5.3 Overview of selected case studies

Various case studies have been published covering food processing technologies that require water and produce waste water. These include, among others, sugar production, beer brewing and fruit juice production.

5.3.1 Sugar plant case studies

Zbontar Zver and Glavič (2005) presented a case study of a sugar plant processing approximately 4300 t of beet in a 24-h period. The beet processing season or campaign lasts approximately 3 months, from October until January. The objective of sugar beet processing is to extract pure sugar from the beet while separating pulp and non-sugars such as minerals and water.

The sugar industry is one of the major water users and wastewater producers. Gutteck (1989) assumed that processing of 1 t sugar beet would require around 20 m^3 of fresh water, supposing that for each stage fresh water would be used. A large sugar plant processing 10 000 t/day of beet therefore requires 2500–4000 m^3/year of fresh water and discharges an even larger stream of waste water (including water contained in the processed beet). Water-saving measures such as water reuse, regeneration and recycling are well known and are widely applied in the sugar industry, see, for example, Grabowski *et al.* (2002) and Urbaniec and Wernik (2002). The present trend in modern sugar plants is to reduce water consumption towards 'zero', implying that all the needs for water in the process are covered by the water contained in the sugar beet (0.75 t of water per 1 t of sugar beet), except for the water required for the boiler house. Grabowski *et al.* (2002) suggested that the water and energy use minimisation in the sugar industry 'caused' – as a result of the process changes – a novel sugar manufacturing process to be established.

The case study results presented by Zbontar Zver and Glavič (2005) have shown the possibility of reducing freshwater consumption during the beet processing season by at least by 54.23 m^3/h, i.e. 69% of all the water consumed, with a payback period of 5 days. This very economically attractive result clearly demonstrates that there is still huge scope in the food processing industry for rapid improvements. The study evaluated several actions, many of which can serve as inspiration to other similar processing plants:

- good housekeeping and regular maintenance (diminished costs on one side and prevention of unnecessary water losses on the other);
- division of wastewater streams with different quality in order to enable more possibilities for water reuse, regeneration reuse or recycling reuse;
- reuse of less contaminated waste water in cooling systems, for pump packing, for equipment cleaning, production area washing, etc.;
- mixing of waste water with fresh water in order to equilibrate contaminant concentration and temperature;
- reuse of steam condensate in boiler house or other process operations;
- wastewater regeneration with less complicated procedures (disinfection, neutralisation, etc.) that do not raise operation expenses considerably, and reuse of the regenerated water in systems that allow such level of water contamination.

5.3.2 Case study of water and wastewater minimisation in a citrus plant

This case study describes a water and wastewater minimisation study carried out for a citrus plant located in Argentina (Thevendiraraj *et al.*, 2003) using pinch technology (Wang and Smith, 1994) and its extensions (Kuo and Smith, 1998). Citrus juice processing plants utilise large quantities of fresh water. The objective of this study was primarily based on reducing the overall freshwater consumption and wastewater produced in this plant.

Background of citrus plants

The main products are citrus fruit juice (in concentrated form) with essential oil and dehydrated peels as by-products. The raw material to the plant is fresh fruit and this is subjected to a series of processes. The citrus processing plant is categorised into the following processes (UNIDO, 1969 and Fig. 5.7): (1) selection and cleaning; (2) juice extraction; (3) juice treatment; (4) emulsion treatment; (5) peel treatment.

Water pinch analysis

Water minimisation was achieved by maximising water reuse and by the identification of regeneration opportunities. Water-using operations were characterised by the maximum inlet and outlet contaminant concentrations, which are dictated by equipment corrosion, fouling limitations, minimum

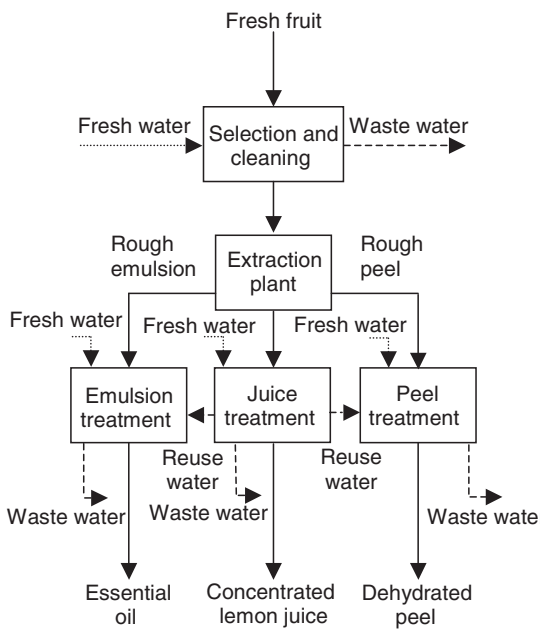

Fig. 5.7 Schematic flow diagram of a typical citrus plant.

mass transfer driving forces and limiting water flowrate through an operation. Targets determined the minimum freshwater requirement using the limiting composite curve and water distribution network. Pinch graphical methods were based on single contaminants and were further extended to multiple contaminants. When dealing with a number of operations, multiple contaminants and multiple water sources, the problem becomes more complex and algorithms using the basic principles have been developed and can be solved using mathematical programming as described by Smith (2005).

Data extraction

The first step is data extraction: each stream is characterised by its contaminant concentration, inlet and outlet concentration levels, and limiting flowrate through each operation. The data are provided by Lithoral Citrus Plant Authorities (2002) in a schematic flow diagram of the citrus plant (Fig. 5.7), a simplified water distribution network and the mass balance of the water streams in the citrus plant. Eleven freshwater-using operations are identified. The main limiting contaminant for this analysis consists of the COD, because it makes up the largest contaminant in the majority of the water streams and exhibits significantly high values. The overall mass balance is closed with a simplistic assumption of 1 t/h as evaporation losses from the steam system to account for an inconsistency of 1 t/h of water. The data extracted included the current inlet and outlet contaminant concentrations together with the water flowrate through each operation accounting for water gained and/or lost from the process. The total mass load picked up by the fresh water through each operation was then calculated. The 11 water-using operations with the water flowrates entering and exiting each operation are represented in the form of a simplified water network as shown in Fig. 5.8.

The freshwater COD concentration level in the plant is 30 ppm. There is existing reuse of water between processes currently in the plant and these water reuse streams have been left unchanged. The simplified water network presented in Fig. 5.8 shows the freshwater-using operations with existing water reuse streams 'in-built' within each identified operation. The current total fresh water consumed and waste water generated in this citrus plant were 240.3 and 246.1 t/h, respectively.

Water pinch targeting

The existing water network provided a base starting point for the water pinch analysis. The freshwater target was evaluated by the composite curves. The maximum concentration levels were based on the constraints and limitations dictated by process conditions and requirements. The data were represented in the WATER software (2002, 2006) with identified constraints. The WATER software creates a solution to the defined problem using mathematical programming based on water pinch technology

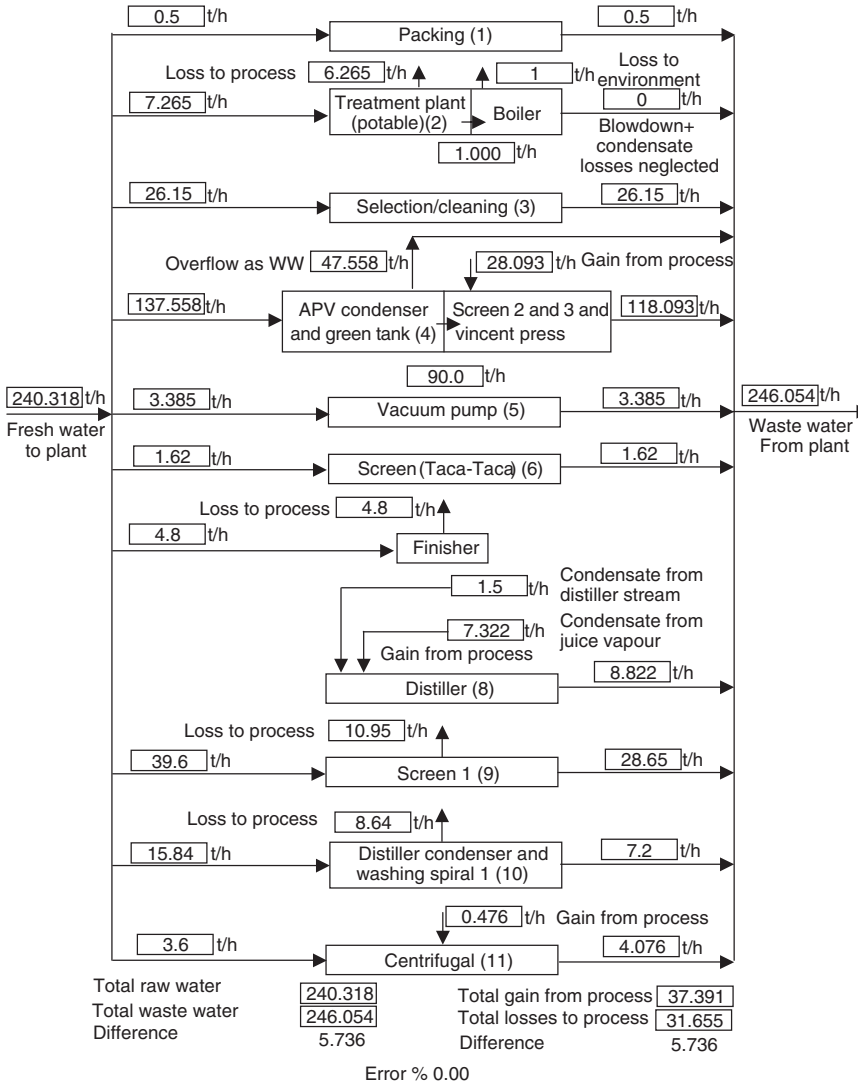

Fig. 5.8 Existing water network (simplified).

principles. The program uses a superstructure that includes all feasible structures and linear programming with feedwater minimisation as the objective function. The automated design allows user interaction through specification of additional constraints that are not part of the formulation. The process restrictions on the water type permissible for each operation indicate that operations 2, 4, 5 and 10 require only fresh water as feed (Fig. 5.9). Consequently the minimum fresh water required by the plant operations is 164.4 t/h.

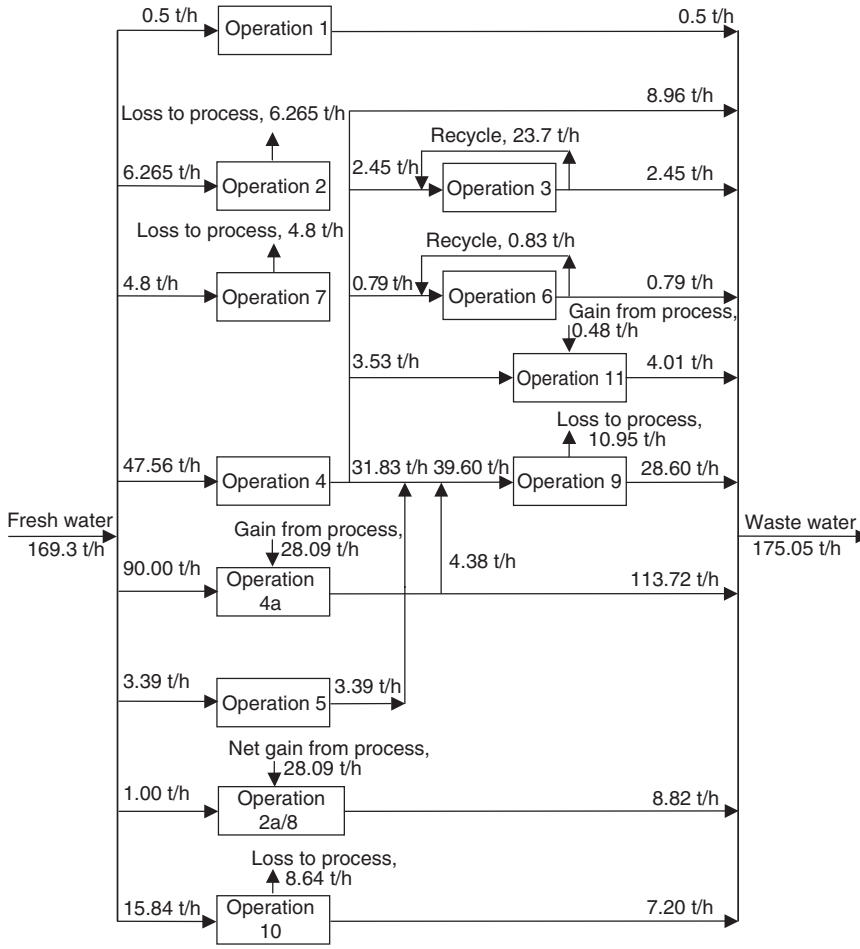

Fig. 5.9 Water network after pinch analysis as a conventional diagram.

Operation 1 is a batch process and although it allows water reuse at its inlet operation, in this analysis it is assumed that fresh water is continuously available for this operation at any one time. The total minimum freshwater requirement for this plant is 164.4 + 0.5 = 164.9 t/h. The current total freshwater feed to the plant is 240.3 t/h. The maximum theoretical freshwater reduction that is achievable is 31.4%.

The water pinch analysis is then carried out for the existing water network with maximum concentration levels. The overall freshwater target is calculated using the maximum reuse analysis. The water network, represented as a conventional diagram, and the limiting composite curve are presented in Figs 5.9 and 5.10, respectively. The freshwater target is compared with the current freshwater consumption to evaluate the overall water and

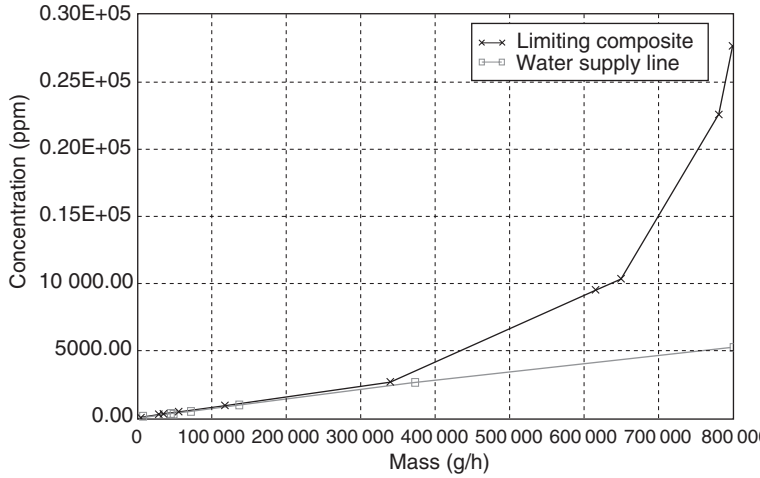

Fig. 5.10 Limiting composite curve generated by WATER software.

wastewater minimisation for this plant. The existing network results in a freshwater demand of 169.3 t/h and a wastewater flowrate of 175.1 t/h. This shows substantial reductions in water and wastewater minimisation, but the design includes reuse of certain streams that require treatment. The analysis is based on COD as the main contaminant in the reuse streams. These streams may contain other contaminants such as solid waste and chemicals in small amounts that may require further treatment prior to their being used for other processes. Further design options need to be developed that deal with process requirements, operating conditions and water reuse suitability.

These reductions may be achieved by introducing further constraints to potential reuse streams, and by utilising the maximum water reuse analysis to obtain optimised designs with improved freshwater targets that meet all process operating conditions and restrictions. The regeneration reuse analysis can also be utilised to explore further design options that allow reuse of regenerated water in some operations. This analysis requires installation of a treatment unit to regenerate waste water, e.g. gravity settling, filtration, membranes, activated carbon system, biological treatment, etc. This further reduces the freshwater target and wastewater generation in the plant compared with the maximum water reuse analysis and was analysed by the WATER software.

Four different design options were generated considering both the maximum reuse analysis and regeneration-reuse analysis. Design options A and B were based on the maximum reuse analysis with both achieving a freshwater consumption of 188 t/h compared with the actual freshwater consumption of 240 t/h, reducing freshwater use/wastewater generation by approximately 22% (Fig. 5.10). There is no further scope for water reuse

due to process limitations and restrictions. The finding at the diagnostic stage indicates that the theoretical maximum freshwater/wastewater reduction is 31%. A further reduction in freshwater/wastewater is achievable by regenerating waste water and reusing it in other operations. Design options C and D are generated based on the regeneration reuse analysis, both resulting in total freshwater consumption of 169 t/h, which is equivalent to a 30% reduction compared with the actual plant consumption.

The reduction in fresh water is achieved by re-routing water streams and therefore requires new pipes. Design option A requires the fewest new pipes (five new pipes), followed by design options B and D with seven pipes each and finally design option C with nine new pipes. These reflect the impact on investment costs. The five new pipes identified for design option A are also required in all of the other options for the same function with similar flowrates. This indicates that design option A requires the lowest investment cost and design option C requires the highest investment cost. Design options C and D both require additional costs for investment in a regeneration unit compared with design options A and B. The results are summarised in Fig. 5.11.

Design options A and D (shown as conventional diagrams in Figs 5.12 and 5.13) are the most attractive options for the maximum reuse analysis and the regeneration reuse analysis. Design option A shows lower freshwater reduction with lower investment cost compared with design option D which results in a higher freshwater reduction and requires much higher investment costs. The waste water generated will also reduce correspondingly with freshwater reduction, hence reducing wastewater treatment operating costs in addition to freshwater savings for each of the options. The cost analysis carried out for design option A shows very attractive financial returns for this low-investment option with a payback period of 0.14 years. The outlet water quality of operation 3 requires further analysis and therefore the investment cost for the regeneration process is not

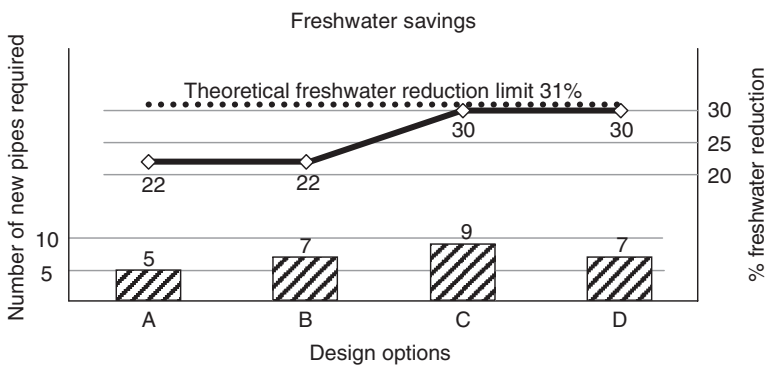

Fig. 5.11 Summary of design options.

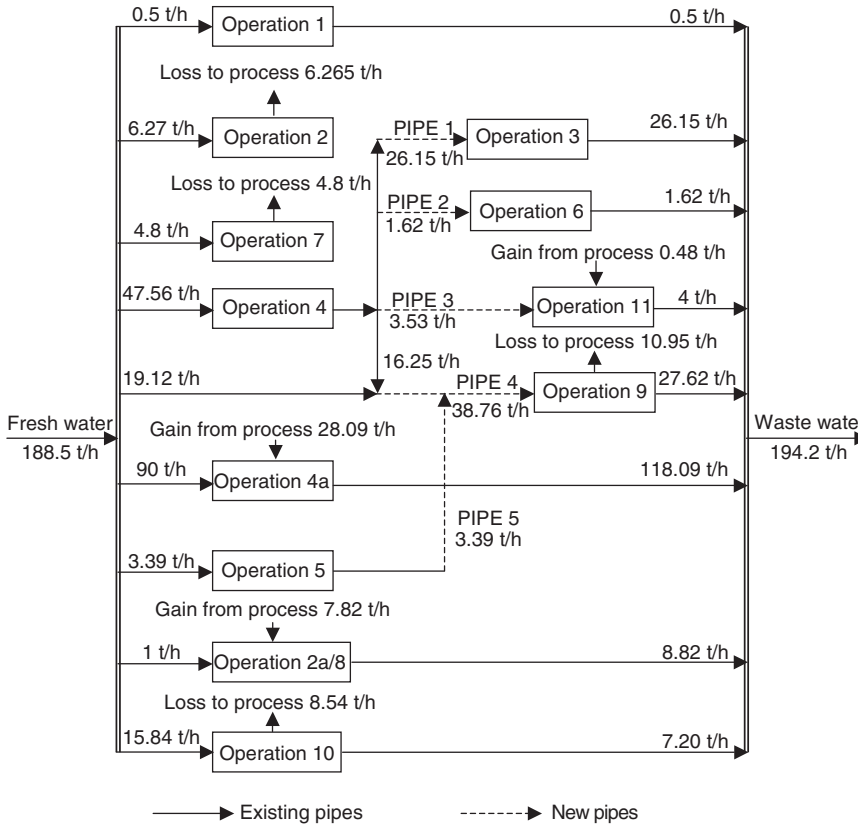

Fig. 5.12 Design option A: simplified water network as a conventional diagram.

available to permit a complete cost evaluation of design option. Further detailed studies need to be carried out to dictate the regeneration process type required and its associated costs in order to fully evaluate this option.

The thermal energy of the reuse water streams proposed in the respective design options are also reviewed to ensure that stream temperatures at the inlet of operations are unchanged. The operating temperatures for the varying operations, with reference to information obtained from the citrus plant authorities, indicate that almost 90% of the operations operate at ambient temperatures with the exception of operation 8 which produces waste water at 90 °C. This stream is highly contaminated which carries some limitations. All the water reuse streams as suggested in the design options are appropriate in terms of temperature requirements and are not expected to affect the operation thermally. The overall hot and cold utility requirements of this plant will not be affected by the changes proposed in the design options.

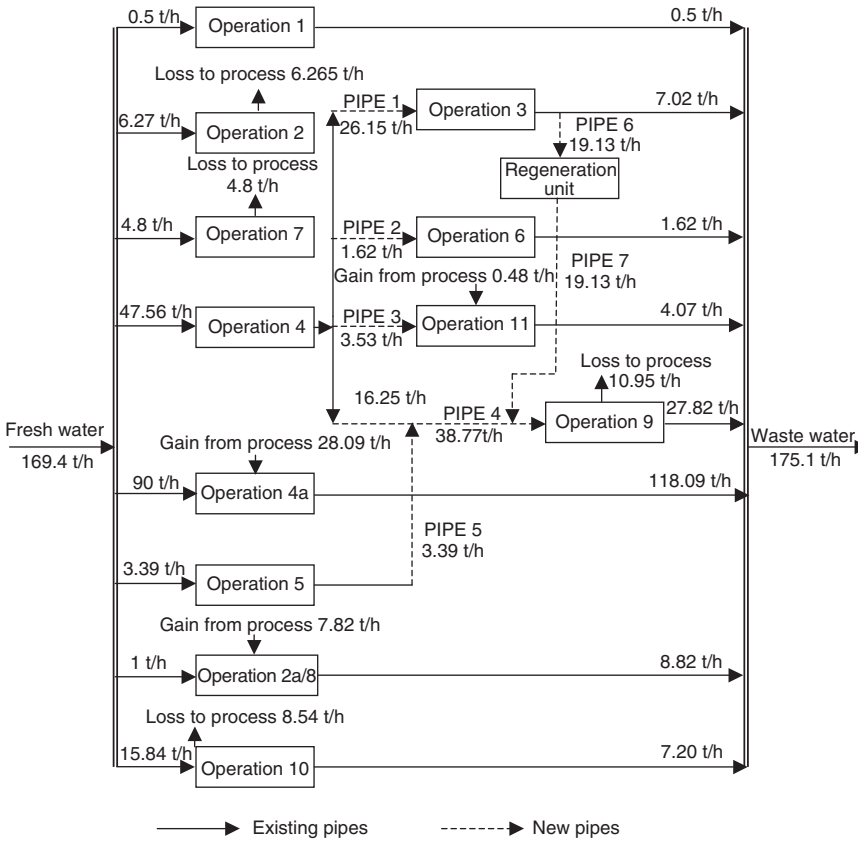

Fig. 5.13 Design option D: simplified water network as a conventional diagram.

Conclusions

The existing freshwater consumption of the plant is 240.3 t/h with a wastewater generation of 246.1 t/h. The design options result in a reduction of freshwater consumption and a corresponding reduction in wastewater generation of 22% for the maximum reuse analysis and 30% for the regeneration reuse analysis. For a practical project the number of modifications is limited. The maximum water reuse analysis requires a minimum of five new pipes, and the regeneration reuse analysis requires seven new pipes. The analysis shows very attractive financial returns with a payback period of 0.14 years. Further detailed studies need to be carried out to specify the regeneration process type required and its associated costs in order to fully evaluate this option.

Water pinch analysis for this citrus plant shows that reductions in freshwater consumption and wastewater generation of up to 30% can be achieved with minimum changes/investments made to the existing plant.

5.4 Sources of further information and advice

5.4.1 Literature and conferences: papers, books, relevant conferences and journals, websites to study with a short evaluation

There are a number of case studies available, for example: Dilek *et al.* (2003), a sugar plant; Hyde *et al.* (2001), food and drink industry – a demonstration project in East Anglia, UK; Vigneswaran *et al.* (1999), a prawn farm.

Wrigley (2004) presented an interesting example of on-farm dairy processing which provides various recommendations for good practices including some interesting cheese production figures. The need for integration of water and wastewater management with farm planning was an essential outcome of this study. It emphasised the need for measurement, performance-monitoring and self-regulation, which could easily be neglected in a busy farming environment.

Andreottola *et al.* (2002) presented pilot-scale experiments that were carried out applying the sequencing batch biofilm reactor (SBBR) process for the treatment of winery waste water. The aim was the evaluation of the SBBR performance and the development of a control strategy based on dissolved oxygen (DO) for the optimisation of the SBBR treatment cycle and the minimisation of the energy supply. The results of the experimentation have confirmed the applicability of the SBBR process pointing out high COD removal efficiencies between 86 and 99%, with applied loads up to 29 g COD/m^2 per day, corresponding to 8.8 kg COD/m^3 per day. The on-line monitoring of DO concentration appeared to be a good indicator of progress in the COD biodegradation.

Linnhoff March (www.linhoffmarch.com) have successfully completed numerous case studies including a whey products factory (Borculo, Netherlands), corn processing (Cestar, UK), food processing (Birds Eye Wall's (UK) and edible oil production (Van den Bergh & Jurgens, UK), etc.

Conferences

There are two main groups of conferences dealing with water minimisation. The first group is related to water and wastewater minimisation generally and includes conferences such as:

- PRES Process Integration, Modelling and Optimisation for Energy Saving and Pollution Reduction has been organised annually from 1999. The recent conferences were organised in Sicily, Italy in May 2005 (PRES '05) and in Prague, Czech Republic, August 2006 (PRES '06), (www.conferencepres.com).
- The IWA (International Water Association) is composed of specialist groups that organise various conferences. The recent conferences were held in June 2005 in Sapporo, Japan (Leading-Edge Conference and Exhibition on Water and Wastewater Treatment Technologies) and in

May 2006 in Moscow, Russian Federation (IWA Specialized Conference – Sustainable Sludge Management: State of the Art, Challenges and Perspectives).
- There are various other related conferences. Water and Wastewater Europe was held in 2004 in Barcelona, Spain; EnviroWater 2006 was held in May 2006 in Delft, the Netherlands (for more information see the website: www.wau.nl/rpv/isomul/envirowater2006/papers.htm).

The second group are specialised conferences related to food processing such as:

- The 3rd International Conference on Sustainable Agriculture for Food, Energy, and Industry. This was held on 22–27 August 2005 at Brock University in St Catharines, Canada (for more information visit the website: www.icsagr-fei.org/conference/).
- Air and Waste Management Association and Water Environment Federation International Conference: Animal Agriculture and Processing: Managing Environmental Impacts. This conference was held on 31 August–2 September 2005 at the Hyatt Regency in St Louis, Missouri (for more information visit the website: www.awma.org/events/confs/Animal/default.asp).
- 2005 National Forum on Contaminants in Fish. This conference was held on 18–21 September 2005 in Baltimore, Maryland (for more information, see epa.gov/waterscience/fish/forum/2005/).
- International Symposium on Water and Land Management for Sustainable Irrigated Agriculture. Conference held on 4–8 April 2006 at Cukurova University in Adana, Turkey (for more information visit the website: symp2006.cu.edu.tr/).
- CIWEM (European Biosolids and Biowaste Conference). The 9th conference was held in November 2004 at Wakefield, UK.
- The Annual Meeting of the Food Industry Environmental Council, US Environmental Protection Agency (www.epa.gov/water/speeches/031703tm.html).

Service providers

Selected service providers from the UK

- Centre for Process Integration, CEAS, The University of Manchester, PO Box 88, Manchester M60 1QD, UK, Tel. +44 (0)161 306 4380, Fax +44 (0)161 236 7439 (www.ceas.manchester.ac.uk/research/researchcentres/centreforprocessintegration/).
- Linnhoff March, Targeting House, Gadbrook Park, Northwich, Cheshire CW9 7UZ, UK; Tel. +44(0)1606 815100; Fax +44(0)1606 815151 (www.linnhoffmarch.com/contact/uk.html and www.kbcat.com).
- Cookprior Associates Limited, Thackie Cottage, Chirnside, Duns, Berwickshire TD11 3XH, UK, Tel. +44 (0)15 35 63 51 28, email enquiries@cookprior.com; www.cookprior.co.uk.

- Grantham Cartwright and Associates Ltd, Europarc Innovation Centre, Innovation Way, Europarc, Great Grimsby, North East Lincolnshire, DN37 9TT, UK, Tel. +44 (0)1472 361361, Fax +44 (0)1472 361362, email grimsby@granthamcartwright.co.uk (www.granthamcartwright.co.uk/contact.html).
- Accepta (water treatment chemicals, wastewater and speciality chemical supplies, test kits, chemical reagents, testing, analysis and control equipment), Quay West, Trafford Wharf Road, Manchester M17 1HH, Tel. +44 (0)161 240 2100, Fax +44 (0)870 135 6389, email info@accepta.com.

A variety of service providers outside the UK can be found on the web. Some of them are listed below:

- Esperanto Environnement, 189 rue de l'EtiageLachenaie, Quebec, Canada, Tel. +1 514 705 3274/1563, Fax +1 450 492 2082, Sales Tel. +1 514 705 1563, Fax +1 514 221 3393, email info@esperantoenv.com.
- CANMET, Natural Resources Canada, Tel. +1 613 995 0947 (www.nrcan-rncan.gc.ca/inter/contact_e.html).
- Frost & Sullivan, San Antonio, Texas, 7550 IH 10 West, Suite 400, San Antonio, TX 78229-5616, USA, Tel. +1.877.GO.FROST (463.7678), email myfrost@frost.com. In Europe, Middle East and Africa: 4 Grosvenor Gardens, London SW1W 0DH, UK, Tel. +44 (0)20 7343 8383, email enquiries@frost.com (www.frost.com/prod/servlet/locations.pag).

EC-supported projects

- AWARENET (Agro-Food Wastes Minimisation and Reduction Network). Provided valuable and comprehensive information on various aspects of waste minimisation, including energy minimisation (de las Fuentes *et al.*, 2002a,b).
- SUCLEAN (Research on minimisation of energy and water use in sugar production by cooling crystallisation of concentrated raw juice) (Klemeš *et al.*, 1999).
- ENALT 2C (Treatment of Wastewater from a Potato-Processing Factory by a Combined Four Step Anaerobic Stationary Fixed Film Technology). This project ended in 1990, however it still provides some interesting results (Cordis, 1990).
- UDOR (technology for treatment and recycling of the water used to wash olives). This project deals with another important water and wastewater-related issue – washing food (Cordis, 2004).
- SEWUM (water pinch: simultaneous energy and water use minimisation). This project significantly contributed towards the development of the process integration methodology reviewed in this chapter and increasingly applied in food processing industry (Cordis, 1998; Klemeš *et al.*, 1998).

- LIFE 1 (integration of environmentally friendly production technologies within the processing of slaughterhouse blood). Dealing with an important water and wastewater problem related to slaughterhouses. The main steps were: (1) decreasing energy and water use; (2) modification of certain production processes; (3) revaluation of certain waste streams; (4) clarification of waste water using ultrafiltration and reversed osmosis (Cordis, 1996).
- WATERMAN (intelligent management system for water and energy minimisation in Latin American food industries), INCO-DC Project.
- LIFE 1 (waste reduction and recycling through animal feed in the agri-food industry – 94/IRL/A13/IRL/00342). The main aims were the development of techniques for the collection, storage, recycling and disposal of waste, particularly toxic and dangerous waste and waste water (Cordis, 1997).

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Part III

Key issues and technologies for food waste separation and co-product recovery

6

The importance of microbiological risk management in the stabilisation of food processing co-products

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6.1 Introduction: importance of microbiological risk management in the stabilisation of co-products

Microbiological stabilisation is an imperative that should be designed into co-product processing systems. This is essential in order to avoid the growth of micro-organisms, which could have two major implications. Firstly, the micro-organisms may utilise or convert some of the co-products and hence diminish yields or corrupt the process (essentially, act as spoilage organisms). Secondly, the organisms may be capable of causing illness to workers involved in the process, or cause illness in consumers for whom the co-product is intended. Accordingly, eradication of micro-organisms or control of their growth should be an integral part of co-product processing. This chapter assumes that the co-product stream is derived from food waste and that the co-products produced from this stream are intended for consumption. Accordingly, the premise adopted is that the co-products can be stabilised by using processes or preservation systems used for food.

The strategies described below are designed to decrease the risk from microbiological contamination of co-products. These risks are that micro-organisms may cause spoilage of the co-products or waste streams, or that the micro-organisms are a hazard to health and may cause disease.

Firstly, therefore, we must define the difference between a hazard and a risk:

- A hazard can be defined as a biological agent with the potential to cause an adverse health effect. In the context of this chapter, however, the potential to cause an adverse spoilage effect will also be considered.

- A risk can be defined as a function of the probability of an adverse effect derived from the hazard and the severity of that effect, consequential to that hazard. Severity might be related to the extent to which the organism could corrupt the purity of the products in the waste stream, or the seriousness of an illness derived from ingestion of the hazard.

Strategies for control of microbiological risk, therefore, necessitate dealing with the hazard. Hazards are the micro-organisms; these might be bacteria, yeasts or filamentous fungi that are either natural components of the waste streams or are introduced by virtue of the co-product processing. If present in sufficient numbers, all three kinds of micro-organisms can result in spoilage, and some types of bacteria, yeasts and filamentous fungi can cause disease in humans.

Classically, the strategies for microbiological risk management involve three key approaches, which are described below.

6.2 Strategies for microbiological risk management

6.2.1 Introduction

The risks to be controlled are the possibilities that the hazards (the micro-organisms) might enter the co-product stream, that they might grow and that they might cause changes to the co-product or result in disease in a consumer. Control of micro-organisms is typically achieved by applying one or more of the three major tiers of microbiological safety (after Schlundt, 2002), which are:

- Good Hygienic Practices;
- Hazard Analysis and Critical Control Points (HACCP);
- Risk Analysis.

6.2.2 The three tiers of microbiological safety

Good hygienic practices

This is very much a hands-on concept and involves general hygiene in production, preparation and processing of the co-product streams and of the end-products derived from them. It involves ensuring that the product remains 'microbiologically clean' throughout and that no additional microbiological contamination occurs during the processing. However, it does not quantify the initial numbers of contaminating micro-organisms, does not determine the risk presented by them, and nor does it introduce procedures capable of controlling their growth or eradication. It is a good first step and should be applied irrespective of the use of HACCP or Risk Analysis approaches.

In the context of co-products, the application of good hygiene practices would begin with the segregation of separate waste streams. This should

ensure that microbiological contamination is minimised. For example, waste streams intended for co-product processing should not be channelled as if they were to be discarded, but should be identified as a specific entity. In this way their microbiological quality can be maximized and not compromised by mixing with other material that may be regarded only as 'waste'.

HACCP

This is a practical, yet systematic approach to the identification, evaluation and control of hazards, and focuses on micro-organisms in the co-product streams and end-products. It designs methods into the process by which the survival and growth of micro-organisms can be controlled. HACCP is, therefore, a tool to control hazards operationally. It concerns the delivery of processing variables that might be determined as requirements for processing. For example, where a heat treatment is required, it is HACCP that controls the time and temperature to which the co-products are exposed. HACCP is based on seven principles, which define the systematic approach to the identification, evaluation and control of hazards as follows (see Table 6.1).

Principle 1. Conduct a hazard analysis

This involves an understanding of the organisms likely to be found within the co-product stream or the end-product, or that may be introduced into the stream throughout processing. Once the hazard has been identified it can be controlled.

Principle 2. To determine the Critical Control Points (CCPs)

These are the methods or processes by which the hazards (micro-organisms identified as being contained within the co-product) can be controlled. For example, the micro-organisms may be susceptible to a particular pH, they may be susceptible to a particular heat and time combination, or to a particular pressure treatment or degree of dehydration. These applied processes or preservative systems contained within the process are the CCPs, and these must be attained if the hazards are to be controlled.

Table 6.1 The seven principles of HACCP

1	Conduct a Hazard Analysis
2	Determine the Critical Control Points (CCPs)
3	Establish critical limits
4	Establish monitoring procedures
5	Establish corrective actions
6	Establish verification procedures
7	Establish record-keeping and documentation procedures

Principle 3. To establish critical limits.
This stage involves identifying the acceptable operating limits of the CCPs. For example, it may be appropriate to control the pH between 2.55 and 3. These then become the limits of the CCP. Equally, a time and temperature treatment may be defined as that required to reduce the numbers of micro-organisms by between 6 and 7 orders of magnitude within the co-product or processing stream. The treatments necessary to achieve these decreases then become the limits.

Principle 4. To establish monitoring procedures
Once the CCPs have been identified and their critical limits defined, it is vital that these are monitored constantly. Preferably these should be built in as on-line measurements and monitoring such that each batch of the process or co-product should be recorded as having experienced a specific CCP regime.

Principle 5. To establish corrective actions
The monitoring of adherence to CCPs will indicate when failure to achieve the specified CCPs occurs. Ideally the monitoring procedures should contain alarms that indicate when CCPs have not been met. Corrective actions should then be applied. This might involve simply reworking the co-products or the end-products through the same process, ensuring that the CCPs are met.

Principle 6. To establish verification procedures
The procedures confirm that CCPs had been met successfully and that where corrective actions needed to be applied, these were adequate.

Principle 7. To establish record-keeping and documentation
Recording and documentation should be retained as evidence that HACCP had been applied successfully to the co-product stream. This is an essential element of demonstrating that the requirement for due diligence has been met.

CCPs might involve applied processing or they might involve the use of preservative compounds, procedures or systems within the co-product stream, the co-products or the end-products themselves. Section 6.3 is an introduction to methods of preservation and deals firstly with preservation systems where agents might be included within the prepared co-products or end-products; these agents are intended to protect against microbial growth during processing, storage, distribution and sale. Secondly, it deals with applied processing, where processes are applied to a batch or continuum of co-product.

Quantitative Microbiological Risk Assessment (QMRA)
This third tier of microbiological risk management was developed under the auspices of The Codex Alimentarius Commission. It is a broad and

overarching framework that is primarily for governmental safety management. QMRA can be considered as a means of imposing safety policy and is one part of a three-part Risk Analysis approach to microbiological safety (Schlundt, 2002). QMRA is the first part of the process, and overlaps with the second and third components of risk management and risk communication, respectively.

Risk Assessments were developed in the chemical industry and in chemical toxicology, where they have been established for managing chemical risks. This was enabled by the availability of dose–response models for chemical toxicology data. However, an analogous system has recently been applied to microbiological safety. This has grown from an increasingly precise ability to model the inactivation and growth of micro-organisms, but has also benefited from the availability of dedicated software and increases in computing power. However, it cannot be overstressed that QMRA is a large-scale global approach that is intended for international application. It is derived from drivers of international trade (principally The Sanitary and Phytosanitary Measures Agreement of the World Trade Organisation), which require measures based on scientific principles that can be assessed by independent experts and that form an objective reference point for international agreement.

Although principally a global and governmental exercise, it will be beneficial to describe here activities involved in QMRA, because these can inform about the microbiological quality of a co-product stream and end-product. The concepts are extremely complex, although worthy of discussion because Risk Assessment provides an estimation of health risk. However, it can equally be applied to an estimation of the risk of microbiological spoilage and provide decision support relating to the need for product recalls. It should not be confused with HACCP, and although both concepts use similar terminology, they are used for different purposes (see Fig. 6.1).

In principle, HACCP is a tool to control hazards operationally. For example, the definitions above show how HACCP involves the control of time and temperature. These are the CCPs. However, it is the role of Risk Assessments to define what extent of control should be imposed, and hence designed into a process. For example, the Risk Assessment might necessitate that to control the safety of a product globally it is necessary to reduce the numbers of micro-organisms by a number of orders of magnitude. The Risk Assessment therefore dictates, as a matter of policy, that a time and temperature should be built into the process to ensure such a decrease in numbers of micro-organisms. It is HACCP that ensures that this requirement is built into the process in a practical way in the form of appropriate CCPs.

The broad application of QMRA (see Table 6.2) to co-products or end-products necessitates an understanding of the types of micro-organism in the co-product, their numbers and fate, and the consumption or use pattern of that co-product or end-product.

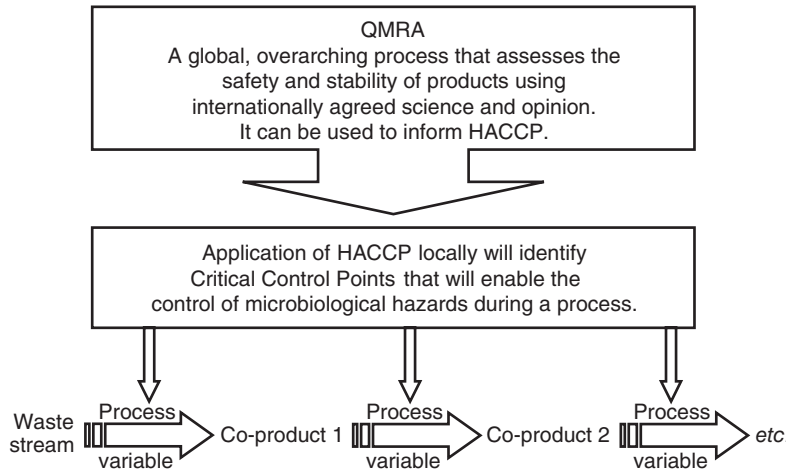

Fig. 6.1 A scheme to describe the difference between the global, overarching process of QMRA and the application of HACCP, which will identify CCPs that will enable the control of microbiological hazards locally at each stage throughout a process.

Table 6.2 The component stages of QMRA

Hazard identification
Hazard characterisation
Exposure assessment
Risk characterisation

Methodology of QMRA

1 *Hazard identification*. This can be either reactive or proactive. Reactive hazard identification is our response to a microbiological hazard that has been identified as a consequence either of an outbreak of disease or of spoilage. It is a response to a problem and to cases where the micro-organisms have been identified and confirmed as the causal agent.

Proactive hazard identification is preferable. It arises where the presence of a microbiological hazard in a particular product may be suspected, but where a link between the product and disease or spoilage has not definitely been established. Nevertheless, this gives us an opportunity to anticipate and control that hazard. The information needed for hazard identification might be derived from databases developed for the purposes of epidemiology or food spoilage, where micro-organisms associated with a particular commodity or co-product derived from that commodity are well documented. The raw materials may thus be used to inform the hazard identification because they

might classically be associated with particular spoilage or food-borne pathogenic micro-organisms. Equally, expert information may be used in order to obtain additional data about the likelihood of occurrence of particular micro-organisms in the waste stream or the end-product. The micro-organisms can then be prioritised: some pathogens or spoilage organisms will be more important than others and therefore require urgent control. Pathogenic or spoilage micro-organisms that might emerge given novel marketing strategies must also be anticipated. For example, co-products intended to be stored or marketed in a chilled distribution chain could be susceptible to growth of psychrotrophic bacteria whose involvement in the product had not been anticipated.

2 *Hazard characterisation*. This is the evaluation of the nature of the adverse effects resulting from the presence of micro-organisms in the co-product. It may be qualitative or quantitative. The hazard characterisation can be influenced by the composition of the co-product stream. For example, a co-product stream that contains nutrients and water will promote the growth of micro-organisms, whereas an alcoholic solution or suspension will not. An additional factor is the numbers of organisms present. This is termed the dose and its importance is a function of the status of the consumer or the co-product which might render each more vulnerable to disease in the case of the consumer, or spoilage in the case of the co-product. The dose of organisms depends in part on the initial number contaminating the waste stream. However, it is influenced by the ability of the waste stream to support multiplication. This will increase the number of micro-organisms within the waste stream during processing. Equally important as an influence on dose, is the effect of any final preparation. For example, where the co-product is intended for inclusion within a food, any influence of treatment by the consumer (such as time and temperature of storage) should also be included in the dose–response, and an estimate made of the effects of such variables on numbers of spoilage or potentially pathogenic micro-organisms.

An additional concept that must be included in hazard characterisation is the status of the consumer. Consumers may be considered to be either normal populations who are fit and well, or susceptible individuals. These susceptible individuals are defined as the young, the old and the diseased; those who are pregnant, immuno-compromised or malnourished; and additionally include tourists who move from one culture and dietary regime to another, which might make them vulnerable to infection. Vulnerability to infection is described by dose–response curves, which express the sensitivity of individuals. These, of course, will differ between the susceptible individuals and the normal population, and the correct relationship must be used for any assessment of the vulnerability of that part of the population. Dose–response curves essentially define the likelihood that ingestion of a given number of potentially pathogenic micro-organisms will result in disease.

However, it could be proposed that a dose–response concept can be developed to inform about the likelihood that spoilage micro-organisms in a product could result in spoilage of that product or products into which it is incorporated. This necessitates a simpler information stream than is required for a disease dose–response curve. Essentially, it can be generally assumed that numbers of bacteria or yeasts in excess of 10^6 colony-forming units per gramme could result in microbiological spoilage.

3 *Exposure assessment*. This description can also be qualitative or quantitative, and is an assessment of the likely intake by a consumer of the hazard in the co-product. Equally, it could assess the likely presence of micro-organisms able to cause spoilage within that co-product. Exposure assessment can be regarded as a function of the prevalence of the hazard in the raw materials or the co-product stream, and its survival during the processing, but also the potential for re-contamination by organisms likely to cause spoilage or ill health. It includes the potential for the survival or growth of those organisms, or contamination and growth after the co-products have been processed, but prior to the consumption by a consumer.

4 *Risk characterisation*. This final aspect of QMRA is an integration of all that has gone before. It is thus an integration of hazard identification, hazard characterisation and exposure assessment. It may again be quantitative or qualitative and it is an estimate of the probability of an occurrence and the severity of the potential adverse effects derived from processing of the co-products or from consumption of the co-products. Therefore risk characterisation combines the identification of the micro-organism concerned and its virulence, either in terms of the risk to the consumer or its ability to cause spoilage. The specific dose–response information about how many organisms are required to either cause spoilage of a product or cause disease by consumption of that product, should then be related to the consumption habits (if the material being produced from the co-product is to be consumed) and, of course, the fate of micro-organisms during the production of the co-products or the storage, distribution, preparation, sale and use of the end-products.

Risk Assessments might be deterministic or probabilistic. Deterministic assessments calculate the risks as a function of a single value and generate a point estimate of risk. This is typically regarded as somewhat conservative and does not take into account variability and uncertainty.

Probabilistic methods for determining risk are more complex, and involve extensive mathematical computation. Software to calculate these effects is widely available. They fall into two major categories: those that calculate risk using Monte Carlo simulations (e.g. @Risk software), and those that combine individual probabilities using Bayesian belief networks (e.g. Hugin software).

Provided that the correct micro-organisms are targeted, it should be possible for the outcome of QMRA to be the likelihood of (a) spoilage of a co-product stream due to the presence of specific organisms or (b) illness due to the consumption of a specific end-product. The estimated number of spoilage incidences or illnesses, and the outcome, may also provide risk estimates for processing, distribution and consumer-use scenarios. For example, it should be possible to assess the risk associated with an entire processing route and to determine the influence of each stage of that route on the overall risk. In this way it will be possible to minimise risk, and hence to substitute routes or processes of high risk with lower risk alternatives.

6.3 Strategies for controlling micro-organisms: methods of preservation

In order to use the range of food-grade preservation methods available optimally, it is important to understand their characteristics, and any interactions that occur between them. Preservation is intended to prevent or retard deterioration that may otherwise be inevitable in co-product streams or end-products due to the effects of changes in their biochemical, chemical, physical or microbiological state. However, what follows deals with measures to control micro-organisms. The methods of preservation described aim to either kill the micro-organisms, or prevent or retard their multiplication. By so doing, the methods of preservation should aim to prevent microbiological spoilage, but should also prevent the growth (and preferably survival) of, and production of toxins by, food-poisoning micro-organisms.

Typically, substrates that support microbial growth have a naturally occurring microflora, the growth of which may be desirable (for example in cheese or yoghurt). If the growth of these associated micro-organisms is not controlled then microbiological deterioration, causing subtle changes in the characteristics of the product, may occur (e.g. increased acidity in dairy products). Microbiological spoilage is often much more in evidence, however, when contaminating micro-organisms grow in, or on the surface of, products, and produce characteristics that are typical of the particular substrate and micro-organism concerned. This combination is then clearly recognisable as spoilage. Examples are the growth of moulds or surface films of yeasts and bacteria, and the production of gas due to the growth of fermentative yeasts.

The presence and multiplication of potentially pathogenic bacteria are frequently less obvious. For example, food-poisoning bacteria may multiply to infective concentrations without causing any noticeable change in the organoleptic properties of products. In some cases the multiplication of pathogenic micro-organisms is paralleled by the growth of spoilage micro-organisms, which results in spoilage and deters consumption of the product,

although this is by no means always so. Inadequate food preservation, therefore, may result either in significant public health hazard, due to the growth of potentially pathogenic micro-organisms, or in wastage of products due to the growth of spoilage micro-organisms.

6.3.1 Commonly employed methods of preservation

This section describes preservation strategies that can be regarded as 'in-pack' methods of preservation. These include the use of chemical additives (organic and inorganic), adjustment of pH, control of the reduction–oxidation potential, control of water activity, use of natural preservatives, microbial antagonism, low temperature and modification of the gaseous atmosphere.

These methods of preservation may be used singly or in combination – in which case they are termed a 'preservation system'. This approach gave rise to the 'hurdle' concept (Leistner and Rödel, 1976). Here, the prevention of growth of micro-organisms is accomplished by using a combination of preservative factors that would not be inhibitory if used singly.

In practice, the formulation or method of manufacture or packaging of many co-products or end-products – and the conditions used in their storage, distribution and sale – lead to the application of just such a preservation system. For example, acidulants (such as acetic, lactic or citric acid) may be combined with other preservatives (such as benzoic acid or sorbic acid). Additionally, sodium chloride and sucrose may be present. These ingredients each have the potential to act as a preservative and can act singly or in combination, and their effectiveness will vary according to the storage temperature of the product.

In order to use such methods of preservation in the most beneficial way, however, it is important to understand their characteristics, and how they interact with each other and with other components.

pH

This is a measure of the concentration of protons, $[H^+]$, in a system, expressed as a negative logarithm: $pH = -\log[H^+]$. When water dissociates, the acidic portion (H^+) is present in an equal proportion to the alkaline portion (HO^-):

$$H_2O \rightleftarrows H^+ \text{ and } HO^-$$

and hence pure water is neither acidic nor alkaline, but neutral. Its dissociation constant indicates that it has a concentration of H^+ of 10^{-7} moles/litre. Expression of this as a negative logarithm gives the pH as 7.0, and this is, therefore, the pH of a neutral system. The further the pH falls below 7 the more acidic it is, and as the pH rises above 7 it becomes alkaline.

In many complex matrices, such as those found in foods, it is difficult to appreciate what a measured pH really means. Heterogeneity of the matrix

can result in localised differences in pH, and certain food components can exert a considerable buffering capacity which prevents changes in pH. Although pH is frequently cited as a major characteristic in relation to microbiological stability, the pH should not be considered in isolation, but together with the type and concentration of acidulant used to adjust the pH, and the interaction with other preservatives if applicable.

Organic acids

Preservatives used in food matrices are predominantly weak acids, such as acetic, lactic, citric or sorbic acids (Fig. 6.2). Their preservative action is a combination of their effect on pH and the antimicrobial properties of the undissociated form of the molecule. Their antimicrobial effect is modulated by the thermodynamic characteristics of dissociation and partition, which are discussed below.

For any given concentration of these acids a proportion exists as the acidic undissociated form, and a proportion as the dissociated anionic form. It is the undissociated form that has the predominant antimicrobial effect (Baird-Parker, 1980; Sofos and Busta, 1981; Eklund, 1983).

Dissociation

This is the characteristic of a chemical compound to separate into certain component parts. In the case of weak organic acids it is an ionisation reaction. This is important in preservation because the preservatives that undergo these reactions are usually more active in one form than the other. A general equation for compounds that dissociate by ionisation in this way is:

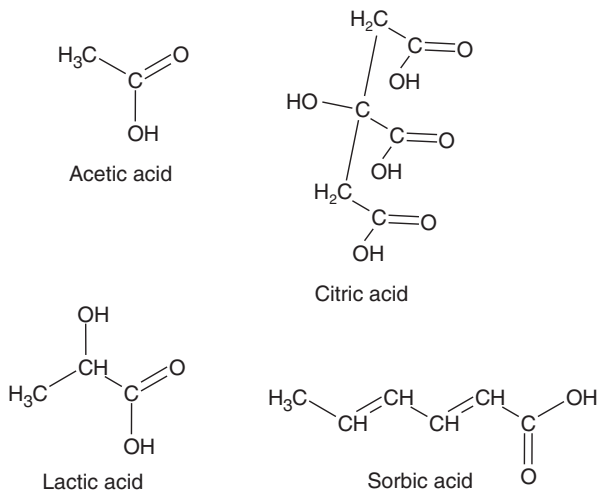

Fig. 6.2 Structural chemical formulae for acetic, citric, lactic and sorbic acids.

$$HA \rightleftarrows A^- \text{ and } H^+$$

where A^- is the anion and H^+ the proton. For example, acetic acid dissociates according to the equation:

$$\underset{\substack{\text{Acetic acid}\\\text{(undissociated)}}}{CH_3CH} \rightleftarrows \underset{\substack{\text{Acetate}\\\text{(dissociated)}}}{CH_3COO^-} \text{ and } \underset{\substack{\text{Hydrogen}\\\text{ion}\\\text{(proton)}}}{H^+}$$

The antimicrobial form of weak organic acids is the undissociated form, and it is the concentration of this form that is important in the processes that rely on these acids as preservatives.

The dissociation is an equilibrium, and the concentration of each side of the equilibrium equation is dictated by the dissociation constant (K_a) that is a characteristic of the compound. The K_a is typically a small number, and published values are available (Windholz, 1983). For convenience we tend to express the dissociation constant in the form of its negative logarithm and give it the term pK where

$$pk = -\log K_a$$

Hence, the K_a of acetic acid at 25 °C of 1.76×10^{-5} moles/litre gives a pK of 4.76 (Table 6.3). The pK is characteristic of each compound. It varies with temperature, but only slightly (Robinson and Stokes, 1959), and the equation to determine this variation is:

$$pK_a = \left(\frac{A}{T}\right) - B + (CT) \qquad [6.1]$$

where T is the temperature in degrees Kelvin (K); the constants A, B and C for some common food acids are shown in Table 6.4. The effect of tem-

Table 6.3 pK values of some organic acids used as preservatives (from Windholz, 1983)

Acid	pK at 25 °C
Acetic	4.76
Citric	
K_1	3.13
K_2	4.76
K_3	6.40
Lactic	3.86
Benzoic	4.20
Sorbic	4.76

perature change on pK is shown in Table 6.5. When the pH is equivalent to the pK, the proportions of the acid and its salt are equal. If the pH is decreased, then the concentration of the undissociated form is increased. If the pH increases, however, the concentration of the undissociated form declines. Some compounds possess more than one functional group that is able to dissociate. For example, citric acid has three carboxylic acid groups. Each of these dissociates, and each has a different pK.

Calculation of dissociation

The Henderson–Hasselbalch equation can be used to calculate the proportions of the undissociated and dissociated forms of weak acids. The equation states that the pH is equal to the sum of the pK of the acid and the logarithm of the ratio of the proportion of dissociated acid to undissociated acid (Wilson *et al*., 2000), thus:

$$\mathrm{pH} = \mathrm{p}K_a + \log_{10} \frac{[\mathrm{acid}]_{\mathrm{dissociated}}}{[\mathrm{acid}]_{\mathrm{undissociated}}} \qquad [6.2]$$

Table 6.4 Values of A, B and C for use in equation [6.1] for the calculation of the effect of temperature on pK

Acid	A	B	C
Acetic	1170.48	3.1649	0.013 399
Citric			
K_1	1255.6	4.5635	0.011 673
K_2	1585.2	5.4460	0.016 399
K_3	1814.9	6.3664	0.022 389
Lactic	1286.49	4.8607	0.014 776
Benzoic	1590.2	6.394	0.017 65

Table 6.5 Calculated effect of temperature on pK using equation [6.1]

Acid	pK	
	At 25 °C	At 4 °C
Acetic	4.76	4.77
Citric		
K_1	3.13	3.20
K_2	4.76	4.81
K_3	6.40	6.39
Lactic	3.86	3.87
Benzoic	4.20	4.23

This equation can be rearranged to give the concentration of weak acid in its undissociated form:

$$[HA]_{aq} = \frac{[HA]_T}{1+10^{(pH-pK_a)}} \quad [6.3]$$

where $[HA]_{aq}$ is the concentration of undissociated organic acid in the aqueous phase and $[HA]_T$ is the total concentration of organic acid. As a 'ready reckoner' Table 6.6 gives the precentage undissociation for acetic acid over the range of pH 4.0–5.6, assuming a pK of 4.76 (20 °C).

A combination of low pH and organic acids can be used to inhibit the growth of, or kill, micro-organisms. The cell membrane of micro-organisms has a gradient of H^+ across it. The concentration is low inside the cell and high outside it. If the pH outside the cell falls (and hence the concentration of H^+ increases) the gradient of H^+ across the membrane increases, and the H^+ leaks into the cell. The lower the pH, the steeper the gradient becomes, and the more H^+ leaks into the cell. The micro-organism must expend energy in the transport of H^+ out of the cell. As the gradient of H^+ increases, a large amount of energy is expended, and the cell's metabolism will slow down, and eventually stop. Undissociated, lipophilic organic acids – such as acetic acid – function as antimicrobial agents because of their solubility in the cell membrane where they may aid in the passage of H^+ into the cell.

Sorbic acid and benzoic acids have the additional ability to inhibit a variety of metabolic processes directly (Leuck, 1980). Citric acid is inhibitory to the growth of micro-organisms because of its ability to chelate divalent cations such as calcium (Rammell, 1962).

Buffer systems

Acidulants and their salts can be combined to construct buffer systems so that any tendency of the pH to shift is resisted. Typically, the buffer system

Table 6.6 Effect of pH on the calculated proportion of dissociated and undissociated acetic acid in solution at 20 °C

pH	Calculated % undissociated acetic acid
4.0	85.19
4.2	78.41
4.4	69.61
4.6	59.11
4.8	47.71
5.0	36.52
5.2	26.64
5.4	18.64
5.6	12.63

is composed of a weak acid and its salt (e.g. acetic acid and sodium acetate; citric acid and trisodium citrate). These buffer systems function according to the equation:

$$\underset{\text{Acid (undissociated)}}{R{-}COOH} \underset{H^+}{\overset{HO^-}{\rightleftharpoons}} \underset{\text{Anion/salt (dissociated)}}{R{-}COO^-} \quad \text{and} \quad H^+$$

If the acidity increases (i.e. an increase in the concentration of H^+ occurs), then the H^+ combines with the anion to produce undissociated acid. If the system becomes less acidic, then the acid dissociates to release the anion and H^+, and hence the pH remains constant. In order to achieve the desired pH, a known quantity of acid and salt are combined so that a reservoir of both the undissociated and dissociated molecules is established. The pH is then a function of the p*K*.

However, changes to the chemistry are reiterative. For example, in aqueous solution organic acids will dissociate. This is dependent upon local pH, but dissociation will then perturb this pH. Dissociation is dependent on the local buffering capacity of the system, which is extremely difficult to predict. Wilson *et al*. (2000) developed calculations describing the reiterative dissociation of organic acids, and these calculations can be used to predict the true chemical composition. The method requires a characterisation of the buffering behaviour of the system by titration with a strong (i.e. completely dissociating) acid, and then using knowledge of the dissociation constants of weak acid preservatives to predict their concentrations and the pH.

The quantity of acid and salt on each side of the above equation can be quite high in order to increase the size of the reservoir. This can allow the formulation of a product in such a way that it contains a high concentration of weak acid in a form that is microbicidal at a given pH (Debevere, 1987), providing that the organoleptic properties of the co-product or end-product do not suffer.

Partition

Organic acids in their undissociated form are lipophilic, although to differing extents. The oil : water partition coefficient is the ratio of the solubility of a compound in oil to its solubility in water. It is important to consider this coefficient in the application of co-products and end-products in systems that contain both a lipid and an aqueous phase, such as emulsions (e.g. mayonnaise). Here the micro-organisms are restricted to the aqueous phase (Tuynenburg Muys, 1971). Preservatives that have a high partition coefficient, however, are present in their lipophilic undissociated antimicrobial form in the aqueous phase at lower concentrations than in the lipid phase. Their effective concentration is, therefore, decreased. This is particularly true of sorbic and benzoic acids, which have partition coefficients of 3.1 and

between 6 and 13, respectively (Von Schelhorn, 1964). Acetic acid, however, has a partition coefficient of between 0.03 and 0.07 (Gordon and Reid, 1922; Bodansky, 1928; Leo *et al.*, 1971), and therefore exists in its antimicrobial undissociated form predominantly in the aqueous phase.

If the pH of the system to be preserved using organic acids is in a region where weak organic acids are present in both the undissociated and the dissociated form, then calculation of the residual concentration of the undissociated form following partition is difficult. This is because its concentration is subject to partition, and to the dissociation equilibrium dictated by the new pH of the system and its new residual concentration of undissociated acid.

The Henderson–Hasselbalch equation can be modified to take these effects into account. It gives the proportion of the total weak acid in an aqueous phase/lipid-phase system that is present in the undissociated form in the aqueous phase, given the pH, the volume fraction of oil and the partition coefficient for the undissociated weak acid. The equation is:

$$\frac{[HA]_{aq}}{[HA]_T} = \frac{1}{1 + K_p\left(\frac{\phi}{1-\phi}\right) + 10^{(pH - pK_a)}} \qquad [6.4]$$

where K_p is the partition coefficient and ø is the fraction volume of the oil phase (Wilson *et al.*, 2000).

Partition can be affected by a variety of factors including: soluble components – such as glucose, sucrose and sodium chloride – which influence the solubility of the organic acid in the aqueous phase; and acidulants which influence pH and hence affect the concentration of the undissociated molecule.

Inorganic preservatives

The most well known inorganic preservatives are probably sulphur dioxide and nitrite, which are used widely. The inhibitory effect of nitrates is derived from their conversion to nitrite in foods. Both sulphur dioxide and nitrite are inhibitors of the growth of micro-organisms and of certain sensory changes, particularly those due to oxidation or enzymatic deterioration. Sulphur dioxide exists in various forms according to the pH of the matrix, and may be present as dissolved sulphur dioxide gas, or it may ionise to form undissociated sulphurous acid (the reaction product of sulphur dioxide in water).

$$\underset{\text{Sulphurous acid}}{H_2SO_3} \rightleftarrows \underset{\text{Bisulphite}}{HSO_3^-} \quad \text{and} \quad H^+ \qquad pK = 1.76$$

$$\underset{\text{Bisulphite}}{HSO^-_3} \rightleftarrows \underset{\text{Sulphite}}{SO_3^{2-}} \quad \text{and} \quad H^+ \qquad pK = 7.21$$

This is the most active antimicrobial form but it exists in equilibrium with bisulphite and sulphite ions. The inhibition of the growth of micro-organisms is largely as a result of the inhibition of respiratory enzymes (Lueck, 1980), particularly those enzymes that contain sulphydryl groups, with which sulphur dioxide and its associated products are particularly reactive. The reactivity with aldehydes produced as integral parts of metabolic pathways is an additional site of inhibition (Gomez and Herrero, 1983).

Nitrite is thought to be inhibitory to the growth of micro-organisms due to the formation of nitrous acid and associated oxides. Although the biochemical effects have not been fully determined, they include inhibition of metabolism by reaction with dehydrogenases, with other respiratory enzymes, with cytochromes and with sulphydryl groups (Lueck, 1980; McMindes and Siedler, 1988). Nitrous acid has a pK of 3.4 (Windholz, 1983), and its effectiveness increases as the pH declines, and where it exists predominantly as the undissociated form.

As is the case for organic acids, the antimicrobial properties of each form of any compound that dissociates in these ways will differ. The pH, therefore, has a considerable influence on the state and the effectiveness of the chemical preservatives used. A disadvantage of the use of such inorganic compounds as preservatives in co-product streams is that both sulphur dioxide and nitrite can react with food ingredients. Accordingly, the residual concentration is often considerably lower than the initial concentration. However, when nitrite is heated with a culture medium that contains meat, the products of the reaction with the meat have antimicrobial properties greater than those due to nitrite alone (Perigo *et al*., 1967).

Importantly, it should be noted that both sulphur dioxide and nitrite, and some of their reaction products, raise serious toxicological questions.

Reduction–oxidation potential

Otherwise known as redox potential, or Eh, the reduction–oxidation potential is a measure (cited in millivolts) of the balance of chemically reduced and oxidised compounds within a system, rather than of the partial pressure of oxygen within or around the system. The Eh is influenced by pH and temperature, and may change as micro-organisms multiply or metabolise. In combination with pH and the composition of the gaseous atmosphere it may influence the ability of micro-organisms to grow.

Aerobic micro-organisms grow best at a positive Eh (an oxidized environment), and anaerobes at a negative Eh (a reduced environment), although the range of Eh in which a given organism (including anaerobic bacteria) can multiply is usually wide.

In oxidized environments (i.e. positive Eh) an accumulation of free radicals produced by interaction with oxygen (e.g. peroxides, superoxides) can prevent the growth of those bacteria that do not possess enzymes (catalase, superoxide dismutase) capable of destroying these radicals. Exclusion of

oxygen, and a decline in the Eh, decreases the threat to these bacteria, and strict anaerobes may begin to grow.

Total exclusion of oxygen, however, may not result in a negative Eh (for example, in plant tissues as noted below). Then, the anaerobic bacteria must use their own chemical reducing power to decrease the Eh of the environment in order to grow. This stress may not be sustainable for some bacteria and they will fail to multiply. Some clostridia, however, can multiply at a positive Eh, providing that oxygen is absent (Jones, 1989).

The Eh of matrices varies widely. The Eh of minced fresh meats, packed loosely and hence oxygenated, is about +200 mV. That of cheeses ranges from –20 to –200 mV. The liquid in plant tissues has Eh values of around +300 to +400 mV at a pH of 4–5. Some chemical additives have a marked influence on Eh; for example, ascorbic acid decreases Eh and nitrite increases it (Brown and Emberger, 1980).

Water activity

There has been a recent tendency to move away from the use of the term 'water activity' and to instead use the term 'water potential'. This term describes the difference between the chemical potential of water at any point in a system and that of pure water under standard conditions. It involves a calculation using the temperature and the ideal gas constant together with a knowledge of the vapour pressure of water in the system and the vapour pressure of pure water at the same temperature. Water potential can be expressed in terms of energy or pressure units and is a more precise description of the state of water in a system than is water activity.

Nevertheless, the term 'water activity' is still used widely and is generally accepted as being a useful tool in the understanding of the principles behind the interaction of water and solutes in preservation. As so many descriptions of foods and the growth of micro-organisms are constructed in terms of water activity this terminology is retained here. Water activity is a measure of the concentration of the available water in a system and can be defined as the tendency of water to escape from a solution relative to its ability to escape from pure water at a specific temperature. It is equal to the equilibrium relative humidity divided by 100. Pure water, therefore, has a water activity (a_w) of 1.00, and an environment where water is absent has an a_w of 0.00 (Troller, 1983).

It is possible to calculate a_w from first principles using a variety of equations, such as Raoult's law (Christian, 1980; Labuza and Bell, 1984), which was derived by Christian (1980) as:

$$\text{Log}_e\, a_w = \frac{-vm\phi}{55.51} \qquad [6.5]$$

where m is the molal concentration of the solute, v is the number of ions generated by each molecule of the solute and ø is the molal osmotic coefficient.

All biochemical reactions inside the microbial cell take place in an aqueous system. Any decrease in the amount of available water in the cell will decrease the efficiency of these reactions; a severe loss of water will cause metabolism to cease and may cause structural damage, such as the collapse of the cell membrane due to plasmolysis (Troller, 1983). Synthesis of compatible solutes inside the cell (solutes produced and retained by the cell) is required in order to attempt to balance the osmotic effect of the external environment and equalise the osmotic gradient across the membrane. This is an energy-requiring process that places further stress on the micro-organism (Christian, 1981; Troller, 1983).

Most micro-organisms require a high water activity in order to grow. For most bacteria, an a_w in excess of about 0.9 is required. Some yeasts and moulds can multiply at an a_w of about 0.80 (Leistner *et al.*, 1981), although osmotolerant yeasts and xerophilic moulds are capable of growth when the a_w is decreased to 0.6 (Lueck, 1980).

The a_w can be decreased by the addition of solutes, such as sodium chloride, sucrose or glycerol. In some cases the solute (humectant) itself may have toxic effects, and the inhibition of growth when sodium chloride is used to adjust a_w can be greater than when glycerol is used, due to the toxicity of high concentrations of sodium chloride (Baird-Parker and Freame, 1967; Gomez and Herrero, 1983; Troller, 1983). In practice, an appreciable decrease in a_w can only be achieved by the use of high concentrations of solute (Table 6.7). For example, the concentration of sodium chloride, and of sucrose, in water that is required to effect an a_w of 0.90 is 16.54 g per 100 g H_2O and 144 g per 100 g H_2O, respectively (Robinson and Stokes, 1959; Seiler, 1969; Lueck, 1980). Consequently, in systems that contain large quantities of water, the tool of decreased a_w is often used as one component of a preservation system (Tuynenburg Muys, 1975).

Table 6.7 Quantities of sodium chloride and sucrose required to poise an aqueous solution to a given a_w (from Lueck, 1980)

a_w	NaCl (g/100 g H_2O)	Sucrose (g/100 g H_2O)
0.99	1.75	11
0.96	7.01	25
0.95	8.82	78
0.94	10.34	93
0.92	13.50	120
0.90	16.54	144
0.88	19.4	169
0.86	22.21	194
0.85	23.55	208
0.84	24.19	220
0.82	27.29	243

However, a high concentration of solutes, and hence a low a_w, in the aqueous phase of emulsions or other two-phase foods decreases the solubility of some preservatives (e.g. sorbic acid, benzoic acid), and increases their partition into the lipid phase. This can have the effect of decreasing the degree of preservation of the food. An important effect of low a_w is to increase, sometimes quite markedly, the heat resistance of micro-organisms, and it can also lessen the inhibitory effect of low pH (Beuchat, 1973; Smith *et al*., 1982; Atherton and White, 1985).

Natural preservatives

Natural preservatives can include inhibitory compounds found naturally in plant tissues, or produced by micro-organisms. Many herbs and spices have antimicrobial properties, as do the extracts of many other plant tissues (indeed sorbic acid is found in, and was originally manufactured from, rowanberry oil (Lueck, 1980)). The active components vary in type considerably and include thiocyanates, sulphoxides, cinnamates, and a range of acids and phenolic compounds (Atherton and White, 1985). The concentration of these compounds in the tissues may vary considerably, and many have limited usefulness as preservatives because of their sensory qualities. For example, attempts to inhibit the growth of yeasts in coleslaw by using onion tissue required unacceptably high concentrations of onion (Brocklehurst *et al*., 1982).

Enzymes can also be inhibitory and contribute to preservation. Egg white lysozyme breaks down the cell wall of certain bacteria, and has been used to replace sorbate in food preservation (Atherton and White, 1985). The lactoperoxidase system found in milk requires the presence of the enzyme glucose oxidase, of glucose and of thiocyanate in order to function, and liberates hydrogen peroxide which is toxic to some micro-organisms.

The by-products of microbial metabolism can be inhibitory, but often quite specifically. Examples of inhibitors are the bacteriocins and antibiotics. Probably the most commonly employed example is nisin, an antibiotic produced by *Lactococcus lactis*, which has a narrow spectrum of activity against Gram-positive bacteria.

Microbial antagonism

The growth of certain micro-organisms can be inhibitory to the growth of others. The most closely studied are the lactic acid bacteria – including *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostoc* – which can be inhibitory to a range of food-poisoning and food-spoilage bacteria (Daly *et al*., 1972; Park and Marth, 1972; Babel, 1977). The mechanism of inhibition is thought to be due to a number of effects in combination, including depletion of substrate, decrease in the redox potential, production of antibiotics and the decrease in pH due to the production of organic acids (Branen *et al*., 1975; Hurst, 1983).

Low temperature

Storage at refrigeration temperatures can be fundamental to the stability of co-products and end-products. The effect of a decrease in temperature is a retardation of chemical deterioration and of growth rate of micro-organisms, thereby prolonging the period of microbiological stability.

Micro-organisms can multiply over a very wide range of temperatures. Those capable of growth at low temperatures are described as either psychrophilic or psychrotrophic. The former description requires an optimum temperature for growth of about 15 °C or lower, a maximum temperature for growth of about 20 °C or lower and a minimum temperature for growth of 0 °C or lower (Olson and Nottingham, 1980). Psychrotrophic micro-organisms, however, are described as having a minimum temperature for growth of between –5 and +5 °C, but do not meet the optimal and maximal temperature requirements of psychrophiles.

There is some risk in the control of the growth of micro-organisms by using low temperature alone. Some food-poisoning micro-organisms (e.g. *Clostridium botulinum* type E, *Listeria*, *Yersinia* and *Aeromonas*) can multiply in conditions of refrigeration (Schmidt *et al*., 1961; Alcock, 1983; Walker and Stringer, 1987). This may result in increased risk when the storage of a product at low temperature has retarded the growth of a product's normal spoilage flora, and prevented the development of visible signs of spoilage. Growth of the food-poisoning bacteria may be further enhanced if the competition afforded by the normal spoilage flora has been decreased.

Small changes in temperature can have a large effect on the growth rate of micro-organism at chill temperatures. Whereas at temperatures close to the optimum for their growth a change in temperature of 10 °C is generally reflected by a two-fold change in growth rate (i.e. temperature quotient, Q_{10}, of 2), as the temperature approaches the minimum for growth the temperature quotient becomes larger. Thus, a small increase in storage temperature during refrigeration can lead to large differences in the growth rate of micro-organisms or, indeed, may allow growth of some micro-organisms that had previously been unable to grow.

Table 6.8 displays the time taken for a population of the spoilage bacterium *Pseudomonas fluorescens* to double (doubling time) at a range of temperatures. By using the equation:

$$d_t = \frac{(t - t_o) \times 0.301}{(\log N - \log N_o)} \qquad [6.6]$$

(where d_t is the doubling time, $t - t_o$ is total time, N is 10^6 and $N_o = 1$), it is possible to determine the time taken to give a population of 10^6/g of co-product, end-product or food from an initial population of 1/g.

If we assume that at 10^6/g this organism can cause spoilage then we can use these data to predict the shelf-life at these temperatures (Table 6.9). These factors emphasise the importance of well-controlled chill

Table 6.8 Approximate doubling time of *Pseudomonas fluorescens*

Temperature (°C)	Approximate doubling time (hours)
25	0.8
20	1.2
15	2.2
10	4
8	5
5	8
3	10

Table 6.9 Calculated time for an increase in the number of *Pseudomonas fluorescens* from 1/g to a concentration of 10^6/g (the number of micro-organisms likely to result in spoilage), calculated from the doubling times in Table 6.8

Storage temperature (°C)	Time to 10^6/g (hours)
25	16
20	24
15	44
10	80
8	100
5	160
3	200

temperatures throughout the preparation, storage, distribution and sale of co-products and end-products.

Modification of the gaseous atmosphere

This involves the introduction of a gas or mixture of gases in order to supplement or replace the existing mixture of carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2). The modification may be deliberate, often achieved by evacuation followed by flushing with the appropriate gas or gases, or by virtue of the respiration of a packaged commodity causing an increase in the concentration of CO_2 and a decrease in the concentration of O_2.

In non-respiring products, the atmosphere is poised at the required composition, and will not be modified further, unless the packaging material is sufficiently permeable to allow exchange of gases with the outside environment. Packaging of systems that are living and hence that contribute to the modification of the atmosphere by their respiration, leads to a dynamic state of modification. The composition of the atmosphere in such products changes continuously. The packaging material is usually chosen to allow exchange with the outside environment in order that an equilib-

rium can be reached, although this is not achieved easily, if at all, in products that have a high rate of respiration.

Atmospheres that consist solely of CO_2 and hence replace O_2 in contact with the product, have an antioxidative effect, and are used in the extension of the shelf-life of fruit juices and carbonated beverages, and in the prevention of microbiological and sensory changes in some dairy products. Similarly, atmospheres that consist solely of N_2 have a preservative effect based on the exclusion of O_2. These atmospheres are also inhibitory to the growth of obligately aerobic microorganisms.

The pH of an unbuffered medium can be decreased in an atmosphere of CO_2, due to the production of carbonic acid. Although this decrease can be as much as 1 pH unit in the presence of 20% CO_2, this effect does not fully explain inhibition due to CO_2 (Clark and Takács, 1980). Some degree of inhibition due to modification of the gaseous atmosphere is probably due to partial inhibition of enzymes used in oxidative metabolism (Gill and Tan, 1980). It is important to note that an absence of O_2 in foods may represent a hazard, in that it presents the risk of growth of anaerobic food-poisoning bacteria, such as *Clostridium botulinum.*

6.3.2 Applied processing

Common treatments or processes that can be applied to kill micro-organisms or retard their growth include chilling, freezing, heating, dehydration or a combination of these processes, such as occurs during extrusion. The effects of chilling and freezing on micro-organisms are to retard the biochemical processes that occur within the cell and that are necessary for metabolism or growth. The application of low temperature has been discussed earlier in this chapter. Equally, the application of dehydration processes changes the water activity of the system in which the micro-organisms are found and the effects of water activity on micro-organisms have also been explained earlier in this chapter.

The effects of heating on micro-organisms result in damage to the cell. Typically, damage occurs at a number of sites on the cell membrane or the cell wall, or within the cell; in order for a cell to die a large number of sites must be damaged irreparably. If the cell is not killed and only a fraction of these sites have been damaged then the cell is able to repair this damage (resuscitation). This must be considered in all methods of enumeration of heat-treated micro-organisms since many micro-organisms cannot form colonies on agar plates that contain some selective components. Accordingly enumeration of micro-organisms on these agar plates might give deceptively low numbers of organisms. Equally, once the micro-organisms have resucitated they are free to grow within the system. Therefore, failure to enumerate sub-lethally injured micro-organisms that have not sufficiently resuscitated to grow on conventional microbiological culture medium can provide a false sense of security as these organisms can resuscitate and subsequently grow and produce spoilage of the system or result in infection.

The effect of heat on bacteria is modelled through the well known system of *D* and *Z* values. The *D* value is derived from the gradient of the line of the number of micro-organisms declining with time of exposure to a given heat process (Fig. 6.3). The *D* value is defined as the time required for a ten-fold reduction in the number of viable micro-organisms. The *D* values can be extracted from such a primary model and be plotted against their corresponding temperature. This creates a secondary model where the gradient of the line relates to the influence of temperature on the *D* value. The gradient of this line can be used to derive the *Z* value, which is defined as the temperature change required for a ten-fold reduction in the *D* value (Fig. 6.4).

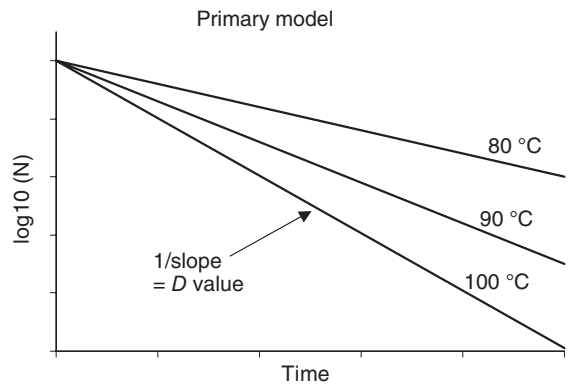

Fig. 6.3 A representation of a primary model of the effect of time on the change in numbers of micro-organisms which can be used to derive the *D* value when modelling the inactivation of micro-organisms at a range of temperatures.

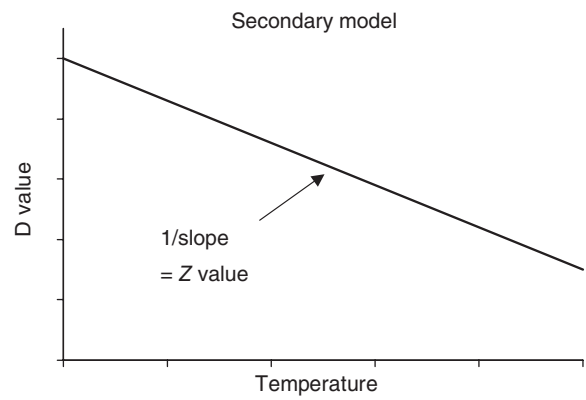

Fig. 6.4 A representation of a secondary model of the effect of temperature on *D* value which can be used to derive the *Z* value when modelling the inactivation of micro-organisms.

An additional preservation method that can sometimes be applied to co-products is the application of pressure. This may involve the use of high-pressure hyperbaric systems or lower pressure extrusion systems. However, the extent of pressure required to cause inactivation of micro-organisms is really very high, largely because bacterial spores are extremely resistant. For example spores of *Clostridium sporogenes* may require pressures of 680 MPa for one hour at ambient temperature to achieve a 5-log reduction (Crawford *et al*., 1996). However, other workers have found that for a similar reduction in numbers, in excess of 1000 MPa were required and even at 1500 MPa a reduction of only 1.5 log cycles of spores was achieved Rovere (1996). The most serious form of food poisoning is that associated with toxin production by *Clostridium botulinum*. Therefore, if there is a danger of this organism being present within co-products in the form of spores then these spores must be eliminated if there is a subsequent chance that they would germinate, and form cells that would then produce toxins. However, spores of this organism are among the most pressure tolerant. Non-proteolytic *C. botulinum* type E required about 830 MPa at 40 °C for 10 minutes to effect a 5-log reduction in number and about 230 MPa at 50 °C (Reddy *et al*., 1999). Spores of proteolytic *C. botulinum* type A required over 800 MPa at up to 88 °C for 9 minutes to effect a decrease of 3 log cycles (Reddy *et al*., 2003). However, in co-product streams we must consider not just harmful effects of organisms but spoilage effects, and the spores of some spoilage micro-organisms have even greater resistance to pressure and heat than *C. botulinum*. It is considered that the most resistant organism is *Bacillus amyloliquefaciens*. This forms highly pressure resistant spores and it has been suggested that this be adopted as the target organism for the development of ultra-high-pressure processes (Margosch *et al*., 2004).

6.4 Future trends

6.4.1 New thinking in QMRA

It has recently been suggested that the QMRA approach for the global control of microbiological hazards be fine-tuned, and two additional definitions have been introduced. One is the Appropriate Level of Protection (ALOP), which is a level of protection of human health established for a specific food-borne pathogen and this must be applied to the co-product stream or to the product that contains the co-product. An additional definition is the Food Safety Objective (FSO) which is the maximum frequency of the hazard in a food at the time of consumption that provides the ALOP (Havelaar, 2004). These definitions are complex and must be applied only in the context of the QMRA approach which, as described above, is a global approach to microbiological risk management (see Fig. 6.1).

6.4.2 Predictive modelling

Over the last decade a major advance in understanding the interaction between the chemistry and physics local to the growth or survival of micro-organisms is that demonstrated by predictive microbiology. This approach builds models that predict the growth or inactivation of micro-organisms given a constant local environment.

Inactivation models

These are the oldest models (established around the 1920s) and are the models on which the canning industry is based. The original models were developed for thermal inactivation, which is described above, but models relating to non-thermal inactivation such as the effects of water activity, pH, organic acids, ozone and ultraviolet light have been developed.

Growth boundary models

These are otherwise known as habitat domain or growth domain models and they define the chemical environment within a system where micro-organisms are capable of growing. Equally, of course such models define the chemical environment where organisms are not capable of growing. These types of model, therefore, enable us to adjust the chemistry of a co-product or co-product stream to that which is outside the habitat domain of specific micro-organisms, and hence at a chemical composition that will impose microbiological safety or stability in respect of specific micro-organisms (Fig. 6.5).

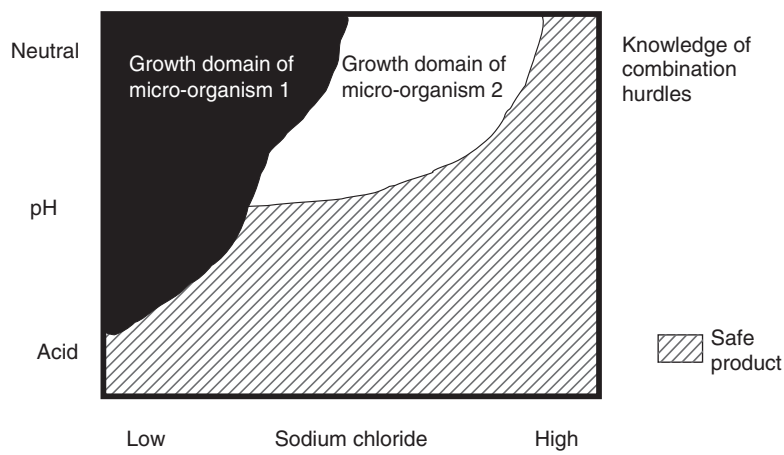

Fig. 6.5 A schematic representation of habitat domain modelling showing that chemical domains can be identified where micro-organisms might be able to grow, or where the chemistry of co-products, co-product streams or end-products can be poised to ensure microbiological stability.

Growth rate models

Where the growth of micro-organisms might be tolerated in food the growth rate can be modelled. The standard approach is to develop a primary growth model which is the relationship between the number of bacteria and time. The primary model is then used to extract parameters such as lag time or specific growth rate. These are then built into secondary growth models where the effect of environmental conditions (such as temperature, pH and sodium chloride concentration) on those growth parameters can be modelled. A wide range of types of secondary model exists such as the Arrhenius model, square root models, polynomial models, cardinal models or artificial neural networks. Essentially, growth models function on the basis that organisms can be controlled by three major factors. These are temperature, pH and water activity. Most of the models that have been developed and exist in software rely upon definition of these three conditions within a system; the potential for growth of organisms in relation to these factors can then be predicted. Such predictive models are available as software and include the Pathogen Modelling Programme (www.arserrc.gov/mfs/PMP6_CurMod.htm) and Growth Predictor (www.ifr.ac.uk/safety/growthpredictor/).

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7

Effects of postharvest changes in quality on the stability of plant co-products

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7.1 Introduction

Plants are obviously alive since we can see that they grow and develop over time. What may not be as obvious is that harvested plant tissues remain alive and continue to respire, develop and respond to their environment long after they have been detached from the parent plant. Many physiological changes alter the quality of trimmings and other food wastes; often at an accelerated rate due to injuries imposed during and after harvesting. Postharvest treatments are applied to control the rate of desirable changes while reducing the rate of deleterious changes, and to thereby maintain quality and market life of the commodity. These postharvest treatments range from the control of temperature and humidity, to chemical and gaseous treatments, to control of atmospheric composition, to cleaning and sanitation, and to packaging for transport and marketing.

As with any complex chain of sequential events, each step from harvest to consumption can have a significant impact on the product's final quality. Products diverted from the usual marketing chain because of defects or as waste from processing are typically of low economic value and preservation of their quality must be with cost-effective treatments. The management of waste and co-product recovery is becoming increasingly important as they are developed into sources of raw materials for new products.

All living tissues respire, and the respired substrates (e.g. sugars, organic acids) are often major components that determine product quality. Respiration involves the controlled transformation and ultimate oxidation of complex organic molecules. Sugars derived from complex carbohydrates (e.g. starch) and oxygen from air are the usually respired substrates, and

the products are carbon dioxide, water and energy. While most of the energy is released as heat, a portion is captured in the form of high-energy bonds in molecules such as adenosine triphosphate (ATP). A constant supply of this biochemical energy is needed to counter the second law of thermodynamics and to maintain the complex organization and structure characteristic of living things.

The rate of respiration (e.g. consumption of oxygen and production of carbon dioxide) is of major concern to scientists studying the storage of harvested horticultural commodities (e.g. fruits, ornamentals and vegetables) because the rate of respiration is tightly coupled to the rate of many other metabolic reactions. Most endothermic enzymatic reactions that convert substrates into products utilize energy transferred from ATP. Therefore, the availability of ATP will govern the rate of these reactions. Many of these reactions are involved in some aspect of quality retention or loss after harvest. They include the hydrolysis of starch to sugars, the polymerization of sugars to polysaccharides and the synthesis of organic acids, precursors of lignin, pigments, and flavor and aroma compounds.

Other biochemical reactions that are not part of normal metabolism can be induced by the traumas of harvest or by the imposed storage technology. Plants have evolved a limited set of responses to biotic and abiotic stresses. These stresses can include diseases, feeding of insects, drought, high temperatures and physical injuries encountered during harvest and processing. The physical forces used during harvest to remove the plant or plant part from its growing environment, and the forces used during processing to segment or abrade the product, are by necessity sufficient to cause physical injury. The many steps between harvest and consumption offer abundant opportunities for additional injuries that often induce unwanted physical and physiological changes. Foremost among these changes are elevated rates of respiration and ethylene production, and the production and accumulation of phenolic compounds that participate in tissue browning and toughening.

7.2 Changes during fruit ripening

Fruit ripening encompasses both catabolic and anabolic changes. Many fruit store the imported products of photosynthesis (e.g. simple sugars) as the polymerized carbohydrate starch. Bitter- or astringent-tasting phenolic compounds are also often present in immature fruit. Cell walls of unripe fruit are ridged, and adjacent cells are held firmly together by pectic substances in the middle lamella between cells. Immature fruit are therefore not sweet, soft or pleasant tasting to potential herbivores (which include humans). As fruits ripen, starch is hydrolyzed to simple sugars, phenolic compounds are removed either by being metabolized or polymerized, and the structure of the cell wall and middle lamella are altered by specific

enzymes. These catabolic reactions produce a sweet, soft and pleasant-tasting edible fruit.

A series of anabolic reactions also accompanies these catabolic transformations in many ripening fruits. Ripening is often heralded by a dramatic change in color brought about by both a loss of chlorophyll and the synthesis of specific pigments. The underlying yellow pigments in banana fruits become visible as the masking chlorophyll is degraded, while in tomato fruit the loss of chlorophyll is accompanied by the synthesis of the red pigment lycopene. The production of characteristic aroma and flavor compounds also accompanies the final stages of ripening. Poor postharvest handling practices with abusive temperature, humidity and damage can alter these metabolic changes so that poor-quality fruit result.

Fruit and vegetables should be harvested as close as possible to their maximum quality to ensure maximum quality of the processed product. For fruit, this stage is fully ripe, while the optimum harvest quality varies tremendously for vegetables because the stage of development at which they are harvested depends on the use for which they are destined. For example, cucumbers can be harvested immature and at only a few centimeters long for sweet gherkin pickles, longer for slicing pickles, longer still for fresh market slicers and near fully ripe for seed production. In contrast to vegetables, there are specific criteria that most fruit should exhibit before being harvested. Softness is often a major attribute defining fruit quality, yet soft, fully ripe fruit cannot survive the rigors of harvesting, handling, shipping and marketing. Therefore, many fruit are harvested when they have developed to a sufficient level of maturity to continue to ripen once harvested yet retain sufficient firmness to be undamaged during marketing. The decision to harvest is therefore often a compromise between the potential highest quality and the greatest marketability.

The ripening pattern of fruits can be separated into two broad groups: climacteric and non-climacteric (Table 7.1). Climacteric fruit are characterized by a substantial increase in ethylene production and respiration coincident with the onset of ripening (Figure 7.1). As climacteric fruit transition from immature to mature fruit they acquire the ability for ethylene to stimulate the rate of its own synthesis; this positive feedback of ethylene on ethylene synthesis is termed auto-catalytic ethylene production. Prior to this, ethylene inhibits its rate of synthesis so that a low concentration is maintained in the tissue. This inhibitory or negative feedback of ethylene on ethylene synthesis is also characteristic of vegetative tissue, and non-climacteric and immature climacteric fruits.

Exogenously applied ethylene stimulates the respiration of receptive plant tissue. Most plant tissue, even non-climacteric tissue, responds to ethylene exposure with elevated rates of respiration and senescence. Interestingly, exposure to low levels of ethylene, which do not produce any observable change during application, can significantly reduce the commodities' shelf-life. The upsurge in ethylene production at the start of the

Table 7.1 Some common fruits grouped by whether they exhibit a climacteric or non-climacteric respiratory pattern while ripening (Gross *et al.*, 2005)

Climacteric fruits	Non-climacteric fruits
Apple	Blueberry
Apricot	Cacao
Avocado	Cherry
Banana	Cucumber
Fig	Grape
Kiwifruit	Grapefruit
Mango	Lemon
Muskmelon	Lime
Papaya	Olive
Peach/nectarine	Orange
Pear	Pepper
Persimmon	Pineapple
Tomato	Strawberry
Watermelon	Tamarillo

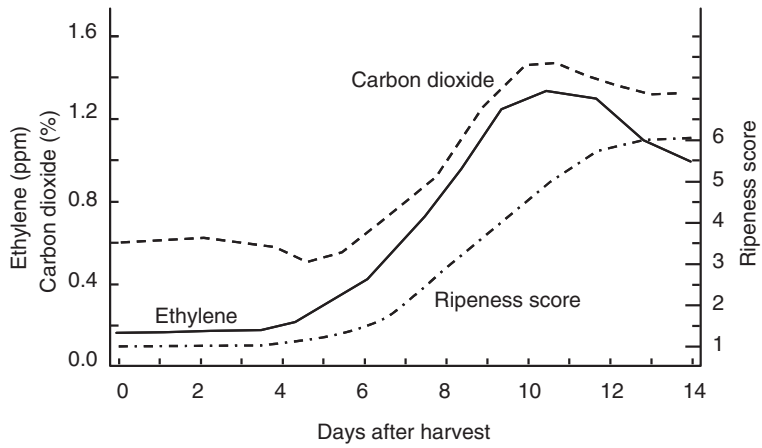

Fig. 7.1 Changes occurring during the ripening of a representative climacteric fruit. Graphs show rates of ripening, and ethylene and carbon dioxide production by harvested mature-green tomato fruit held at 15 °C in air. The rise in ethylene production precedes or is coincident with the rise in carbon dioxide production.

climacteric process produces a rise in the internal concentration of ethylene in the tissue. This rise, coupled with increased sensitivity to ethylene, is thought to produce the observed climacteric rise in respiration.

Nullifying the climacteric rise in ethylene production with chemical inhibitors or genetic engineering reduces the rise in respiration and ripening. In contrast, exposing mature fruits to phyto-active levels of ethylene stimulates both respiration and ripening. Climacteric fruit include apples, avocados, bananas, mangoes, pears, tomatoes, and many melons. These

fruit can be harvested at a mature, but unripe, stage and ripened after harvest. For example, mature-green bananas and tomatoes are harvested and shipped when mature but not yet climacteric. They are routinely treated with ethylene in specially designed ripening rooms at regional distribution centers to promote ripening. These ripening rooms should be isolated from other storage areas to prevent ethylene from escaping and promoting adverse changes in sensitive fruits, vegetables and ornamentals in adjacent storage areas. Since ethylene also stimulates respiration as well as ripening, these rooms should be equipped with sufficient refrigeration and air circulating capacity to maintain the optimal temperature and relative humidity to produce the highest quality ripe fruit.

Shipping mature, but as yet unripe, fruit has a number of advantages. The unripe fruit are firmer, and more resistant to mechanical injury and pathogens. Their longer shelf-life allows shipping to more distant markets, or the use of less rapid and therefore less expensive forms of transport. Unripe fruit may also be more resistant to water loss, but that trait varies greatly among commodities since the permeability characteristics of the epidermis and cuticle are often greatly modified during ripening. Because the unripe fruit is not yet ripe, but only has the potential to produce a high-quality ripe fruit, it is very sensitive to its environment during transit from harvest to the ripening room. Abusive temperatures, low relative humidities, adverse handling, or delays do not usually produce changes that are readily apparent, but the damage they inflict becomes very obvious when the fruit is called upon to perform the complex sequence of metabolic and compositional changes that will turn it into a high-quality ripe product.

A fruit is classified as non-climacteric if it does not exhibit a rise in ethylene production or respiration coincident with ripening (Table 7.1). These fruit will not ripen or improve in quality after harvest and must be left on the plant until they have developed sufficient quality to be marketed. While ethylene will not stimulate further ripening of non-climacteric fruit, exposure to phyto-active levels of ethylene will stimulate respiration and the onset of senescence (e.g. loss of chlorophyll, excessive softening).

Senescence in both climacteric and non-climacteric fruits is usually just a continuation of the changes associated with ripening (e.g. softening). The difference between a ripe and a senescent fruit is often a matter of personal preference, which varies greatly among consumers. For example, the definition of a ‘ripe’ banana varies from a fruit that still retains some green areas on the ends, to a fruit covered with brown spots. Other changes characteristic of senescent tissue are reduced respiration, loss of cellular integrity, loss of turgor and increased disease susceptibility. Wounding, water loss, abusive temperatures and diseases all promote premature senescence in many fresh fruits and vegetables.

Most senescence processes are not simply degradative in nature, but require the activation or synthesis of new enzymes, pathways or compounds. Senescence processes result in the production of lower molecular

weight compounds that can be translocated from the senescing tissue to growing portions of the plant. Export of these molecular fragments is precluded in harvested commodities because of their detachment from the parent plant, and their retention and accumulation in the harvest commodity may contribute to some of the postharvest changes that are characteristic of high quality (e.g. high levels of simple sugars and/or organic acids).

The loss of sweetness or acidity, or a significant change in their ratio, often results in a loss of taste quality. The conversion of stored sugars to starch or the hydrolysis of starch to sugars are two processes that also alter the sugar and acid content and the sugar-to-acid ratio in tissues. Sweet peas and sweet corn are valued for their sweetness, and that is directly related to their sugar content. The conversion of sugars to starch in these commodities is controlled by rapid cooling and holding at 0 °C. Genetic modifications that reduce the activity of the enzymes responsible for the sugar to starch conversion have produced lines of corn (e.g. *Zea mays* L., cv. Sugar Queen) that maintain their sweetness even when stored at room temperature for a number of days.

The contrasting conversion of starch to sugar is a hallmark of fruit ripening and imparts sweetness to many fruit that accumulate starch during their growth and development (e.g. apples). However, this conversion is not always desirable. Potatoes undergo 'sweetening' which is characterized by the conversion of starch to simple sugars when they are stored near 0 °C. When cooked at high temperatures (e.g. frying), these sugars spontaneously react with other components of the cell (e.g. proteins and carbohydrates) in the Maillard reaction to produce unwanted changes in flavor, odor and pigmentation. Dark-colored potato chips and 'French' fries are the result of such reactions during the frying of sweetened potatoes. The content of this 'excess' sugar can be reduced by holding the potatoes at 10 °C for a week so the sugar can be metabolized or re-polymerized.

In addition to changes produced by adverse storage conditions, many normal metabolic reactions produce a variety of deleterious activated oxygen species, such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals. Although the generation of activated oxygen species is a common event during growth and development, their increased production in response to abiotic stresses – such as chilling, heat, drought, pollutants and ultraviolet radiation – can overwhelm the cell's detoxifying capacity.

Plant cells detoxify activated oxygen species with both enzymes and antioxidants. Superoxide radicals are detoxified by the enzyme superoxide dismutase and hydrogen peroxide is destroyed by the enzyme catalase and different kinds of peroxidases (e.g. guaiacol peroxidase). A major hydrogen peroxide-detoxifying system in plants is the ascorbate–glutathione cycle which includes ascorbate peroxidase and glutathione reductase. The

synthesis of lipid- and water-soluble antioxidants – such as ascorbic acid, glutathione, α-tocopherol, flavonols, carotenoids, reduced glutathione and phenolic compounds (chlorogenic, isochlorogenic, caffeoltartartic and dicaffeoltartartic acids) – is part of a complex mechanism that involves both restricting the production of activated oxygen species and protection from the activated oxygen species produced. This protection extends beyond the plant to the consumer. The beneficial effect of eating a diet rich in fruits and vegetables has been partially attributed to the increased consumption of phenolic compounds with antioxidant properties. These compounds reduce the oxidative damage that has been linked to arteriosclerosis, brain disorders and cancer.

7.3 Response to adverse environments

7.3.1 Temperature

Cold temperatures are the foundation of most storage technologies. Chemical reactions proceed at a slower rate at lower temperatures. Changes in reaction rates with temperature are often characterized by their respiratory quotient (Q_{10}). The rate of simple chemical reactions is halved by a 10 °C reduction in temperature and is therefore said to have a Q_{10} of 2. The rate of many enzymatic reactions shows a more pronounced decline with temperature and these have a Q_{10} greater than 2. For example, the rate of respiration of harvested broccoli is reduced by a quarter (a Q_{10} of 4) as the temperature is reduced from 20 °C to 10 °C, and by another third (a Q_{10} of 3) as it is further reduced to 0 °C. Broccoli that would remain marketable for 3 days at 20 °C, would therefore have 12 and 36 days of shelf-life at 10 and 0 °C, respectively. The length of shelf-life is often inversely related to the rate of respiration (Figure 7.2). However, not all fruits and vegetables respond so favorably to reduced storage temperatures.

Fresh fruits and vegetables can be categorized by their sensitivity to cold, chilling temperatures (Table 7.2). Plants indigenous to the tropics and subtropics often suffer from a physiological disorder called ‘chilling injury’ if stored at non-freezing temperatures below 10 °C (50 °F). The level of sensitivity varies greatly among plants and tissues, with some being damaged by less than a day at 0 °C (e.g. avocados, bananas), while others (e.g. cantaloupe, peppers) tolerate many days at 0 °C before exhibiting any chilling-injury symptoms. Symptoms characteristic of chilling injury include altered and abnormal ripening, elevated respiration and ethylene production, increased water loss which often produces surface pitting, tissue and vascular browning, and increased senescence and susceptibility to disease. Tolerance to chilling injury is affected by growing conditions and temperature conditioning before chilling, while symptom development during or after chilling is affected by factors such as humidity, sanitation, wounding, packaging, atmospheric composition and rapidity of use. If the level of

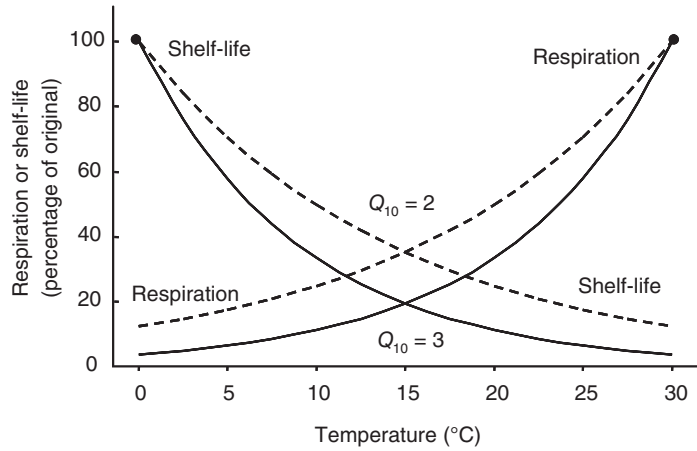

Fig. 7.2 Relationship between the rate of respiration and the shelf-life of a non-chilling-sensitive commodity. Relationships are given for a commodity with a respiratory quotient (Q_{10}) of 2 (dashed lines) and one with a Q_{10} of 3 (solid lines). Shelf-life decreases as the rate of respiration (and other associated metabolic reactions) increases with increasing temperature.

Table 7.2 Some common fruits and vegetables grouped by their chilling sensitivity. Very chilling-sensitive crops should not be stored below 7–10 °C, moderately chilling-sensitive crops should not be stored below 3 °C, while chilling-tolerant crops can be stored at 0 °C (Gross *et al.*, 2005)

Very chilling sensitive	Moderately chilling sensitive	Chilling tolerant
Avocado (e.g. Fuerte)	Apple (e.g. McIntosh)	Apple (other cultivars)
Banana	Asparagus	Apricot
Basil	Cantaloupe	Artichoke
Bean (Snap, Lima)	Cowpeas	Beetroot
Citrus (most)	Cranberries	Berries
Cucumber	Lychee	Broccoli
Eggplant (aubergine)	Olive	Cabbage
Ginger	Orange	Carrot
Jicama	Peach	Celery
Mango	Pepper	Cherry
Melons	Pomegranate	Collards
Okra	Potato	Corn, sweet
Papaya	Tomato (ripe)	Grapes
Pepper	Watermelon	Leeks
Pineapple		Lettuces
Pumpkin		Onions
Summer squash		Pears
Sweet potato		Strawberries
Tomato (mature-green)		
Yam		

chilling is moderate, symptoms may only develop after removal to a warmer, non-chilling temperature. Many chilling-sensitive fruits and vegetables can therefore be stored for a short time at chilling temperatures (the duration depends on the sensitivity of the tissue) if they are rapidly used after removal from storage.

Elevated temperatures reduce the quality of harvested fruits and vegetables. Moderately high temperatures (20–35 °C) increase respiration and the associated reactions that reduce quality, while higher temperatures (>35 °C) denature crucial enzymes and thereby prevent many reactions necessary for producing or maintaining high-quality fruit. Elevated rates of respiration reduce quality because many of the same compounds that contribute to product quality (e.g. sugars and organic acids) are substrates for respiratory metabolism. In contrast, higher temperatures inhibit essential reactions (softening and the production of characteristic pigments and flavor compounds) that contribute to product quality. If the high temperatures have not caused cellular death (i.e. sunburn or sunscald on tomatoes), the tissue may recover normal metabolic processes during storage at proper storage temperatures.

A compromise must therefore be made between the detrimental effects of high and low temperatures (Figure 7.3). Chilling injury can occur if the sensitive product is held at too low a temperature, while the rate of deterioration will increase if the temperature is too high. The optimal storage temperature may vary as a fruit progresses through various stages of ripeness. For example, a mature-green tomato is more sensitive to chilling than is a ripe tomato. Unripe fruit may appear to be more sensitive to extreme

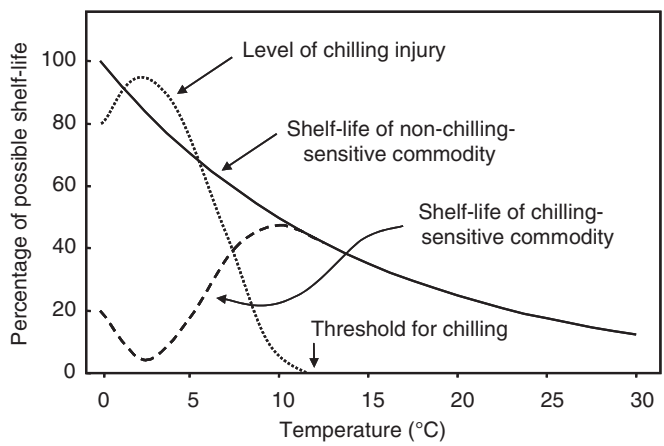

Fig. 7.3 Comparison between the shelf-life of a chilling-sensitive (dashed lines) and non-chilling-sensitive (solid lines) commodity. The level of chilling injury (dotted line) increases as the temperature declines from a threshold at 12.5 °C to 2.5 °C, and then decreases as the temperature declines further to 0 °C.

temperatures because the physiological changes they must undergo during ripening are more sensitive than are the physiological processes involved in maintaining the ripened tissue. However, the increases in ion leakage and respiration induced by chilling are similar in unripe and ripe tissue. It appears that a similar level of physiological damage is produced by chilling in unripe and ripe fruit tissue, but it is not as apparent in the ripe tissue since it has already ripened and any damage that would have occurred to the ripening process is now irrelevant.

7.3.2 Wounding

Wounding plant tissues elicits the production of a wound signal which propagates into adjacent tissue and initiates a number of genetically programmed responses. Over their evolutionary history, plants have acquired a limited number of responses to adverse conditions. Each type of stress does not therefore evoke a unique response, but different types of stresses (e.g. mechanical damage from cutting, insect feeding, or flexing in the wind; exposure to toxic levels of certain chemicals; exposure to phyto-active levels of plant growth regulators like ethylene) often elicit similar responses. While adaptive in their original setting, many of these reactions produce unwanted affects in harvested tissue.

Many stresses induce increased phenylpropanoid metabolism with the synthesis and accumulation of phenolic compounds. Simple phenolic compounds (e.g. cinnamic acid, chlorogenic acid) can directly discourage insect feeding, or provide the substrates for further synthesizing and polymerizing reactions that produce lignin to strengthen cell walls and isolate injured or diseased areas of the plant. Toughening of harvested asparagus is a good example of how harvest-induced injuries promote phenolic synthesis, and the subsequent lignification and toughening of the supportive stem tissue. While the stress-induced increase in the production of phenolic compounds is beneficial for the survival of the plant in the field, it is often objectionable in harvested fresh fruits and vegetables.

The level of phenolic compounds varies greatly among fruits and vegetables, within their various tissues and over time. Young fruit have higher levels of phenolic compounds than ripe fruit, while younger vegetative tissue has lower levels than more mature tissue. Artichokes, apples and potatoes, for example, have relatively high levels of phenolic compounds constitutively, and rapidly brown when cut. In contrast, lettuce has low levels of phenolic compounds and the synthesis and accumulation of wound-induced phenolic compounds significantly increase the development of tissue browning. Development of strategies to control tissue browning must therefore be tailored to the requirements of the specific commodity and form in which it is marketed (e.g. whole or fresh-cut). Antioxidants can lessen the browning of phenolic compounds already present in the tissue, while interfering with phenolic synthesis can prevent the accumulation of

sufficient concentrations to produce browning in tissue with low levels of indigenous phenolic compounds.

Wounding also promotes increased respiration, ethylene production, water loss and senescence. By disrupting the protective epidermal and cuticular layers it also offers an entry point for pathogens. The expansion of the market for fresh-cut fruits and vegetables has increased the need for a better understanding of the processes elicited by wounding and how they can be controlled.

7.3.3 Altered gaseous environments

Air is composed of 78% nitrogen, 21% oxygen, 0.9% argon, 0.03% carbon dioxide and assorted other gases. Oxygen is consumed and carbon dioxide is produced during respiration in air. The diffusion of gases into and out of plant tissue is controlled by the difference in their concentration across a barrier, the thickness of the barrier and the permeability of the barrier; this relationship is summarized in Fick's law of diffusion. As fruit become larger, the surface area through which gases diffuse into and out of the fruit increases slower than the volume of tissue that is dependent upon this exchange of gases. For example, doubling the size of a spherical commodity (e.g. a tomato) will increase its surface area four-fold, while its volume will increase eight-fold. Tissue in the center of large fruit is therefore in danger of being unable to obtain sufficient oxygen or eliminate sufficient carbon dioxide to maintain normal aerobic respiration. This problem can be compounded by enhanced respiration induced by physical injury, ripening or exposure to ethylene, by surface waxing, by packaging, or by the intentional or unintentional modification of the composition of the external atmosphere.

Controlled atmospheres (CA) and modified atmospheres (MA) are used to extend the storage life in a number of crops. The concentration of oxygen is lowered and the concentration of carbon dioxide is elevated in both techniques; they just differ in the level of control exerted over the concentration of the gases. In CA, the concentration of the gases is periodically measured and intentionally adjusted, while in MA the concentration of gases is allowed to maintain itself through judicious selection of temperature, rates of gas diffusion and product respiration. While CA is usually employed in large, commercial-size storage rooms, MA can be scaled down to individual consumer packages in the form of a modified atmosphere package (MAP). The commercial success of the MAP remains limited because the control of temperature (a major factor governing respiration and therefore the atmospheric composition in the package) is often unreliable during marketing. The added expense of the package and the variability of the respiratory behavior of the product also contribute to its limited use. However, the MAP can be an excellent technology when the variables are properly controlled, as in bags of fresh-cut salad mixes.

7.4 Changes in composition

Harvested plant commodities are composed of water, carbohydrates, proteins, lipids, vitamins, minerals and molecules that contribute to flavor and aroma. Changes inevitably take place in the proportion of these constituents after harvest and thereby affect the quality and utility of the stored product. Water not only composes over 80% of most commodities, which contributes to the bulk of the tissue, but its relative distribution within the tissue can cause changes in turgor that alter both texture and enzyme activity. Loss of water therefore can alter not only the size and appearance of the commodity (i.e. weight loss, skin shrivel), but also its texture (i.e. crisp versus flaccid) and its physiological activity.

Water has two avenues of escape from the tissue. One is as water vapor, much like the diffusion of other gases, while the other is as liquid water that migrates through the tissue to the surface of the commodity where it evaporates. Water loss is a combination of both processes and is proportional to differences in the vapor pressure of water between the commodity and the storage environment. Air in void spaces within tissue and near its surface has a relative humidity close to 100% and its vapor pressure is fundamentally determined by its temperature. The vapor pressure of water in air is dependent on the air's temperature, but also on its relative humidity.

The fundamental manner by which storage rooms are cooled necessitates a lowering of the air's relative humidity. Heat is removed by passing warm storage-room air that has a high relative humidity over cold evaporator coils in a mechanical refrigeration system. Condensation on the coils removes water vapor from the air and makes it very difficult to maintain a relative humidity of greater than 80–90% in storage rooms. The evaporator coils are maintained at a low temperature to facilitate the required rate of heat transfer which is proportional to the difference in temperature. Water condenses onto the cold evaporator coils so that the cold air exiting the evaporator has a lower water content than when it entered as warmer air. The relative humidity of the cold air drops as it warms during its absorption of heat from the stored produce. This warmer, dryer air can now absorb water from the produce which it conveys to the cold evaporator coils. The need to have a large temperature differential to remove enough heat to maintain the cold storage temperature often results in the coils being maintained at below freezing temperatures. Water condenses and freezes on the coils and the layer of ice greatly reduces the effectiveness of the coils, so that they must be periodically defrosted.

Water loss could be reduced by using larger evaporators with smaller temperature differentials, but evaporators are expensive. The use of packaging that acts as a barrier to water loss, or the injection of water vapor into the storage environment are two ways to overcome this inherent limitation of mechanical refrigeration. However, packaging that restricts water

loss may also reduce the penetration of cold air into the package and thereby interfere with maintaining the proper storage temperature.

Carbohydrates are constantly being metabolized by the commodity to supply energy (i.e. respiration) and smaller molecules for the synthesis of other macromolecules (e.g. proteins, lipids, hormones, etc.). Most commodities have sufficient carbohydrate reserves that their loss from metabolism during short-term storage does not significantly alter the composition. However, their conversion from complex molecules (e.g. starch, hemicellulose) to simpler molecules (i.e. mono- and disaccharides) can significantly alter the composition of the commodity. Arresting this enzymatic conversion with low temperatures, enzyme deactivation (e.g. blanching) or genetic engineering maintains quality. For example, all three approaches have been used to maintain the quality of sweet corn (preventing the conversion of sugars to starch) and potatoes (preventing the conversion of starch to sugars).

Commodities low in storage carbohydrates (e.g. melons, leafy greens, cut-flowers) can exhibit a significant reduction in quality and shelf-life as their limited reserves are depleted by respiration. The carbohydrate reserves of cut-flowers can be replenished by immersing their stems in sugar solutions. Such remedial action is unavailable for harvested fruits and vegetables, so their quality at harvest must be the highest possible, and their storage environment must be optimal to preserve their limited storage reserves.

While vegetative plant tissues (e.g. leafy greens) are usually low in both proteins and lipids, many reproductive (e.g. fruit and seeds) or storage (e.g. roots and tubers) organs can be excellent sources of these two nutrients. The nutritional quality of plant protein is not only determined by the amount of protein in the tissue, but also by the amino acids that compose the proteins. Proteins are made of 20 amino acids linked together to form long linear arrangements of hundreds of individual amino acids. Humans cannot synthesize all the amino acids we need to make functional proteins, so we must eat proteins that contain those essential amino acids. A 'balanced' diet therefore contains protein in both sufficient quantity and quality. Detrimental changes in protein content are minor, and usually entail the loss of activity of proteins functioning as enzymes. In contrast, there are many detrimental changes than can occur with lipids.

Lipids serve as food reserves in seeds (e.g. walnuts) and fruit (e.g. avocados), and are the major component of membranes (i.e. phospholipids) in all tissues. Their susceptibility to oxidation is dependent on their level of saturation. A fully saturated lipid has only single bonds between its linear backbone of carbon atoms, and is resistant to oxidation. In contrast, the more easily oxidized unsaturated lipids can have one, two or three double bonds between the carbon atoms. These unsaturated lipids are considered to be more healthy for humans than saturated lipids, but are more easily degraded by oxidation during storage and marketing. Oxidation products of lipids give the tissue a rancid taste and aroma.

Plant commodities are significant sources of vitamins A and C in the human diet. These vitamins are susceptible to respiratory loss by the plant after harvest. Their rate of synthesis is low in harvested tissue, and there are few reports that they can actually increase during storage. Postharvest techniques used to control other quality changes are equally capable of reducing the loss of these vitamins.

The mineral composition of tissues does not significantly change after harvest. However, the availability may change dramatically as other components (e.g. phenols) bind the minerals into inaccessible complexes.

The quality of many fruits and vegetables is closely linked to their aroma and flavor. Many of these compounds are volatile and therefore require continued synthesis to maintain the characteristic aroma and flavor of the commodities. Improper handling and storage (e.g. immaturity at harvest, excessive water loss, chilling temperatures) can reduce the capacity of the tissue to synthesize these compounds. Quality can also be lost by the synthesis of unwanted volatile compounds. For example, the oxidation of lipids produces rancid odors, while anaerobic respiration produces alcoholic aromas.

7.5 Future trends

The traditional application of correct temperature and relative humidity management to harvested fresh fruits and vegetables has been thoroughly explored. When combined with handling and marketing practices that reduce injuries, stress and exposure to disease – while fostering rapid handling – these technologies deliver a cornucopia of fresh fruits and vegetables at reasonable prices and excellent quality. Additional improvements using these traditional techniques will be incremental and will involve working with agricultural engineers and economists to optimize wholesale and retail marketing.

As with many other areas of biology, revolutionary changes promise to come from a better understanding of the basic process that controls the maturation, ripening and senescence of fruits and vegetables. Once identified, specific enzymes or pathways can be modified to produce the desired change. However, most researchers are involved in breeding crops for characteristics such as disease resistance and yield; or in formulating cultural practices that involve optimizing fertilizer and pesticide use; or in designing harvesting, processing, and marketing of the primary product. They may not be as interested in how their modifications affect material that is presently considered waste and which requires a recovery process to capture anything of value. In addition, consumer reluctance to accept some practices (such as genetically altered food) may limit the implementation of the more innovative changes and force reliance on the traditional repertoire of postharvest techniques (e.g. low temperatures, high humidity,

sanitation, minimized injuries and ethylene exposure, altered gaseous environments and rapid marketing and use).

The major constraints limiting the implementation and usefulness of these postharvest techniques to maintain the quality of products diverted from the usual marketing chain because of defects or as waste from processing are their low economic value, their increased disease susceptibility and their enhanced metabolism. Because of the wounds inflicted during harvest, handling and processing, these commodities are the very ones in need of the most effective postharvest techniques; yet their low economic value precludes the use of the more expensive technologies.

The appropriate postharvest technology not only depends on the value of the commodity, but also on the duration of storage, and the use to which the product will be put. For example, the texture of waste fruits and vegetables destined for canning is often degraded if first frozen, while the extraction of juice, sugar, pigments, flavors and aroma compounds can be facilitated by freezing. The packing shed or processing plants that generate these waste products are often working at maximum capacity, so any co-product recovery must wait until some of that capacity becomes available and is reconfigured, or until an alternative co-processing line becomes operational. Storage techniques are therefore necessary that quickly arrest deleterious changes and can handle the large amounts of material that are rapidly produced. Short-term storage may be needed if the material is to be transported in bulk to a local processing facility, while prolonged storage may be necessary if it is to last until the end of the current season. In either case large amounts of material must be rapidly treated in a relatively short time at minimal expense. Techniques employed in under-developed counties may be better suited to these requirements than are the energy-intensive technologies used to maintain the quality of the primary products in the industrialized West.

7.6 Sources of further information and advice

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8

The potential for destructuring of food processing waste by combination processing

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8.1 Introduction

Destructuring of plant- and animal-derived materials and waste residues provides a basis for exploiting potentially marketable components. Such an approach can be carried out on a number of length scales; e.g. metres, millimetres, microns, depending on the structural entities.

Plant organs comprise tissues, which can be separated although that is not normally a desired final result. Nevertheless, fruit or vegetable skins may be separated for purposes of recovery of hydrophobic waxes. The tissues consist of cells that might, if separated by specialist thermal and dehydration treatments, provide a suitable final product (such as in the production of dehydrated potato granules). Such destructuring of the tissue must be carried out in a specific way to achieve whole intact cells, otherwise the tissue will merely be fragmented into small tissue pieces containing assemblies of intact cells surrounded by broken ones. Having arrived at this point there are a host of structural and storage-related polymeric species in the cell walls and cells including a combination of lignin, cellulose, hemicelluloses, pectic polysaccharides, starch, protein and lipids. There are also a large number of solutes in the solutions within cells. If the aim is to gain access to the cell contents, the deconstruction strategy would be to prevent cells from separating and to break open as many cells as possible, enhancing juice accessibility.

Although generally much richer in protein, and lacking a polysaccharide-based cell wall, animal cells exhibit a number of similarities with plant cells

(Aguilera and Stanley, 1990). The cell is possibly the most important structural feature in biological tissue.

Processing rarely achieves the maximum benefit from foods in single stages or unit operations. The following operations may be used in isolation or in combination. Heating has an effect on almost all biological materials. Biopolymers have some of the same types of responses as synthetic polymers with respect to temperature, time and stress, although these are usually more complex as a result of irreversible changes as a result of structuring or destructuring at the molecular level. Heat transfer is a key process and a number of options are classically available. Water has many roles and affects the mobility of biopolymers and the processes chosen (dry-milling-type operations are key to separation of flour components and extraction of cell contents in plant materials). The availability of water will also affect the chemical and microbiological stability of food microstructures (Levine and Slade, 1993). Mass transfer, such as water removal, is again a classical operation and can be achieved in a number of ways. Heat and mass transfer are usually undertaken together as in many drying processes.

A destructuring process, which will inevitably involve separation of the biological material, is difficult to deconvolute from the heat and mass transfer associated with the unit processes. Most processes are actually combinations of chemical engineering unit operations and obviously food processes are equally applicable to the treatment of food wastes and co-products that are often sourced in the clean environment of a food factory.

If the food material has already been processed for its primary resources – e.g. grain for brewing, vegetables and fruit for juice, seeds for oil extraction – then a second operation (or often a third in the case of olive oil extraction) is required to deal with the residue, often with the added targeting of chemical or biochemical processes. Brewer's spent grain, oil seed and vegetable pulps can be processed as added bulking ingredients or further processed to remove more components. The challenge in waste utilisation is in using processes in a new way to achieve added-value products, often with the complexity that the time scale is greatly reduced by the onset of spoilage. Enzyme and microbial attack need to be halted or exploited depending on the product(s).

Finally, food wastes can be dealt with for both food or non-food utilisation, the latter opening up a number of additional, but generally low-value, opportunities. Fundamental to process design for destructuring is an understanding of the effect of the base variables, from which decisions can be made to tailor existing processes or their combinations, or to investigate new opportunities for bespoke processes. The economic demands are, however, stringent given the opportunity to use the food factory raw material input directly if a large added-value process is developed, rather than have to deal with damaged, aged or mixed-input material as it is diverted from the factory line.

8.2 Effect of destructuring on foods and their components

8.2.1 Tissues

There is a hierarchy of structures in plants and in meat (Fig. 8.1). Meat includes a number of different tissues (Lillford, 2001). Edible muscle tissues are of interest for food use and consequently for co-product recovery. Muscle cells and fibres approximate to long, small-diameter cylinders. Fibres are surrounded by the sarcolemma comprising cell membrane overlayed with endomysial connective tissue (Aguilera and Stanley, 1990).

Cereals, vegetables and fruit are the principal plant foods in the human diet. Plants contain different tissues although only the parenchymatous tissue is generally edible. The different tissue types can, however, respond differently to environmental factors such as freezing damage in carrot tissue (Saltveit, 2003), or show different propensities for extraction – as in sugar beet (Aguilera and Stanley, 1990). Other plants are grown directly for extraction of useful commodities (e.g. sugar beet, soybean, rapeseed, sunflower) and the post-extraction cake or pomace is the source for further component recovery.

The texture and destructuring of plant tissues has its origins in two extreme modes of failure; the cells can either separate at their boundaries or break through the walls (Fig. 8.2). In practice, a combination of modes can exist (Verlinden *et al.*, 1996). Intercellular air space is also a feature of many tissues.

8.2.2 Cells

The primary elements to consider in destructuring cells are the protein collagen in animal tissue and the carbohydrate cellulose in plant tissue. Parenchyma cells contain solutes in water that are surrounded by a semi-permeable membrane (Waldron *et al.*, 1997). This is important in the context of many processes because water can pass through the membrane but larger molecules like sugars cannot. The outflow of water from the cells makes the tissue flaccid or wilted with a less desirable texture; conversely, hydration of the tissue leads to water flow into the cells, such that they become enlarged or turgid leading to enhanced mechanical properties and a more desirable texture.

8.2.3 Biological molecules

A brief summary is given here as each class of biopolymer has been the subject of numerous studies. In addition, important smaller molecules are lipids comprising glycerides and fatty acids. Phospholipids are important in membranes; these feature strongly in other structures and can be exploited for selective permeability.

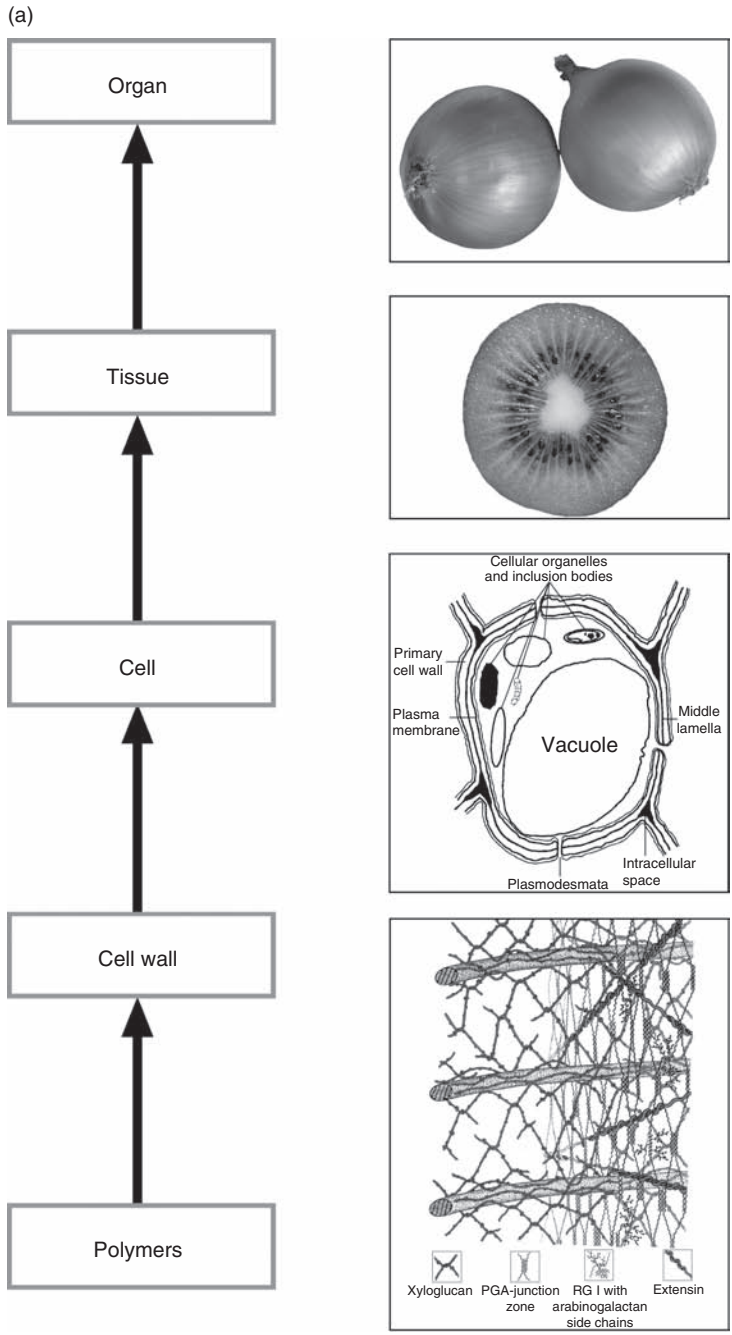

Fig. 8.1 (a) Hierarchy of structures in plants (using material from Jackman and Stanley (1995) and Carpita and Gibeaut (1993)). (b) Hierarchy of structures in meat (from Jolley and Purslow (1988)).

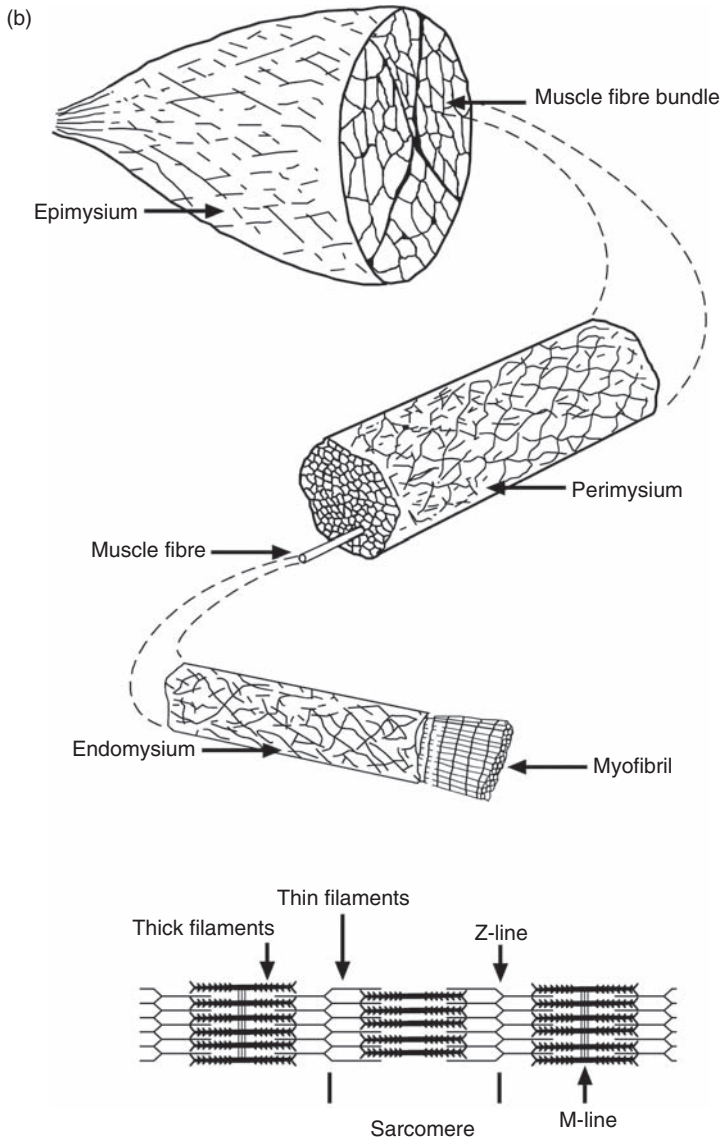

Fig. 8.1 cont'd

Proteins

Proteins provide the structural elements of many foods and their amino acids can be arranged in many ways. Globular, temperature-sensitive proteins are illustrated by whey and soya, random-coil, temperature-insensitive proteins are typified by casein (Visser, 1988). Protein–protein and protein–water interactions lead to functionally important structures. Heat leads to protein unfolding and association with other components such as carbo-

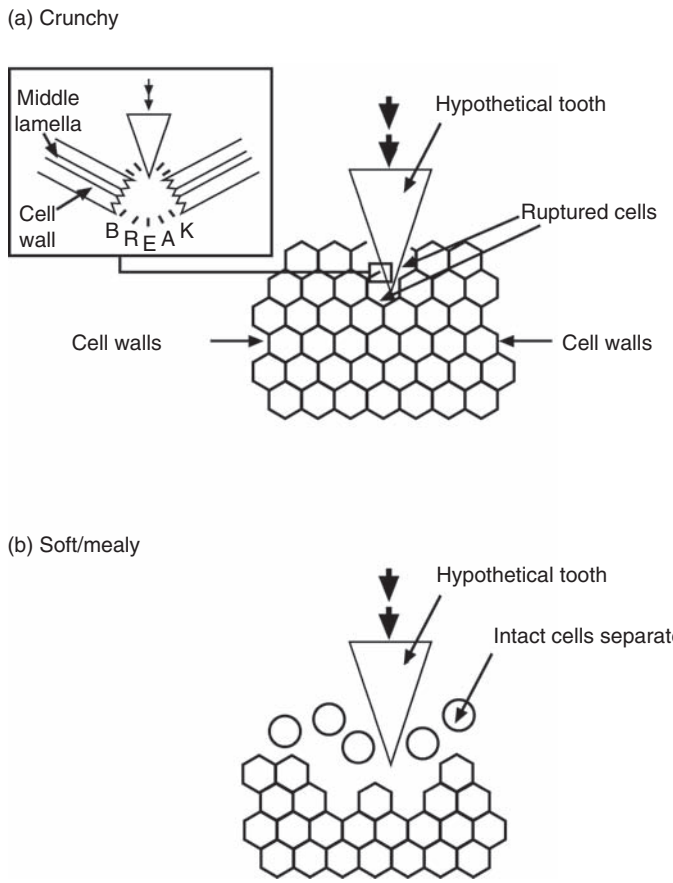

Fig. 8.2 Cell separation/breakage schematic.

hydrates and lipids. Kinsella (1978) has reviewed processes for texturing proteins.

Collagen is significant as a connective, rod-shaped protein in meat tissues. Collagen fibrils are formed from helical molecules and in turn the fibrils associate to form collagen fibres. In plant cell walls there are various proteins, glycoproteins and enzymes.

Polysaccharides

Cell wall polysaccharides comprise cellulose, hemicelluloses and pectins (Waldron *et al*., 2003). Cellulose is the most abundant carbohydrate and the principal structural component of plant tissue and is also fibrillar in structure. Models of the cell wall (Fig. 8.1) show how these polymers interact with proteins (Carpita and Gibeaut, 1993) and these models provide a powerful indicator of the role of composite theory in explaining cell wall deformation (see below). Pectins are also located in the middle lamellae

regions where they can be thought of as the glue that sticks cells together.

Starch comprises two polymers: amylose and amylopectin. In an excess of water starch gelatinises at 60–80 °C. With reducing water content, a second melting transition is observed (Donovan, 1979). The amorphous elements of starch have a glass transition that also varies with moisture content and degree of crystallinity of the starch (Zeleznak and Hoseney, 1987). Starch can be transformed to different extents by processing, with granules being partially swollen and gelatinised, or destroyed (Holm *et al*., 1988).

Lignin

Lignin is a phenolic cross-linked polymer in plant cells; it makes a significant and important contribution to the structure of wood but is less important in food materials (Smith *et al*., 2003). In thermal analysis, transitions were observed for wood due to lignin and hemicellulose, which are both plasticised by water (Kelley *et al*., 1987).

Biopolymer mixtures

In addition to their interactions with other molecules such as lipids and fats – as the building blocks of the cell – structures are also fabricated from biopolymers. They and their mixtures with smaller molecules are exploited in fabricated foods so as to produce new structures unlike those of the original ingredients. The simplest fabricated structures are edible films (Krochta, 1992) and packaging films (Shogren *et al*., 1993), whereas examples of the more complicated structures are the aerated and filled structures such as bread and salami.

8.2.4 Materials science of destructuring

Many food materials have structures that are either cellular, fibrous or particulate-filled, or combinations thereof. They are heterogeneous at different levels of structure, even if average properties are assumed for a given volume. Materials science allows treatment of their underlying mechanical properties.

Flaws

An object will break more easily if the force is concentrated by a flaw or a crack. This is because of the resultant stress concentration which is then a site for fracture initiation. Linear elastic fracture mechanics (Williams, 1984; Jeronimidis, 1991) describes the stability of cracks in stressed isotropic plates. Near the crack tip the stress intensity factor, K_{1c}, varies as the square root of the crack length, a criterion originally established by Griffith. When the stress intensity factor reaches a critical value the crack propagates in an unstable manner. The approach has been modified for plastic

flow at the crack tip where there is a second contribution to the overall crack length (Marshall *et al*., 1973). In a plastically deforming material the crack is blunted and a deformation zone forms around the crack. An 'essential work of fracture approach' (Hashemi, 1997) is more generally applicable to the failure of composites and sums the energies from the different dissipative mechanisms, such as yield and debonding, as well as fracture. The approach has also been applied to biopolymer films (Yakimets *et al*., 2005). The testing of a material to identify these contributions can, however, include energy that is not being used to create new fracture surfaces such as that due to sound.

Foams

Gibson and Ashby (1997) described the use of scaling laws to relate stiffness and strength of a foam, σ_c, to the stiffness or strength of the matrix, σ_m; and the relative density of the foam, ρ_c, to the matrix ρ_m:

$$\frac{\sigma_c}{\sigma_m} = k\left(\frac{\rho_c}{\rho_m}\right)^n \quad [8.1]$$

where k is a constant. They also gave the relationship for the foam fracture toughness, K_{1c}, as:

$$\frac{K_{1c}}{\sigma_m} = k\sqrt{l}\left(\frac{\rho_c}{\rho_m}\right)^n \quad [8.2]$$

where l is the length of a foam cell, all of which are assumed to be identical. The $\sqrt{l}$ term occurs in the classical relationship for K_{1c} for a flaw in a plate (Williams, 1984; Jeronimidis, 1991). Their application to foods has been reviewed (Smith, 1989).

The model of Warner and Edwards (1988) used variations on this type of equation to apply this modelling to liquid-filled foams typical of plant tissues; their model gives limits for modulus corresponding to initial application of stress and later equilibrium.

Filled composites

Fibre-filled composites are often treated by the method of mixtures to obtain the overall stiffness or strength, σ, based on the properties of the matrix, σ_m, and fibres, σ_f, and their relative volume fraction, ϕ_f:

$$\sigma = \sigma_m + \phi_f\sigma_f \quad [8.3]$$

Fibre-filled composites can fail by fibre breakage and fibre slip. Particulate-filled composite plastics are also well-documented where particle size and shape, volume fraction and particle mechanical properties are variables (Phillips and Harris, 1977). Many foods comprise relatively inert fillers in a protein- or starch-based structure; this becomes relevant when using wastes for part or all of a composite structure.

Molecular basis of mechanical behaviour

Polymers are classically described as amorphous (e.g. polystyrene) or semi-crystalline (e.g. polyethylene) structures, the properties of which change dramatically with increasing temperature at the glass transition and crystalline melting temperatures, respectively.

The glass transition marks the change in molecular mobility and is often identified by differential scanning calorimetry and dynamic mechanical thermal analysis (Levine and Slade, 1993). The low-strain mechanical properties, such as the modulus, fall dramatically at the glass transition temperature. The high-strain mechanical properties are not the same but they often undergo a brittle–ductile transition which maps onto the glass transition for synthetic amorphous polymers (Ward, 1983). The origin of the brittle–ductile transition is in the intersection of the weakly temperature-dependent brittle fracture stress and the more strongly temperature-dependent yield stress, such that the latter mode of failure is favoured above a certain temperature. Polymers have time-dependent properties but the yield stress increases more rapidly with strain rate than the fracture stress. This means that the intersection of the two stresses shifts to high temperatures such that substances will remain brittle to higher temperatures when tested at higher rates; this has ramifications for both texture and processing.

Plasticisers, especially water, affect these transitions in biopolymers extensively, in addition to the other role of water in undamaged cells whereby it will affect the properties of materials through turgor pressure. In an example of a plasticised starch, Ollett *et al.* (1991) observed a gradual change from a glassy, shiny broken surface to one with rough morphology as moisture content increased (Fig. 8.3(a) and (b)). Meat also provides an example of the operation of the brittle–ductile transition where a fibre-filled composite model might be applicable. Dobraszczyk *et al.* (1987) tested frozen meat and the scanning electron micrographs of fracture surfaces showed brittle fracture of the matrix giving way to some fibre debonding and pullout. Tensile testing of notched samples of cooked meat (Purslow, 1985) showed debonding between muscle fibre bundles and then fracture (Fig. 8.3(c) and (d)) without debonding or pullout of individual fibres.

Toughness is the mechanical engineering property defined as the energy used to propagate a crack per unit area. Measurements can be made through cutting experiments (Hiller and Jeronimidis, 1995). Toughness increases with turgor and it takes more energy to cut tissues made turgid by hydration (Fig. 8.4(a)). Cooking generally softens vegetables (Fig. 8.4(b)), but this can be due to the cell walls becoming more easily broken or due to a change of failure mode to cell separation, as shown in Fig. 8.2, which requires less energy. In the examples in Fig. 8.4(b), a cell breakage to separation change occurs in potato heated for 20 and 30 min. These data are relevant to various size-reduction processes where energy must be expended to create new surfaces (see below).

(a)

(b)

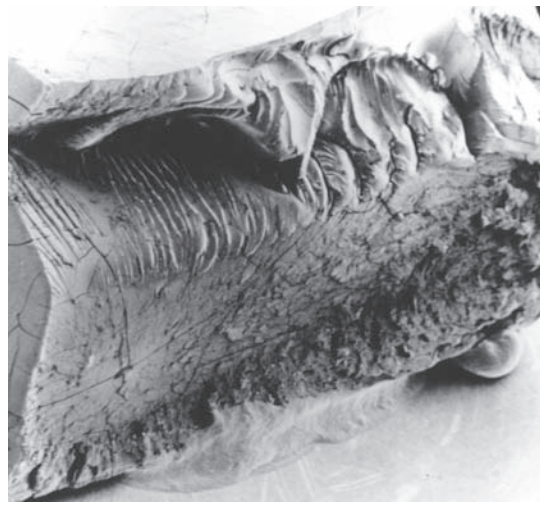

Fig. 8.3 (a) and (b) Failure in samples of starch/glycerol (80:20 weight/weight of non-aqueous components); water content of sample is (a) 6.5% (wet weight basis) and (b) 9.9% (wet weight basis) (from Kirby *et al*., 1993). (c) and (d) Failure in cooked meat (from Purslow, 1985).

Because fracture mechanics involves a dimension, as in the Griffith criterion for semi-infinite plates and the Ashby formula for notched foams, it is not surprising in considering the brittle–ductile transition that the brittle fracture stress depends on the size of the specimen. The observation that it is harder to break objects as they become progressively smaller is in accordance with analysis that there is a brittle–ductile transition indepen-

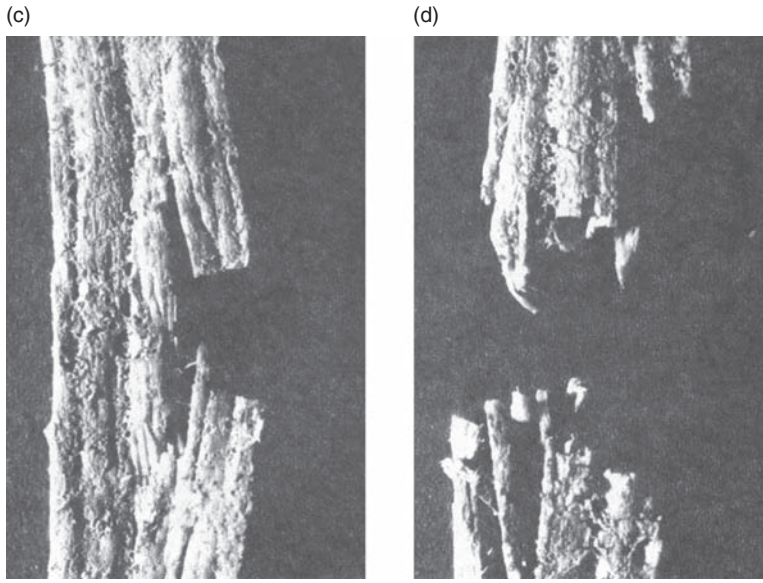

Fig. 8.3 cont'd

dent of temperature. This is elegantly described by Roberts *et al.* (1989) in studies on salt (Fig. 8.5). This science has implications for size-reduction processes such as grinding and milling (see below).

8.3 Lessons from other industries

The retting process (Meijer *et al.*, 1995) demonstrates the role of fermentation in the food industry, albeit that the fermentation is uncontrolled compared with many food industry applications. For bast fibres such as hemp and flax, dew retting is the main process currently used for separating the fibres from other plant tissues. The plant stems are left in the field to 'weather' until the fibre–matrix bond is broken down; a fibre mass is obtained, which is then broken up in a decorticator, a procedure that results in severe damage to the fibre structure. A technique that has more potential use for food waste is enzyme retting (Akin *et al.*, 2000); this allows improved fibre extraction, while reducing the penalty of mechanical deterioration and limiting chemical treatments. Large quantities of many types of enzymes are used in leather processing; low-temperature washing powders usually only contain one type of enzyme, although some have two or three to target protein, starch or fat.

Cutting, chopping and attrition actions are common to many industries for the use and disposal of various materials – from ceramics to plastics to wood. The paper industry has a rich heritage in the study of plant polymers

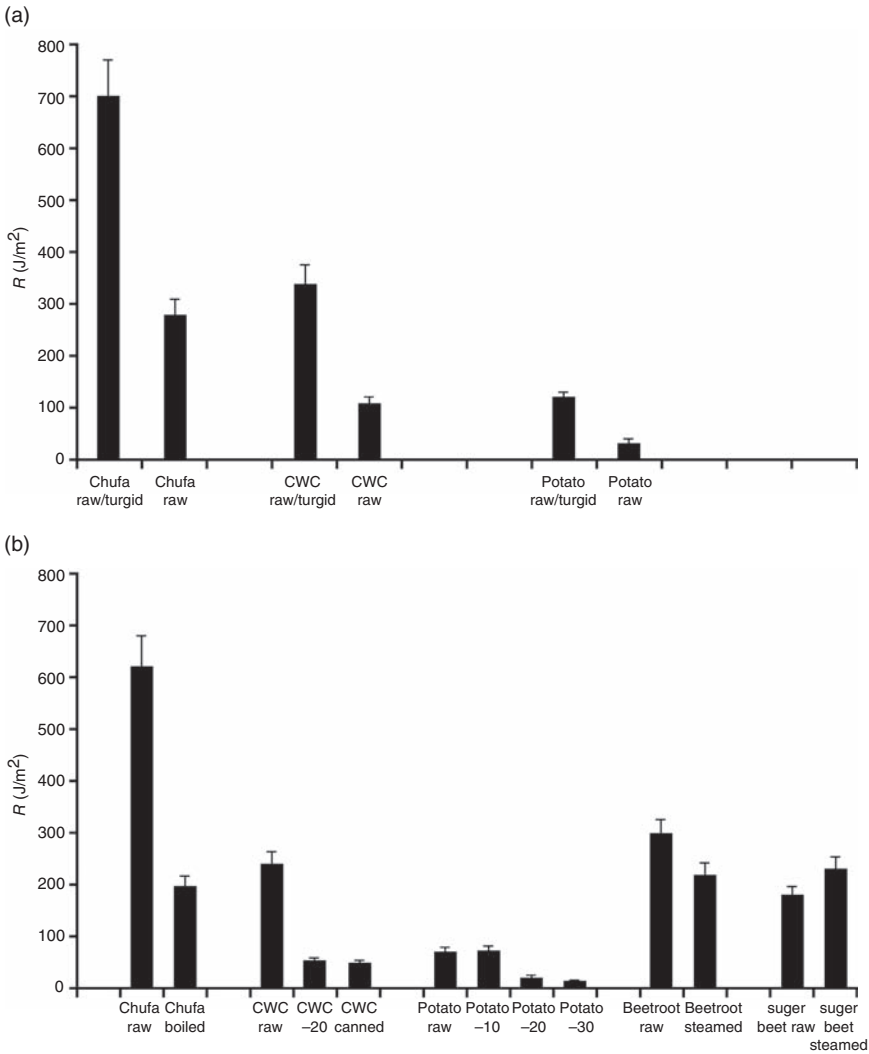

Fig. 8.4 Cutting toughness (R) of plant tissues showing effects of (a) turgor; and (b) boiling/steaming. Boiling times are given in minutes (based in part on Parker *et al*. (2000), with unpublished data from A. J. Harvey, 1996). CWC, Chinese water chestnut.

(Back and Salmen, 1982) and the use of enzymes (Kenealy and Jeffries, 2003) for fibre and lignin treatment.

8.4 Preservation processes

Higher quality of plant material and meat is normally synonymous with less destructuring, therefore the requirements of the waste utilisation processes

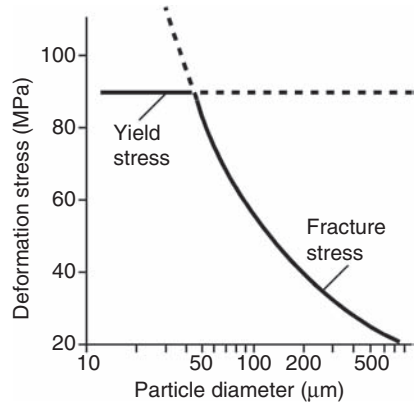

Fig. 8.5 Brittle–ductile transition in salt (from Roberts *et al.*, 1989).

may or may not be aligned with protocols for maintenance of quality. This is a general comment that pervades the transfer of rules from other industries.

Before destructuring, the waste stream may need to be preserved or to have the time scale of any change in properties lengthened. This is more important than it would be for raw material input to a food factory because of possible damage and mixing and removal of preservation methods after input to the food process line. The selection of the preservation technology to stabilise waste streams depends on their structure, moisture content and composition, and also the throughput and the processes downstream that derive components from the waste streams. Proper stabilisation of waste streams, before storage or transportation, is a necessary condition for the valorisation and possible upgrading of the streams, and the separation of valuable components.

Physical methods are preferred and elevated-temperature drying is the most common procedure for stabilisation and storage of these materials, although this process affects the quality of the extracted material. High-pressure treatment is often cited as preserving quality attributes better than thermal treatments but chilling and freezing are also options. In addition, chemical preservatives and sterilising filtration are further candidates for preservation protocols (Loncin and Merson, 1979). However, a barrier or 'hurdle' approach (Alakomi *et al.*, 2002), such as where low pH, high pressure and refrigeration are used, has been observed to produce greatest longevity.

Different process conditions are applicable including freeze-drying (often used to control the stability of the material) and drying using physical techniques such as pressing, vacuum concentration and modified atmospheres. Heating, cooling and drying are described below.

8.4.1 Heating methods

Heating animal tissues is necessary for microbiological safety and to bring about desirable eating textures. Whereas destruction of microorganisms is achieved with rapid, high-temperature treatment, the inactivation of enzymes may require a lower temperature, and longer treatment time. Cooking of meat involves denaturation of structural proteins, and the activation and subsequent deactivation of proteolytic enzymes. Lipids and water are also excluded and there are structural changes in sarcomere tissue and myofibrils, and coagulation of proteins (Aguilera and Stanley, 1990). During heating, myosin, collagen and actin undergo successive endothermal transitions with increasing temperature (Findlay *et al.*, 1986).

Cooking of fruits and vegetables 'softens' them. The mechanisms can be loss of turgor, degradation of cell wall polysaccharides and gelatinisation of starch. In the extraction of useful ingredients, the thermal method may be engineered to yield either intact granules or to release cell contents including starch.

In the controlled dehydration of potato, starch swelling is minimised because cell rupturing is not desirable and separate intact granules are required. Respiration rates also increase with increasing temperature, which has a negative effect on storage life and the rate of loss of quality attributes (Aguilera and Stanley, 1990). Mild heating, such as pre-cooking or blanching, leads to cell walls of enhanced mechanical properties which gives improved texture. An alternative strategy used with heating is to use calcium to crosslink pectic substances which again tends to improve the mechanical properties of the tissues.

8.4.2 Cooling

Normally chilling is the key to the cool chain transport and storage to reduce metabolism and extend shelf life. However, damage as a result of chilling injury can occur in some crops – usually those of tropical or sub-tropical origin – although some temperate crops, such as asparagus and potato, are susceptible at low but non-freezing temperatures (Saltveit, 2003).

Extensive microstructural changes may occur when ice crystallises. The rate of freezing becomes an important variable and affects the crystalline structure of ice and also affects the nucleation of crystals. Slow freezing generally causes extracellular growth as large crystals, while rapid cooling produces more uniform and smaller crystals (Aguilera and Stanley, 1990) with a higher associated quality as a result of lower levels of damage. This is a simplistic account since Ostwald ripening may occur during storage of the frozen material; this is where large crystals grow at the expense of smaller ones. Freezing disrupts tissues and cell walls by the formation of ice crystals; the temperature of freezing depends on the concentration of solutes in the tissue. As with heating, freezing disrupts the means of water transport across cell boundaries and turgor cannot be maintained.

This has consequences for texture loss and also for destructuring since the cells become deflated and the tissue becomes difficult to break open.

8.4.3 Drying

Drying is a separation process and can be carried out using a number of techniques. Removal of water can be by mechanical expression, by evaporation at atmospheric or reduced pressures, and by freezing the water and subliming the ice.

Sorption isotherms provide the base relationship between moisture content and equilibrium relative humidity that underlies much of drying technology. In fact critical processing points may be marked on the isotherm to denote packaging, storage and atmospheric conditions (Gal, 1983). Sorption data are normally given at temperatures close to ambient, in part because of the practical control of humidity through saturated salts. Sorption isotherms have been extensively studied and described by equations such that the constants characterise the various food components.

Freeze-drying confers maximum quality after rehydration although it is the most expensive of the drying methods. It preserves the approximate shape and size of the sample.

8.5 Tools for breakdown/disassembly

Separation techniques based on centrifuging, pressing, distillation or filtration are universal in the food industry and are candidates for part or all of the destructuring process. In terms of direct size reduction, hard brittle materials can be crushed, ground, abraded or broken by impact, although there is a limit to size reduction as described above. Ductile, tough materials are usually cut.

8.5.1 Separation and extraction

Separation and concentration can be carried out at different length scales. Figure 8.6 shows some means and examples of larger-scale separation processes. Separation can also be carried out by sieving, filtration, micropore filtration and ultrafiltration, reverse osmosis and gel filtration in decreasing order of restriction scale (opening size in the barrier) (Loncin and Merson, 1979).

8.5.2 Drying

In addition to preservation, drying brings about structural changes at a range of levels from macroscopic shrinkage, loss of cellular structure to molecular changes (Smith, 1991).

Slow drying reduces mass gradients and uniform shrinkage occurs through the specimen. Moisture gradients induce stresses that can lead to case hardening and trapping of the water, which then escapes by cracking of the surface leading to reduced quality. In general flaws reduce strength

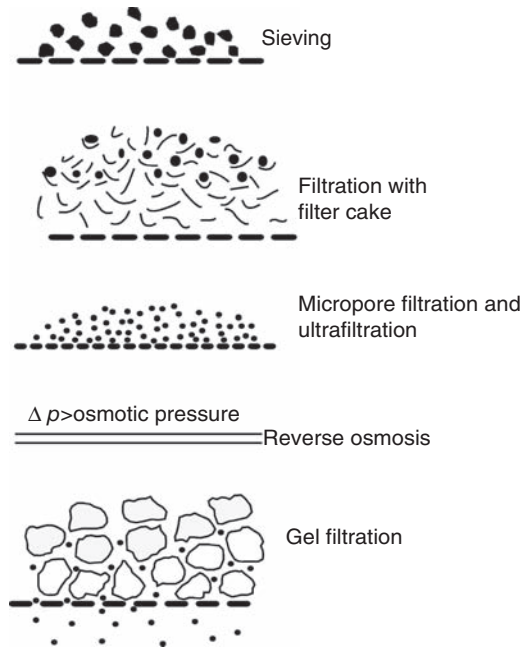

Fig. 8.6 Schematic separation barriers where Δp is the hydrostatic pressure difference applied to the solution (from Loncin and Merson, 1979).

and, again, moisture gradients, e.g. in the case of biscuits, lead to cracks called 'checking', which can lead to easy breakage (Wade, 1987). Controlled changes in moisture and temperature during the drying process – to make the moisture distribution as even as possible – allay these problems and can be 'tuned' to minimise drying times.

In addition, the composite structure of the specimen being dried will affect the structure during and after drying. Although cellular plant structures dry more completely and rapidly if their structures are broken down by heat treatments, this may affect rehydration and post-rehydration structure. While this is important in food texture, the ease of cell content removal will also favour retention of elements of the structure although the economics of drying may favour loss of structural barriers.

Drying may induce crystallisation and aggregation of certain moieties such as sugars, polysaccharides and proteins. The caking temperature, which is related to the glass transition of polymers, will determine the macroscopic behaviour of biopolymeric powders (Levine and Slade, 1993).

8.5.3 Biotechnology

Enzymes, classed as microbial or non-microbial, are used to catalyse chemical reactions but they are specific: proteases break down proteins,

amylases break down starches and lipases break down lipids and fats. Exogenous enzymes are used to break down elements of plant tissues and cell-wall-degrading enzymes are present in fruits and vegetables. Pectic enzymes may be used to increase the efficiency of juice and colour extraction from grapes, citrus fruits and apples. Suspension of intact cells may be achieved with enzymes that promote cell separation without affecting the cell wall. Complete liquefaction of fruit tissues can be brought about by pectic and cellulolytic enzymes (Whittaker *et al.*, 2002). Cellulase and proteinases have been used to hydrolyse the plant cell wall and allow extraction of proteins. Fermentation may be used to modify structure for a subsequent process such as extraction (Aguilera and Stanley, 1990).

More broadly, total liquefaction or partial solubilisation will enhance extractability of phytochemicals or biopolymers. Enzyme use can be before, during or after other processes.

An important factor determining the use of enzymes in a technological process is their expense; they also lose activity with time as a result of denaturation. When used in a soluble form, enzymes retain some activity that cannot always be economically recovered for re-use. This residual activity remains to contaminate the product and its removal may involve extra purification costs; the enzyme may need to be inactivated to halt the reaction.

A process window, defined in terms of pH and temperature, can be found where the reaction can be carried out with optimal yield and minimal biocatalyst costs. The maximal yield is then only defined by the catalysed reaction. Once the process conditions and the end-point have been chosen in the process window, the enzyme costs per kilogram of product are influenced by the type of reactor (batch, or continuous stirred tank or fixed-bed reactor) selected to carry out the process.

An alternative technique to conventional enzyme processing is enzyme immobilisation using a substrate, for example porous particles that can easily be filtered off at the end of the process. In these systems, the kinetics differ from those of systems with free enzymes, as the mass transfer inside and to and from the particles with the biocatalyst causes the formation of concentration and pH gradients that influence rates and yields. The plant size needed for continuous processes is two orders of magnitude smaller than that required for batch processes using free enzymes (Buchholz *et al.*, 2005).

Table 8.1 describes some advantages and disadvantages of cells and enzymes as biocatalysts in comparison with chemical catalysts. In summary, the application of enzyme technology is diverse as a process adjunct in the food industry. Enzymes are associated with (Uhlig, 1998): (1) juice and wine production; (2) sterile filtration of plant extracts; (3) cheese ripening; (4) malting in beer production; (5) starch processing; (6) improved preservation of juice concentrates.

Table 8.1 Advantages and disadvantages of cells and enzymes as biocatalysts in comparison with chemical catalysts (from Buchholz *et al.* 2005)

Advantages
• Low temperatures (0–110 °C) required
• Low energy consumption
• Active at pH 2–12
• Less by-products
• Non-toxic when correctly used
• Can be re-used (immobilized)
• Can be degraded biologically
• Can be produced in unlimited quantities
Disadvantages
• Cells and enzymes are
– unstable at high temperatures
– unstable at extreme pH values
– unstable in aggressive solvents
– inhibited by some metal ions
– hydrolysed by peptidases
• Some enzymes
– are still very expensive
– require expensive co-substrates
• When inhaled or ingested enzymes are potential allergens

8.6 Processes

Size-reduction processes include crushing, grinding, milling, pulping and cutting.

8.6.1 Milling methods

Wet milling

Corn refining is a two-step process that involves the wet milling and processing of corn. Shelled yellow dent corn, which has been removed from the cob during harvesting, is cleaned and steeped (softened) in tanks with a water-based solution, releasing starch. Steepwater is drawn off and the corn is milled, breaking the germ loose. Mechanical and solvent processes during germ separation extract oil from the germ. The corn leaves the germ separator in a water suspension for further grinding to release starch and gluten from the kernel. The starch and gluten slurry is piped to starch separators. The fibre and germ residue (spent flake) are traditionally sold as animal feed. Protein, which is lower in density than starch, is removed in a centrifuge. Starch is collected and washed to produce a high-quality material that can be onward processed into specialty starches or converted into corn syrups (Pomeranz, 1987). Similarly, wheat starch can be produced from whole wheat by wet milling (Lineback and Rasper, 1988).

Dry milling
In dry milling, cereal components are separated by rolling; for example, wheat is converted to separate endosperm, bran and germ (Pomeranz, 1987). The mechanical properties of germ, bran and endosperm vary differently with moisture content such that germ and endosperm are easily segregated.

8.6.2 Thermal treatments

Thermal technologies, including retorts and heat exchangers, are the foundation of food preservation and production methods (Richardson, 2001). Among the newer thermal technologies, radio-frequency processing causes direct heating within the volume of the food material (Rowley, 2001). Energy is absorbed by a dielectric material although the mechanism is different for radio-frequency processing compared with that using microwave frequencies. The radiation produces more uniform temperature and moisture profiles for drying and heating, with associated higher quality products; however, the equipment cost mitigates against its use. Microwave technology produces more rapid heating rates for the same power and the technology is more compact although the complexity is greater and the smaller penetration depth of the lower wavelength limits its use.

Ohmic heating (Ruan *et al.*, 2001) is a high-temperature, short-time process more suited to liquid–particulate mixtures than scraped surface heat exchangers which cannot achieve sterilisation temperature in the centres of larger particles. A continuous flow ohmic heater is not restricted by heat transfer from the outside of particulates and heats the food as an electrical resistance in a circuit connected to an AC power supply. In contrast to radio-frequency or microwave heating it requires contact with electrodes, and temperature non-uniformities can arise through heterogeneity of the heating medium.

8.6.3 High-pressure treatments

High-pressure treatments have been reported to cause inactivation of microorganisms and spoilage enzymes (Ludikhuyze *et al.*, 2001). A pressure of 350 MPa has been cited by Knorr (1995) as the threshold in plant systems for an effect on structure and texture. Severe texture loss occurs through rupture of cellular membranes and consequent loss of turgor pressure. Pressure affects individual components such as starch, proteins and polysaccharides. Fruit and vegetable enzymes such as polyphenoloxidase and pectin methylesterase have been widely studied and just as their heat resistance varies considerably so does their pressure sensitivity. Inactivation or enhanced activity have been observed depending on source and conditions. Studies on eating have shown beneficial textural effects on meat that is

subsequently cooked, although in some cases a combination of pressure and temperatures is effective (Cheftel and Culioli, 1997).

For a number of foods, dehydration is an unsatisfactory way to preserve them. Many dried foods rehydrate slowly in boiling water, remaining in part tough and unappetising. Kozempel *et al.* (1989) described modified and improved dehydration that included a step they called 'explosion puffing'. A partially dried piece of apple, for example, is subjected briefly to high temperature and pressure, then released into the atmosphere, where it expands instantly, or explodes. The result is a lightweight, porous piece of apple that can undergo further drying more quickly than an unexploded one. Researchers found that apples, celery, carrots, and potatoes so processed will reconstitute in water quickly, fully and evenly. The technique was claimed to have many applications. Explosion-puffed blueberries (Sullivan *et al.*, 1982) are suitable for inclusion in cereals and muffin mixes. Sliced mushrooms also can be explosion-puffed (Sullivan and Egoville, 1986), retaining their nutrients and delicate flavour. The mushrooms can be stored for more than a year, then rehydrated in only 5 minutes. The puff-dried mushrooms can be used in dehydrated soup mixes and similar products, and can also be eaten in salads in place of conventional croutons.

Onion waste example

In many food processes onions are heated to modify their texture and flavour. As an example of a prototype process and using the white, outer fleshy layers of onion waste as an illustration, pressure cooking is one step in pursuit of useful cell wall material (Le Cain *et al.*, 1999). At the tissue level there was cell separation and at the cellular scale there was swelling of the cell wall. Sequential extraction of the cell wall material showed an increase in the water-soluble pectic polysaccharides accompanied by a small reduction in their peak molecular weight.

8.6.4 Separation processes

Pressing and solvent extraction

Oilseeds contain oil, protein and starch in plant cells. Destructuring processes include cracking with corrugated rolls, dehulling, decorticating and flaking (Galloway, 1976). Seeds vary in oil content and the equipment required differs. Soybeans are solvent extracted whereas cotton seed, rapeseed, peanut, sunflower, palm and copra are examples where pressing followed by solvent extraction are undertaken (see Fig. 8.7). The main requirement is to avoid excessive damage, although cell and membrane breakage is required to open up the structure to release oil quickly. However, combination processes are actually used in secondary extraction after the majority of the oil has been removed. In addition to application of forces, elevated temperatures are used to denature proteins, decrease the oil

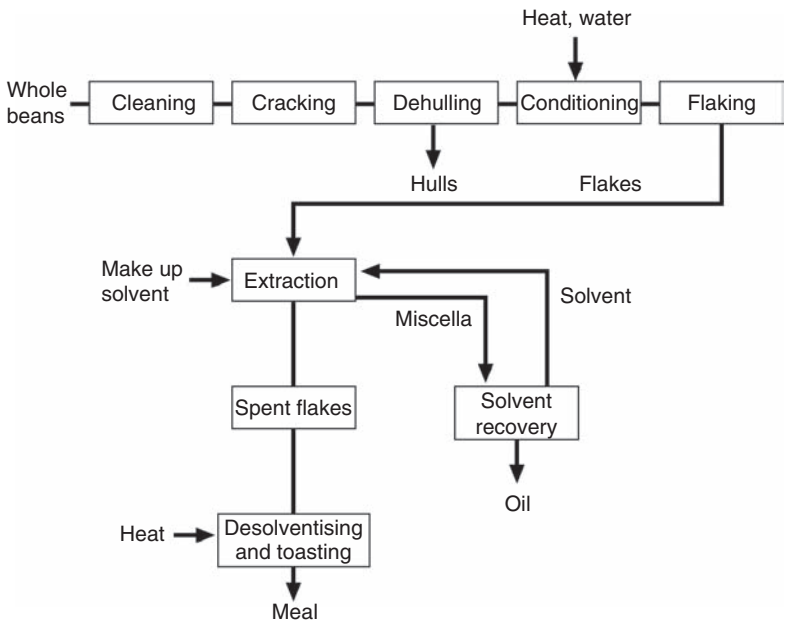

Fig. 8.7 Flow diagram for soybean oil extraction (from Aguilera and Stanley, 1990).

viscosity and soften for flaking. Distillation is required to separate the solvent and extracted oil since solvent extraction also results in a carry over of impurities leading to a need for further oil refining. Solvents for extraction include hydrocarbons, alcohols, ketones and water.

The plant cellular structure is disrupted progressively by crushing and flaking. Pressing is an alternative process that can be carried out hydraulically or in a screw press, although while the pressed material is free of solvent contaminants the efficiency of extraction is less. Following defatting, the protein in the meal can be removed by water extraction using similar approaches to those used in oil extraction.

In beet extraction for sugar, beet is cut into thin slices or cossettes. Heating above 50–60 °C causes plasmolysis leading to the cell membrane and cytoplasm becoming permeable.

Fruit juice extraction occurs by pressing or squeezing. Primary extraction again involves heat treatment of fruit slices to achieve plasmolysis. Secondary extraction involves treatment of the residue or pomace. Spices, flavour and colours are also extracted using variations of the same approaches.

In terms of animal residues, a process of rendering is employed involving pressure cooking, then centrifugation followed by pressing of the residue to obtain solid fats.

Supercritical fluid extraction
The behaviour of a fluid in the supercritical state can be described as that of a very mobile liquid. The solubility behaviour approaches that of the liquid phase while penetration into a solid matrix is facilitated by the gas-like transport properties. As a consequence, the rates of extraction and phase separation can be significantly faster than for conventional extraction processes. Supercritical fluid extraction (SFE) is known to be dependent on the density of the fluid, which in turn can be manipulated through control of the system pressure and temperature. A supercritical fluid can be used to extract a solute from a feed matrix as in conventional liquid extraction. However, unlike conventional extraction, once the conditions are returned to ambient the quantity of residual solvent in the extracted material is negligible.

A principal advantage is that the dissolving power of the supercritical fluid is controlled by pressure and/or temperature and it is easily recoverable from the extract because of its volatility. Carbon dioxide is the most commonly used supercritical fluid, due primarily to its low critical parameters (31.1 °C, 7.38 MPa), low cost and non-toxicity. Separations that are not possible using more traditional processes can sometimes be effected and thermally labile compounds can be extracted with minimal damage, since low temperatures can be employed in the extraction.

The disadvantages of SFE arise from the high capital investment required for equipment, largely as a result of the elevated pressures involved and the need for compression of the solvent, which requires elaborate recycling measures to reduce energy costs. Notwithstanding, the special properties of supercritical fluids bring certain advantages to chemical separation processes (Rozzi and Singh, 2002).

Food and flavouring
The advantage of SFE here is that the residual solvent can be easily removed from the product no matter whether it is the extract or the extracted matrix. The biggest application is the decaffeination of tea and coffee. Other important areas of use are the extraction of essential oils and aroma materials from spices. The brewery industry uses supercritical fluids for the extraction of flavours from hops. The method is also used in extracting some edible oils and producing cholesterol-free egg powder.

Pharmaceutical industry
SFE is used to produce active ingredients from herbal plants while avoiding thermal or chemical degradation. The elimination of residual solvents from the products applies equally to wastes and co-products.

8.6.5 High-temperature, short-time extrusion

Extrusion cooking is arguably a combination process in its own right and has been demonstrated to be a useful process for the production of foods

and feeds. The process converts biopolymers, e.g. proteinaceous and starchy materials, into a 'melt' for forming through dies (Smith, 1992). In the case of starchy materials the breadth of processing possibilities dictates that the extruder may merely compact the flour, grit or starch, or bring about disruption and degradation. Water solubility increases with specific mechanical energy (SME) input for maize grits (Kirby *et al*., 1988), although above a certain value of SME the maize comprises completely disrupted granules. With increasing SME, the water absorption index reaches a peak before decreasing with further solubility and SME increase. Similar studies on maize starch show that as the SME increases, the intrinsic viscosity, which is proportional to molecular weight, decreases (Parker *et al*., 1990). Figure 8.8 shows microstructure and molecular size changes in pure starch extrusion cooking (in different equipment).

Extrusion cooking was quickly exploited to produce textured protein from defatted soybean flour and isolates (Frazier *et al*., 1983; Stanley, 1989). Denaturation and association steps lead to the formation of a 'melt' as with starchy materials. Texturised proteins can be produced as meat analogues by extrusion cooking and formation through an appropriate die assembly. Conventionally, spinning processes are used to form textured proteins. Cheese and fat analogues have been formed from caseinate (Cavalier *et al*., 1990; Queguiner *et al*., 1992).

Another use of the extruder has been in fruit leathers (McHugh and Huxsoll, 1999). Using fruit as an input in this type of process indicates what could be done with the factory-grade off-cuts from fruit and vegetable processing. Extrusion cooking has also transformed materials that would otherwise be of limited usefulness such as hard-to-cook beans (Stanley and Aguilera, 1985); it is now possible to produce an extrudate that can be used to make an 'instantised' food (Martin-Cabrejas *et al*., 1999).

Mitchell and Areas (1992) summarised the effect of extrusion cooking on different animal proteins and compared this with effects on soya isolate. Their observations have implications for the upgrading of meat or mixed waste. Proteins from animal by-products have been texturised after partial defatting by extrusion; Areas and Lawrie (1984) showed that phospholipids stabilise the proteins.

Another extrusion area relevant to co-product use is the extrusion of pet foods and fish feeds (Rokey, 1994). Fish feeds comprise fishmeal, wheat and various vitamins, minerals and binding agents (Oliveira *et al*., 1992). The extruded product needs to have a structure and density and be able to absorb fat or oil. Its water absorption can be modulated so that the particle sinks at a predetermined time.

Onion waste example

In a complementary experiment to the pressure cooking of onion waste (section 8.6.3), extrusion cooking was carried out on cell wall material from

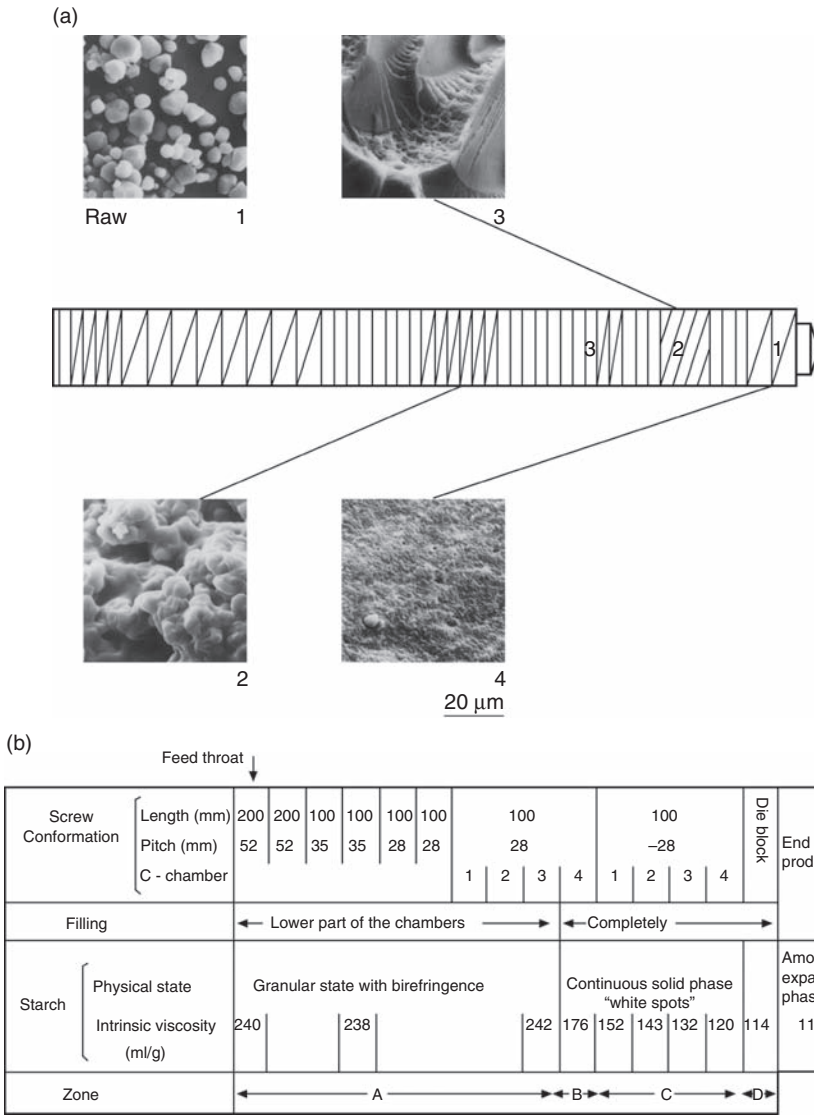

Fig. 8.8 Changes in (a) microstructure and (b) molecular size in extrusion cooking of maize starch ((b) from Colonna *et al*., 1983).

the same white, fleshy outer leaves. The solubility of pectic polymers and hemicelluloses was increased and there was increased swelling and some depolymerisation of the cell wall material (Ng *et al*., 1999). The difference compared with pressure cooking was that extrusion cooking degraded the arabinose side chains more and the galactose side chains less.

Potato outgrades example
Waste includes outgrades that do not meet retail standards for size and shape. They are often not even collected from the field, a problem that was recognised in the 1980s. One approach is to use a twin-screw extruder to deal with the outgrades as a second feed stream with potato granules as the primary and more conventional feedstock. Potato granules are used as the source for domestic mashed potato and for industrial extrusion of potato-based snack products. They are, however, produced by energy-intensive processes that involve cooking and drying. The extrusion of potato granules rehydrates and heats them again to form snack pellets. By this time any original potato flavours have been largely lost. One concept is to use the potato outgrades as a source of unprocessed potato to be cooked once rather than twice and to supply its intrinsic water content to the dehydrated granules (Ferdinand *et al.*, 1989). Granules are conventionally fed gravimetrically into the feed end of the extruder but the addition of diced potato was achieved with an auger device feeding in part-way along the barrel against the pressure developed at that stage. An alternative is to configure the twin-screw extruder so that the pressure falls to atmospheric levels; however, some form of forced pumping would still be required.

Destructuring of Brewer's spent grain (BSG) example
The extrusion cooking process is often used to incorporate dietary fibre into expanded cereal-based products (Smith, 2003). Lue *et al.* (1991) added sugar beet fibre and Hu *et al.* (1996) added soy fibre to maize in both cases. Spent grain was used in extrusion cooking by Wampler and Gould (1984). Brewers' spent grain (BSG) is a major by-product of the brewing industry and is mainly used as cattle feed. The composition of BSG includes arabinoxylan, protein and residual starch, and therefore it could be more economically used. To investigate this, BSG has recently been subjected to milling and sieving to produce palea and lemma rich in arabinoxylans, and also to produce protein- and starch-rich material (Jay *et al.*, 2006).

8.7 Examples of combination processing

8.7.1 Enzyme–filtration combination processes

Hydrolysis of lactose
Whey is a by-product in cheese production, where mainly protein and fats are precipitated in the milk by addition of a 'coagulating' enzyme (chymosin or rennin, a carboxyl acid peptidase). The remaining liquid phase (whey) contains ~5% sugars, mainly lactose. Previously, whey was used as a feedstock but a more economic use involves hydrolysing the lactose to produce glucose and galactose. A high substrate content is favourable in order to reduce downstream processing costs. In milk, the lactose content cannot be changed, but in whey it can be increased by nanofiltration (Buchholz *et al.*, 2005).

8.7.2 Extrusion combination processes

Extrusion cookers are often fed from pre-conditioners; this effectively increases the process time, which is desirable in matching conventional longer-duration processes. Pre-conditioners mix dry feed with water and/or steam for better distribution of moisture. Steam is also injected directly into the extruder and enables better processing of high-lipid mixtures. Supercritical fluid extrusion technology (Rizvi *et al*., 1995) combines two high-pressure processes – supercritical fluid processing and extrusion cooking – to extend the capabilities of conventional extrusion technology.

Caseinate

Casein from milk becomes soluble in water when neutralised with caustic soda. In conventional processing, agitating vessels and drum driers are required but the process has also been shown to be a candidate for the use of the extrusion cooker, followed by a continuous drier. The mixing capability of the twin-screw extruder avoids local soda concentrations (Wiedmann and Strobel, 1987).

Paper industry

The pulp and paper industry is applying new, ecologically sound technology in its manufacturing processes. The technologies implemented tend to change the existing industrial process as little as possible. Commercial applications include xylanases in pre-bleaching kraft pulps and the use of various enzymes in recycling paper. In the future, value-added products could be built around enzyme processes (Kenealy and Jeffries, 2003).

A related example is that of starch derivitisation for non-food use. Della Valle *et al*. (1990) report twin-screw extrusion of wheat starch with 3-chloro 2-hydroxypropyltrimethyl ammonium chloride, with sodium hydroxide as a catalyst, to produce cationic starches. These have affinity for cellulose and produce high-viscosity translucent pastes that are suitable for the paper industry. Wiedmann and Strobel (1987) showed that in the production of cationic potato starch the degree of reaction increased with not only caustic soda concentration but also at the lowest water contents. The latter factor argues in favour of equipment like the extrusion cooker.

Starch syrups and bioethanol

Starch syrups are conventionally produced by acid or enzymic hydrolysis. The latter actually needs gelatinised starch so this could be a sequential process using unprocessed starch as the starting material. Linko *et al*. (1980) demonstrated the use of the twin-screw extruder as a continuous bioreactor for gelatinisation and liquefaction of starches to make syrups. A related process is actually to make bioethanol, a fuel used as a petrol substitute for road transport vehicles (Linko *et al*., 1984).

Linko *et al*. (1979) reported that starchy materials could be pretreated so that as a follow-on process glucoamylase could saccharify the extrudate.

Linko *et al*. (1980) showed that cereal alpha amylase was stable enough to survive extrusion cooking, although more severe conditions inactivate the amylase. This led to the addition of thermostable amylase to starches in the extrusion cooker to produce low-dextrose equivalent (DE) extrudates. If the enzyme is not inactivated immediately after extrusion, enzymic hydrolysis continues and the DE increases. Glucose syrups were produced by adding glucoamylase during or after extrusion cooking with alpha amylase. Maltose syrups were produced with beta amylase and pullulanase with a low-DE liquefied starch.

Simultaneous saccharification and fermentation were achieved by adding yeast or *Zymomonas mobilis* bacteria (Linko, 1992). In the case of yeast, Linko *et al*. (1984) give an example of a yield of 28.3 kg of 100% ethanol from 100 kg of barley. The ethanol, which is produced from the fermentation process, still contains a significant quantity of water, which must be removed using the fractional distillation process.

8.7.3 Ultrasonics and extraction

Ultrasonic energy causes cavitation, the formation of gas bubbles and turbulence. It can be used to aid solvent extraction of oilseeds. The efficiency of both oil extraction and protein extraction are increased by energy input through sonication.

8.8 Future trends

A fundamental difficulty in whole waste exploitation concerns the complexity of the structures; for plants and meat this is the cell matrix and the multicomponent interactions therein. A hierarchical approach is needed to tailor manipulation of isolated polymers and residues, in conjunction with the extraction of cellular contents including phytochemicals and proteins.

It is desirable to integrate bioprocesses with advanced physical processes, thereby developing hybrid processing systems incorporating closed-loop water-recycling activities. The following technologies, which have already proven their technical applicability in fields such as food, chemical and pharmaceutical processing, are candidates for consideration in the development of novel hybrid processes:

- Extrusion-extraction: in combination with enzymatic treatment this will open up the structure of the material to be extracted, or convert the raw material into valuable components (e.g. peptides, oligosaccharides).
- Supercritical CO_2 extraction: a proven technology for several industrial applications (e.g. decaffeination, drug extraction from plants), though

not currently used for extraction of value-added components from food processing by-products. This safe and clean technology in conjunction with enzymic pre-treatments has potential for extraction of small molecules (e.g. secondary metabolites, phytochemicals). The combination with extrusion cooking is also a recent development, as is the possibility of using enzymes with supercritical fluids as the solvent medium.

- Membrane filtration: this is widely used for *water purification* and also in the chemical, food processing and pharmaceutical industries. Membrane filtration can be applied in product pre-concentration, clarification, sterile filtration and fractionation. The nature of the products and their susceptibility to deterioration make preservation, such as drying at low temperatures, necessary. Freeze-drying and some forms of spray-drying are candidates but may be too costly.

Many technologies have proven practical and economic relevance for other applications, but they need to be developed for the upgrading of food processing co-products.

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9

Enzymatic extraction and fermentation for the recovery of food processing products

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9.1 Introduction and key issues

The history of food processing technologies is as long as the history of mankind itself. Our ancestors used different techniques to preserve and convert foods. One of the most important conversions is biocatalysis using enzymes or cells in food processing. From a historical perspective, acid proteases have been considered as the oldest enzymes used (Hofmann, 1974). One isolated enzyme of the group – *chymosin*, in the form of rennet – has been used for thousands of years. People in the twenty-first century – being more and more concerned about environmental problems – intend to use these 'ancient' methods not only in the processing of foods, but also in waste management and co-product recovery in the food industry. Great efforts have been made to find and develop proper and effective biocatalytic techniques in this area. Since biocatalysis – in general – is a mild, highly selective and environmentally safe tool in processing technologies, its application is sensible in handling wastes and co-products formed in the food industry. In addition to these key reasons, it is important to note that in many cases the methods involving enzymes and microbial cells are waste-free and produce (sellable) value-added compounds, while solving the environmental problems caused by wastes and co-products. Thus biocatalytic methods can contribute significantly to higher effectiveness in food technologies (Birch *et al.*, 1981; Tucker and Woods, 1998).

9.2 Biocatalytic methods

The application of biocatalytic methods to the handling of waste and co-products coming from food technologies can be divided into two main groups:

- methods without product formation;
- methods with product formation.

Firstly there are procedures existing where no product is formed during the treatment of solid waste or waste water from food processing. In these cases, degradation of acid content or simply reduction of the waste's volume are the main purposes (Elmaleh *et al*., 1999). Similarly, preservation – and not product formation – may be the aim of ensilation processes, e.g. shrimp waste ensilation by lactic acid bacteria (Shirai *et al*., 2001). On the other hand, product(s) may be obtained from the waste or co-product; this is considered to be a more sophisticated, enhanced method. Energy and/or any kind of material (compounds) can be produced in this way. The energy production can be realised by, for example, microbial fuel cells (Grzebyk and Pozniak, 2005; Ieropoulos *et al*., 2005). This kind of equipment, however, currently only exists at laboratory scale and the scientific fundamentals are being studied in detail; thus, its application is expected in the next decade. Biogas production – as an energy source – on the other hand, is widely realised by anaerobic fermentation, using mixtures of microbe populations. However, this method is not restricted to waste management in the food industry and is therefore not discussed here.

The production of various compounds from food wastes and co-products by biocatalytic methods has been investigated for a long time and many interesting and surprising examples can be listed. Some of these are presented below to illustrate the extremely wide spectrum of uses; these uses are arranged according to the particular product of the fermentation and enzymatic procedures. Although a number of different products, biocatalysts and methods are given here, the list is far from complete.

9.2.1 Fermentation

Single cell protein (SCP)

SCP is the dried form of various microorganisms, such as bacteria (sometimes called bacterial protein), fungi and algae grown on different kinds of substrates. SCP production on solid as well as liquid food wastes is one of the well-known fermentation processes. Numerous raw materials are suitable for the process (Ozyurt and Deveci, 2004), e.g. molasses (Nagy, 1998), starch processing waste water (Jin *et al*., 1998), lemon pulp and peel (De Gregorio *et al*., 2002) or whey (Moeini *et al*., 2004) are equally appropriate initial substances. In the procedure based on beet molasses, *Saccharomyces cerevisiae* yeast is applied and pure oxygen is supplied which prevent

bacterial multiplication as well as foam formation. The protein content of the SCP is approximately 70%. *Aspergillus oryzae* was selected for SCP fermentation from starch waste, while *Aspergillus niger* and *Trichoderma viride* species were applied in the slurry-state fermentation of citrus pulps. Using whey as a substrate, a mixed yeast culture was found suitable and effective for improved SCP production.

In certain cases it is also possible to simultaneously produce enzymes during SCP production. An interesting example of this was a study where extracted grape waste material and pressed apple pulp were tested as carbon sources for growing *Penicillium funiculosum*, *Myrothecium verrucaria* and *Aspergillus niger*, with the aim of producing cellulolytic enzymes (Kuzmanova *et al*., 1991). Crude protein (35%) and a high level of cellulase activity was produced during the test.

Production of enzymes

A wide variety of enzymes are produced by the fermentation of solid and/or liquid wastes and co-products. Table 9.1 shows the range of hydrolytic enzymes, the microorganism applied and the food waste/co-product used. In the class of hydrolases (EC 3), amylases, cellulases and hemicellulases, pectinases, proteases and lipases are the most important enzymes in the processing of various food wastes. The feed mixture of these processes would usually contain the main compound(s) targeted for degradation or convertion by the enzyme. For example, in the case of pectinase enzymes, a pectin-containing mixture is required as feed in the fermentation (enzyme induction).

The majority of these hydrolytic enzymes act on *O*-glycosyl compounds (EC 3.2.1) and are used for degradation of polysaccharides (cellulose, starch, xylanes, pectin, chitin), while lipases (EC 3.1.1.3) and peptidases (EC 3.4) hydrolyse carboxylic ester bonds (triglycerides) and peptide bonds (proteins), respectively. These substances occur in almost all types of wastes and co-products from food processing. Therefore their degradation is extremely important and highlights the need for commercial quantities of these enzymes. The most cost-effective way to obtain these enzymes is to synthesise them 'on-site', using the same substrate; i.e. simultaneous conversion of biomass and the production of biocatalysts is undoubtedly beneficial, moreover these systems allow much higher flexibility in processing (Gao *et al*., 2002). The other benefit is the possible adaptation of the species to the particular substrate, resulting in higher enzyme activity (Juhász *et al*., 2005).

Food wastes and co-products are also suitable materials for the production of other (non-hydrolytic) important industrial enzymes. A few examples are: fumarase (EC 4.2.1.2) and aspartase (EC 4.3.1.1) production on molasses by *Erwinia* species for the biotransformation of fumaric acid into L-malic acid and L-aspartic acid, respectively (Bagdasaryan *et al*., 2005); laccase (EC 1.10.3.2) produced by *Botrytis cinerea* (Howard *et al*., 2003);

Table 9.1 Production of hydrolytic enzymes on waste or co-products from the food industry

Enzyme	Microorganism	Waste/ co-product	Reference
α-Amylase	*Bacillus coagulans*	Wheat bran	Babu and Satyanarayana, 1995
α-Amylase	*Bacillus subtilis*	Banana fruit stalk	Krisha and Chandrasekaran, 1996
α-Amylase	*Cellulomonas* sp.	Wheat bran	Emtiazi and Nahvi, 2004
Amylases	*Botryodiplodia theobromae* *Rhizopus oryzae*	Cassava starch residue	Ray, 2004
Cellulases	*Phanerochate chrysosporium*	Soy hull	Jha *et al.*, 1995
Cellulases	*Trichoderma reesei*	Rice straw	Kaur *et al.*, 1998
Hemicellulases	e.g. *Botyris cinerea*	Wheat straw	Thygesen *et al.*, 2003
Xylanases	*Trichoderma harzianum*	Rice/wheat straw, sugarcane bagasse	Abdel-Sater and El-Said, 2000
Pectinases	*Aspergillus niger* *Aspergillus carbanerius*	Sugar beet pulp Wheat bran	Naidu and Parda, 1998
Pectinases	*Thermoascus aurantiacus*	Orange bagasse Sugar cane bagasse Wheat bran	Martins *et al.*, 2002
Pectinases	*Aspergillus niger*	Apple pomace	Berovic and Ostroversnik, 1997
Lipase	*Penicillium simplicissimum*	Babassu cake, sugarcane molasses, corn steep liquor	Gutarra *et al.*, 2005
Lipase	*Penicillium restrictum*	Babassu oil cake	Gombert *et al.*, 1999
Acid proteases	–	Surimi wash water	DeWitt and Morrissey, 2002
β-*N*-Acetylhexos-aminidase (chitinase)	*Verticillium* silage *lecanii*	Shrimp waste	Matsumoto *et al.*, 2004

lignin peroxidase (EC 1.11.1.7) and manganese peroxidase (EC 1.1.1.13) production by *Phanerochaete chrysosporium* on lignocellulose wastes (e.g. straw) for removal of toxic phenolic compounds from industrial waste water (Fujian *et al*., 2001).

Acid compounds

Various acid compounds – used as acidifying agents in the food industry – are synthesised based on food wastes or co-products. Two of the most important are citric acid and lactic acid. Citric acid was manufactured, for example, by *Aspergillus niger* on apple pomace in a packed bed bioreactor (Shojaosodati and Babaeipour, 2002), but it was found that ram horn pepton is also a suitable protein source using the same strain (Kurbanoglu and Kurbanoglu, 2004). Lactic acid production from the dairy co-product whey is a 'traditional' technique, where lactose content is converted by bacteria species (Nagy, 1998). Glucose is similarly a suitable substrate for lactic acid formation (Tucker and Woods, 1998). Lactic acid may also be produced on canned pineapple syrup, by *Lactococcus lactis*, applying grape invertase enzyme to improve the utilisation of sucrose from the syrup (Ueno *et al*., 2003). Starch-containing food wastes are also applicable for lactic acid production, although saccharification of starch is required beforehand. Saccharification and fermentation may be achieved simultaneously using amylolytic enzyme preparations and, for example, *Lactobacillus delbrueckii* (Kim *et al*., 2003).

Biodegradable polymers

Biodegradable polymers (Smith, 2005) are possible products in glycerol processing (glycerol is the by-product of, for example, fat splitting or biodiesel production). Among these green polymers, an important group are the polyesters, including the polyhydroxy alkanoates (PHAs) and polyesters of 1,3-propanediol (PD) which can be produced not only from glycerol, but also from other food wastes and co-products. In the case of PHAs, the length of the alkyl chain may be varied but butyrate is the most favourable compound. Its fermentation (Table 9.2), for example on sugar beet molasses, can be achieved by *Pseudomonas cepacia* (Celik *et al*., 2005); or on cheese whey by *Azotobacter vinelandii* (Dhanasekar *et al*., 2001); or on molasses and whey by *Rhizobium meliloti*, *Rhizobium viciae* and *Bradyrhyzobium japonicum* (Mercan and Beyatli, 2005). It is also possible to use palm oil effluent for the production of PHAs. Japanese researchers described a two-stage process (Hassan *et al*., 1996). In the first stage, anaerobic treatment of the waste by palm oil sludge was carried out to obtain organic acids, particularly acetic and propionic acid. The acids were then converted into PHA by a phototrophic bacterium, *Rhodobacter sphaeroides*, in the second stage.

The microbiological production of PD from glycerol is of great interest worldwide since its polycondensation with dicarbonic acids also results in

Table 9.2 Production of biopolymers

Substrate(s)	Microorganism	Product	Reference
Sugar beet molasses	*Pseudomonas cepacia*	Poly(3-hydroxybutyrate)	Celik *et al.*, 2005
Cheese whey	*Azotobacter vinelandii*	Poly(3-hydroxybutyrate)	Dhanasekar *et al.*, 2001
Molasses and whey	*Rhizobium meliloti, R. viciase* and *Bradyrhizobium japonicum*	Poly(3-hydroxybutyrate)	Mercan and Beyatli, 2005
Palm oil sludge	*Rhodobacter sphaeroides*	Poly(3-hydroxyalkanoate)	Hassan *et al.*, 1996
Food wastes (whey, cane sugar)		Poly(lactic acid)	Datta *et al.*, 1995

Table 9.3 Microbiological production of 1,3-propanediol

Substrate(s)	Microorganism	Product	Reference
Glycerol	*Clostridium butyricum*	1,3-propanediol	Papanikolaou *et al.*, 2000
Glycerol	*Klebsiella pneumoniae*	1,3-propanediol and 2,3-butanediol	Biebl *et al.*, 1998
Glycerol and dihydroacetone	*Klebsiella aerogenes*	1,3-propanediol	Streekstra *et al.*, 1987
Glycerol	*Citrobacter freundii*	1,3-propanediol	Homann *et al.*, 1990
Glycerol	*Clostridium pasteureanum*	1,3-propanediol	Heyndrickx *et al.*, 1991

biodegradable polyesters. Two of the microorganisms used are *Clostridium butyricum* (Papanikolaou *et al*., 2000) and *Klebsiella pneumoniae* (Biebl *et al*., 1998) (Table 9.3). Beyond polyesters, poly(lactic acid) is another biopolymer with high potential in environmentally friendly packaging materials, it can be manufactured from lactic acid based on food wastes (Datta *et al*., 1995).

Microbial fat

Microbial fat provides an alternative to plant and animal fats, and oils, and can also be manufactured from food wastes. Bednarsky *et al*. (1986) used molasses supplemented with whey as a broth and fermentation was carried out by *Candida curvata* yeast species. The biomass contained triglycerides (11%) and proteins (23–34%).

Miscellaneous compounds
Of the miscellaneous compounds, one of the most interesting groups is phytochemicals; for example, ellagic acid was synthesized by *Lentidus edodes* on cranberry pomace (Vattem and Shetty, 2003). A similarly remarkable and peculiar example is the production of bacterial cellulose by *Acetobacter xylinum* on untreated beet molasses in a loop airlift reactor (Bae and Shoda, 2005).

9.2.2 Enzymatic processes and enzymatic extraction

Glucose
Glucose is the most 'popular' conversion product from food wastes, since glucose is the monomer for cellulose and starch. Cellulose degradation is usually carried out by complex cellulolytic enzyme preparations (like Celluclast, or Rapidase, etc.), containing several types of cellulases. *Trichoderma reesei* (earlier *Trichoderma viridae*) are considered to be the best sources of cellulase enzymes. However, the mixture should often be supplemented with β-glucosidase enzyme to enhance the hydrolytic process. Any kind of cellulose-containing waste or co-product is considered for glucose production, e.g. rice, wheat and rye straw, corn cob (Vlasenko *et al*., 1997; Kaur *et al*., 1998; Hang and Woodams, 2001; Sun and Cheng, 2005). These substrates need pre-treatment (acidic or steam treatments) in most cases.

Starch is much easier to degrade than cellulose, thus the majority of the glucose comes from various starch-containing waste sources, as produced in potato, cassava and corn manufacturing (Jin *et al*., 1998; Gao *et al*., 2002; Del Re *et al*., 2003). Amylases: mainly alpha- and beta-amylases (sometimes glucoamylases) from various sources (often *Aspergillus* species) are used for enzymatic starch hydrolysis. Recently, selection of thermostable amylase enzymes has been the focus of the research in this field. According to the cost analysis of these processes, enzymes are the most expensive materials. To reduce the cost, 'on-site' enzyme production is worthy of further study and development, as mentioned above.

A special bioreactor system is indicated from a practical point of view, due to the strong inhibition phenomena occurring in enzymatic polysaccharide hydrolysis. One of the most promising solutions to the problem is the utilisation of membrane bioreactors (Bélafi-Bakó *et al*., 2002a, 2006). In these bioreactors the enzymatic reaction and the separation of the inhibitory product (glucose) takes place simultaneously in one unit. The differences in size of the substrate (polysaccharide) and the product make separation possible by a suitable porous membrane (ultrafiltration range); this is able to reject long polysaccharide chains as well as the biocatalyst, while the product passes through the membrane easily. In such a system, continuous uptake of substrate and release of product without loss of

enzyme can be achieved. Moreover, higher effectiveness can be obtained in this particular system because of the lack of product inhibition.

Ethanol

Ethanol is one of the most important glucose-based products and is either available as an ingredient of wastes and co-products in the food industry (such as in molasses from the sugar industry) or can be obtained by hydrolysis from wastes (such as corn stover) containing polysaccharides. Several techniques have been developed according to the raw material used. One of the simplest examples is the direct fermentation of molasses by *Saccharomyces cerevisiae*. If polysaccharides are the raw materials, saccharification and fermentation can be applied as separate steps or simultaneously (Varga *et al*., 2004).

Protein hydrolysates

Protein hydrolysates (mixtures of amino acids and oligopeptides) can be produced by protease enzymes. A detailed method for the manufacture of gelatine and gelatine hydrolysates is described by Birch *et al*. (1981), using collagen-rich wastes from abattoirs. An Alcalase enzyme preparation is used at a temperature of 28 °C and the conditioning time is 6–24 h. Gelatine hydrolysates are produced by hydrolysis using Alcalase and Neutralase preparations. Recently, keratinases from various sources have been the focus of many investigations. Certain keratinases (for example from *Paecilomyces marquandii*, *Doratomyces microsporus*, or *Chryseobacterium* sp.) are able to degrade feather with an acceptable reaction rate (Brandelli and Riffel, 2005; Gradisar *et al*., 2005); this is regarded as an extremely high-impact finding due to the huge amount of feather produced as waste worldwide. Although feather in its original form is considered as a low biological value protein source – due to its deficiencies in nutritionally essential amino acids like methionine, lysine, histidine and tryptophan – the feather meal obtained by thermal processing has been incorporated into the diets of certain animals (Onifade *et al*., 1998). Microbiological degradation of feathers, however, may enhance the nutritional value of feather protein hydrolysate, since the biomass could autolytically contribute to the protein and amino acids content of the feather meal. Additionally, certain keratinophilic bacteria (e.g. *Kocuria rosea*) also synthesise carotenoids, which are useful compounds in salmonid feed or egg yolk pigments (Bertsch and Coello, 2005).

Proteases are also used for bioprocessing of another enormous waste mass, i.e. crustacean (shrimp, crab, lobster, etc.) shells. Deproteinisation of shrimp and crab shells has been carried out using the Alcalase enzyme preparation and resulted in a valuable chitin source (Oh *et al*., 2000). Recently, an enhanced method was presented where protein hydrolysate

can be recovered as well (Synowiecki and Al-Khateb, 2000; Gildberg and Stenberg, 2001); thus the effectiveness of the process is improved.

Other types of animal wastes may be similarly good sources of protein hydrolysates. Atlantic cod viscera (Aspmo *et al.*, 2005), chicken intestinal waste (Jamdar and Harikumar, 2005), shrimp heads (Ruttanapornvapeesakul, 2005), hake filleting waste (Martone *et al.*, 2005) and ram horn (Kurbanoglu and Algur, 2004) are just a few examples of the wastes available from animal processing. The protein content of these materials can be hydrolysed by various commercial protease enzyme preparations (such as Alcalase, Neutrase, or Papain) or by different endo- and exopeptidases from *Aspergillus* and *Bacillus* species.

Utilisation of these protein hydrolysates (Table 9.4) can be divided into two main areas according to whether the nutritional or the biodegradable properties are exploited. Protein hydrolysates (concentrates) can be used either as nutrient sources in media for microorganisms and feed supplements for higher animals, or as components for polymer modifications (improving biodegradability).

Natural flavour compounds

Natural flavour compounds can be synthesised from a co-product formed in distilleries (alcohol production). It is called fusel oil, and contains ethanol and short chain alcohols. Volatile, flavour ('fruit') esters can be manufactured if these alcohol compounds are reacted with short chain acids,

Table 9.4 Utilisation of protein hydrolysates

Sources	Utilisation	Reference
Ram horn	Medium for aerobic bacteria	Kurbanoglu and Algur, 2004
Hake (*Merluccius hubssi*) filleting waste	Nutrient source in media for bacteria and archae	Martone *et al.*, 2005
Shrimp (*Pandalus borealis*) wastes	Feed supplement for salmonid fishes	Gildberg and Sternberg, 2001
Shrimp (*P. semisulcatus*) head wastes	Animal/aquaculture feed formulations	Mizani *et al.*, 2005
Sardinella aurita fish wastes	Nitrogen source for *Rhizopus oryzae* (lipase production)	Ghorbel, 2005
Leather waste of tanning	Modification of linear-low-density polyethylene polymer	Saha *et al.*, 2003
Leather waste (chrome shaving)	Modification of polyvinyl alcohol films	Kresalkova *et al.*, 2002
Shrimp head silage	Protein source for Nile tilapia (*Oreaochromis niloticus*)	Plascencia-Jatomea *et al.*, 2002
Fish wastes and Feather	Protein sources for sea bass fry (*Dicentrarchus labrax*)	Langar *et al.*, 1993

resulting in low molecular weight esters. The reaction is catalysed by lipase enzyme and can be carried out in organic solvent or in a solvent-free system (Gubicza *et al*., 2000; Ehrenstein *et al*., 2003).

Biolubricants

Biolubricants can also be manufactured from fusel oil. In this case, longer chain acid compounds (fatty acids from, for example, hydrolysis of plant oils) should be used in the esterification reaction. The process can be carried out by acidic (Özgülsün *et al*., 2000) or enzymatic catalysis (Fig. 9.1). The drawback of acidic catalysis is that acid traces may remain in the

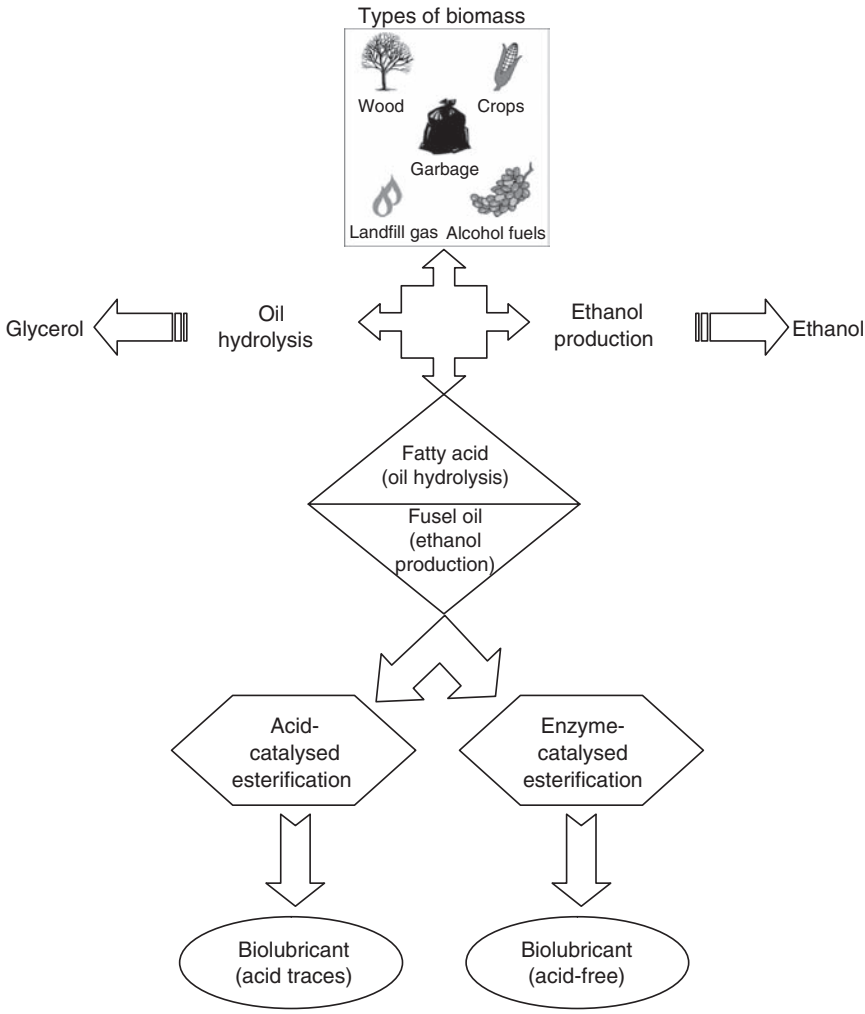

Fig. 9.1 Production of biolubricants.

product, which causes corrosion during utilisation. By applying enzymatic catalysis (e.g. lipase), this can be avoided. The biolubricants obtained are not only derived from natural renewable sources (biomass), but can also be degraded biologically (Dörmő *et al*., 2004).

Similar biolubricants can be produced by *enzymatic extraction* of ethanol (from food wastes) by fatty acids (Csányi *et al*., 2004). Ethanol recovery from the aqueous fermentation broth can be realised by extraction using, for example, oleic acid, with simultaneous esterification by lipase enzyme. In this way, ethyl oleate is manufactured, while ethanol inhibition is avoided.

According to tribological tests, these (ester) types of biolubricants can be characterised as lubricants having a low flash point, low pour point, high viscosity index, and low acid number which is a result of the enzymatic catalysis (instead of acidic catalysis). Thus, it is suggested that they are used mainly at high-speed, and low-load tribological regimes. In industry they can be applied as, for example, cooling lubricant compounds for metal-working processes, and also in particular processes where lubricant loss may occur, e.g. mist lubrication, chain lubrication and launch engine lubrication.

Galacturonic acid

Galacturonic acid is an acidifying agent in foods and the monomer of pectin molecules. Thus pectin-containing co-product and waste can be processed to recover the pectin. Sugar beet pulp is one of the raw materials most often used (Jördening *et al*., 2002). When pectin is extracted from sugar beet pulp by hot water, the process can be promoted by using pectinase enzymes. In this way pectin becomes more soluble, its viscosity is decreased and the yield of the enzymatic extraction is much higher. If the aim is recovery of galacturonic acid, complete degradation of pectin can be carried out. However, controlling the enzyme action may result in partial degradation, where soluble pectin can be obtained.

9.2.3 Combination approaches – a case study: glycerol production from oil waste

Glycerol production from oils provides a special case where both fermentation and enzymes are used in the processing of a food technology co-product. The process forms a closed circle, or cycle (Fig. 9.2), and biocatalysis is involved in almost every step of the procedure. Oils and fats are produced from renewable sources (e.g. oil plants, i.e. biomass) and are used in cooking, particularly frying. The waste (used) frying oils – usually selectively collected – (chemical name: triglycerides) can be converted either into biodiesel (transesterification with methanol) or fatty acids (enzymatic hydrolysis). In both cases glycerol is formed as a co-product.

Biodiesel is mainly produced industrially by transesterification with methanol using alkali as a catalyst (Mittelbach and Tritthart, 1988).

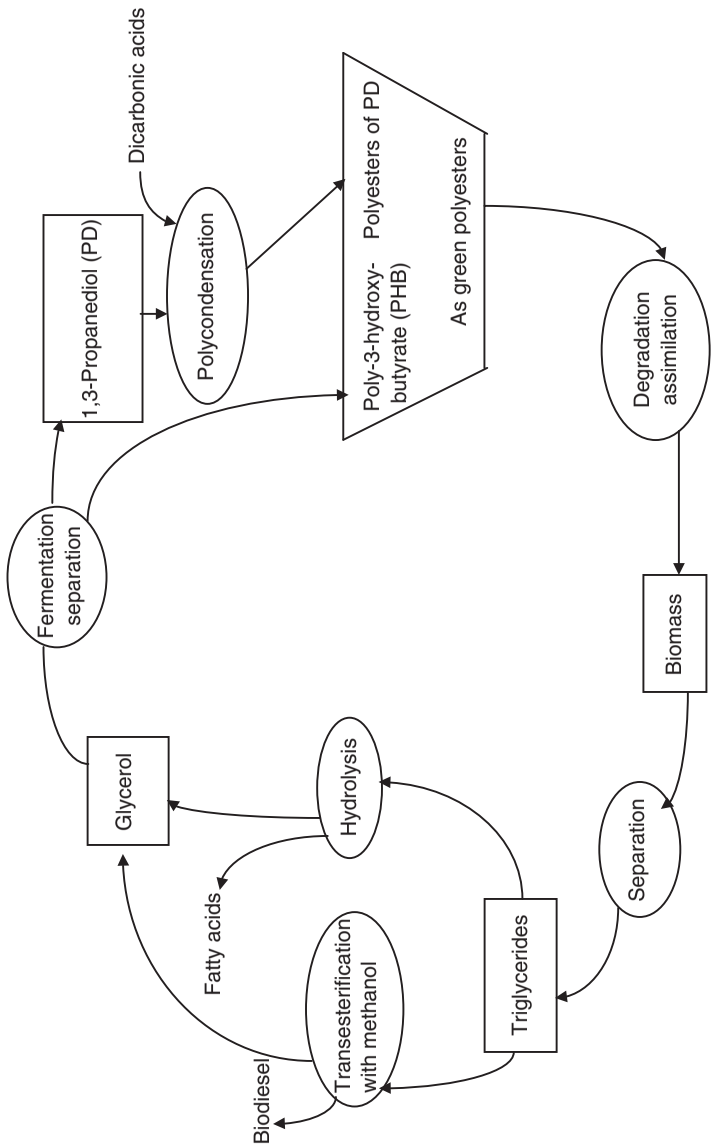

Fig. 9.2 Glycerol cycle.

However, it is a hazardous material and should be treated after usage. By applying enzyme catalysis, a 'green (waste-free) process' can be realised by, for example, using lipase enzyme in a solvent-free system (Bélafi-Bakó *et al*., 2002b). Although the high price of the enzyme currently makes the process unprofitable, intensive research work is being carried out to develop cost-effective industrial enzyme preparations.

Hydrolysis of oils results in fatty acids and glycerol. The main difficulty in the process is caused by the distinct solubility of both substrates (triglycerides and water) and products. Enzymatic hydrolysis by lipase has several advantages compared with conventional methods. Instead of high pressure and temperature, the enzymatic reaction takes place under mild conditions resulting in energy savings and products of better quality. If the hydrolysis is carried out in a *membrane bioreactor*, further advantages are added to the process: the two phases remain separated during the reaction, thus there is no need to use an emulsion system, and the separation of the products can also be solved by the membrane. Moreover, the membrane offers a suitable surface for immobilization of lipase, resulting in a more stable biocatalyst, which can be used for a longer time (Bélafi-Bakó *et al*., 1994).

Glycerol can be processed further into PD (Papanikolaou *et al*., 2000) or PHA (Doi, 1992) compounds that are raw materials for green polyesters (as mentioned previously). These polymers can easily be degraded biologically and in this way – after use – they become part of the biomass. Thus the circle is closed.

9.3 Future trends

This chapter has presented some utilisation possibilities for the wastes and co-products being generated by the food industry. Remarkable development is expected with the introduction of genetically modified microorganisms, where certain enzyme activities are 'tailored' according to the substrate to be converted. Another possibility to enhance the effectiveness of biocatalytic processes in waste conversion is to apply hybrid systems, where a fermentation or enzymatic reaction is coupled with, for example, a separation step, such as an extraction or membrane technique (Bélafi-Bakó and Gubicza, 2000) – as mentioned in polysaccharide hydrolysis. Thus, the footprint of the technologies can be reduced, implying lower investment cost, etc. Regarding the usage of the valuable product obtained, it is surely beneficial if the product formed from food industrial waste could also be applied in the food processes, i.e. it is kept in the same sector. A possible example of this is galacturonic acid obtained from extracted sugar beet pulp, which can then be used as an acidifying agent in foods.

These types of utilisation have, however, always had limitations. The best possible way would be to use waste-free processing technologies. Although it is not often feasible, in some areas waste and co-product

formation can be almost completely avoided. An example of this is a special fruit juice processing method called '*total liquefaction*'. The concept is to achieve a complete breakdown of the fruit cell walls using cellulases and hemicellulases in addition to pectolytic degradations (Tucker and Woods, 1998). In this way, a high yield can be achieved, pressing is no longer required since all the pulp becomes juice and there is virtually no residue. Of course this kind of process cannot be applied in many areas, however it is very important to search for and to find similar, waste-free processes in the food industry.

9.4 Sources of further information and advice

Full-length papers cited (see Reference section) can give further details on the processes mentioned in this chapter. Review papers on various topics within the subject may be found similarly useful (e.g. Doi, 1992; Naidu and Panda, 1998; Kim *et al*., 2003). Relevant journals include *Process Biochemistry*, *Bioresource Technology* and *Applied Microbiology and Biotechnology*. The suppliers of the enzymes, microorganisms are in general reliable sources of relevant information. They usually have feedback data and extensive knowledge on the bio-processes. Some of the major commercial suppliers are: Calbiochem-Novabiochem, USA (www.calbiochem.com); Novozymes, Denmark (www.novozymes.com); Amano Pharmaceutical, Japan (www.amano-enzyme.co.jp). More information on the behaviour of enzymes can be found in various databases, for example:

- BRENDA (www.brenda.uni-koeln.de);
- ENZYME (www.expasy.ch/enzyme);
- Protein Data Bank (www.pdb.org);
- Enzyme Structure (www.biochem.ucl.ac.uk/bsm/enzymes);
- LIGAND (www.genome.ad.jp/dbget/ligand.html).

A complete list of enzyme suppliers and databases was published recently in the journal *Biotechnology Advances* (Krishna, 2002).

Technical and other opportunities for biocatalytic methods of waste management and co-product recovery in the food industry have been regularly surveyed by the Food and Agriculture Organization (FAO) and some of results are published. For example, Rolle (2005) summarised the possibilities for these technologies in developing countries. Thus, readers are advised to consult the FAO website (www.fao.org/ag).

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10

Supercritical fluid extraction and other technologies for extraction of high-value food processing co-products

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10.1 Introduction

Supercritical fluid extraction (SFE) with carbon dioxide (CO_2) is already established as a process for the decaffeination of coffee beans and tea. Recent studies have focused on other applications, especially with botanical materials and thermally liable substances (Walker *et al.*, 1999; Rizvi *et al.*, 1994; Rozzi and Singh, 2002; Brunner, 2005; Moura *et al.*, 2005; McHugh and Krukonis, 1994; Mukhopadhyay, 2000). Many excellent review articles of supercritical fluid technology exist. Subjects covered include: a general overview (Chester *et al.*, 1998), the food industry (Brunner, 2004; Rozzi and Singh, 2002), Chinese herbal medicine (Chen and Ling, 2000), pharmaceutical research (Vasukumar and Bansal, 2003), biotechnology (Williams and Clifford, 2000) and natural product studies (Lang and Wai, 2001; Sovova, 2005).

The development of new separation techniques for pharmaceutical and food industries has received a lot of attention due to rising concerns about the health, environmental and safety hazards associated with traditional solvent techniques. Organic solvent residues can remain in the final product as well as promote oxidative degradation of these sensitive nutraceutical compounds during processing; these disadvantages are of special concern in the purification of pharmaceuticals, nutraceuticals and food products. Additional set-backs in the use of these potentially toxic organic solvents include the high initial investment and regeneration. SFE with CO_2 is a focus of interest due to the advantages of limiting auto-oxidation and thermal decomposition of the products and offers complete removal of the non-toxic, inexpensive and inert solvent. SFE with CO_2 is used in the

extraction of desired components from natural materials for eventual use in food, perfumery, pharmaceutical and nutraceutical industries (Goto *et al.*, 1998; Mukhopadhyay, 2000; Rozzi and Singh, 2002).

The SFE process in solids is based on the contact between a solid raw material and pressurized solvent, which removes the compounds of interest from the solid phase. After this removal, the extract separates from the solvent through a pressure reduction. The kinetics of the solute extraction consists of releasing the solutes from porous matrices into the supercritical fluid via mass transfer mechanisms. The extraction of the solute involves the dissolution of the solid component, diffusion of the solute to the solid surface and external mass transfer around the solid particle. Mathematical models have been proposed to correlate overall extraction curves (OEC) during the SFE process (Goto *et al.*, 1993). However, no single model has been universally accepted.

CO_2 is an ideal, alternative, extraction solvent because it prevents harmful oxidation reactions and can enter a supercritical state under low temperature and pressure conditions (31.1 °C and 7.38 MPa) (Rizvi, 1994; Krukonis, 1988). Supercritical CO_2 has the additional advantages of low cost, non-toxicity, high diffusivity and low viscosity. Solvent separation from the extract is easily accomplished by reducing the pressure and returning the CO_2 to a gaseous state. SFE using CO_2 is a promising technique with the advantages of limiting the auto-oxidation, decomposition, and polymerization of polyunsaturated fatty acids (PUFAs) found in animal, fish, and fungal oils (Amano *et al.*, 1992; Cohen and Heimer, 1992; Leman, 1997). Application of SFE with CO_2 has increased since CO_2 is a non-toxic, non-flammable, inexpensive, 'green' and generally regarded as safe (GRAS) solvent (Rizvi *et al.*, 1994; Rozzi and Singh, 2002; Letisse *et al.*, 2006). The application of SFE to fish oil fatty acids has been studied by many authors (Stinson *et al.*, 1991; O'Brien and Senske, 1994; O'Brien *et al.*, 1993; Bajpai *et al.*, 1991; Yazawa, 1996; Letisse *et al.*, 2006) and recent detailed studies have focused on other applications, especially botanical materials and other thermally labile substances (Yongmanitchai and Ward, 1989; Certik and Sajbidor, 1996; Singh and Ward, 1997; Kendrick and Ratledge, 1998; Vazhappilly and Chen, 1998; Molina Grima *et al.*, 2003).

SFE utilizes the ability of normally gaseous chemicals to become excellent solvents for certain solutes under a combination of tunable properties in terms of temperature and pressure. The solvent becomes supercritical when it is raised above its critical point for both temperature and pressure (T_c and P_c, respectively). For CO_2, the T_c is 31.1 °C and P_c is 7.38 MPa. Only one phase exists in the critical region (hash marks in Fig. 10.1) that possesses both gas- and liquid-like properties. A supercritical fluid has liquid-like densities and a viscosity close to that of normal gases. The diffusivity for a supercritical fluid is about two orders of magnitude higher than that

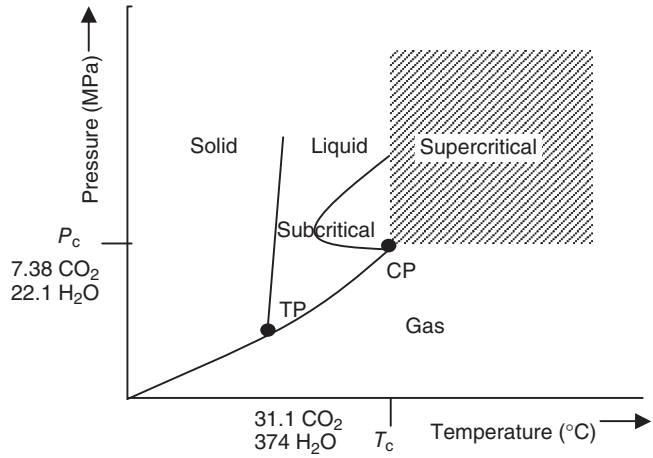


Fig. 10.1 Supercritical state for a pure component. TP, triple point; CP, critical point; P_C, critical pressure; T_C, critical temperature (Brunner, 1994).

for typical liquids: $(0.2–0.7) \times 10^{-3}$ cm^2/s compared with $(0.2–2.0) \times 10^{-5}$ cm^2/s (Brunner, 1994, 2005; Mukhopadhyay, 2000; Rozzi and Singh, 2002). The low viscosity and other 'gas-like' properties allow for the solvent to diffuse more readily through the solid matrix. These characteristics facilitate rapid mass transfer and faster completion of extractions compared with traditional liquid extraction techniques.

McHugh and Krukonis (1994) have given a detailed historical perspective of the developments related to supercritical fluids. SFE technology, after initial ups and downs in its developments, started to become a possible alternative extraction technology in many fields in the late 1980s and early 1990s.

The most commonly used supercritical fluid, as an extraction solvent, is CO_2. The phase diagram given in Fig. 10.1 shows the supercritical region for CO_2. CO_2 is a non-polar fluid, has a solvating power comparable with hexane and is widely used for the extraction of non-polar compounds. Modifiers in the form of appropriate solvents such as ethanol may be used to extract polar compounds using CO_2. The major problems associated with the conventional solvent extraction industry (flammability, possibilities of toxic residues, waste disposal regulations and environmental concerns) have resulted in increased attention for SFE. SFE, apart from overcoming problems associated with conventional solvent extraction, also offers additional advantages such as selective extraction and fractionation of high-value components in the extract under optimized extraction conditions.

10.2 Key reasons for use of supercritical fluid extraction (SFE)

Increasing public awareness about healthy, natural and non-toxic products, and strict environmental regulations have acted as an impetus for the supercritical fluid industry. Moreover, the pharmaceutical, nutraceutical, and food industries have also promoted developments in this area of research (Schneider *et al.*, 1980; Stahl *et al.*, 1988; McHugh and Krukonis, 1994; Rizvi, 1994; Clifford, 1999; Kiran *et al.*, 2000; Mukhopadhyay, 2000).

Interest in SFE for health and environmental reasons has resulted in several detailed contributions on this subject. Chester *et al.* (1998) and Williams (1981) reviewed developments in SFE. Paulaitis *et al.* (1983) summarized chemical engineering and thermodynamic aspects of supercritical fluids. Clifford (1999) and Kiran *et al.* (2000) dealt with the fundamentals of supercritical fluids. Williams and Clifford (2000) described process development using supercritical fluids.

SFE from food, pharmaceutical, nutraceutical and other natural and biological products has received significant attention in recent years. Rozzi and Singh (2002), Mohamed and Mansoori (2002) and Raventos *et al.* (2002) reviewed applications of supercritical fluids in the food industry. Rizvi (1994), Awasthi and Trivedi (1997), as well as Mukhopadhyay (2000), enumerated extraction techniques from natural materials. King and List (1996) dealt with applications for SFE for lipids and oils. Chen and Ling (2000), as well as Lang and Wai (2001) and Catchpole *et al.*, (2004), described applications of SFE technologies for herbal medicine. Apart from these detailed reviews and books, there are several other research publications on supercritical fluids for the extraction of various biological materials (Froning *et al.*, 1990; Peker *et al.*, 1992; Bhaskar *et al.*, 1993; List *et al.*, 1993; Tsuda *et al.*, 1995; Chester *et al.*, 1998; Cheung *et al.*, 1998; Nguyen *et al.*, 1998; Ambrosino *et al.*, 1999; Cheng *et al.*, 1999; Galan *et al.*, 1999; Ibanez *et al.*, 1999; King, 2000; Senorans *et al.*, 2001; Wong *et al.*, 2001; Canela *et al.*, 2002; Rozzi *et al.*, 2002; Prieto *et al.*, 2003). Tehrani (1993) suggested successful SFE strategies. DeCastro and Carmona (2000), after reviewing the advantages and limitations of SFEs, talked about future directions of the process.

10.3 Supercritical fluid extraction

A supercritical fluid is defined as any substance that is above its critical temperature (T_c) and critical pressure (P_c). The critical pressure is the highest pressure at which a liquid can be converted into a gas by an increase in temperature, while the critical temperature is the highest temperature at which a gas can be converted into liquid by an increase in pressure. In 1822, Baron Cagniara de la Tour (de la Tour, 1822) was able to identify the appearance of a supercritical phase in a closed glass container (Clifford,

1999; Clifford and Williams, 2000; Mukhopadhyay, 2000). In the critical region there is only one phase that possesses both gas- and liquid-like properties. A supercritical fluid has both the gaseous property of being able to rapidly diffuse into a solid matrix and the liquid property of being able to dissolve materials into their components. Moreover, the solvating power of a supercritical fluid varies with a change in its density as a result of a change in pressure or temperature. Generally, for supercritical fluids at constant pressure, the solvating power decreases with an increase in temperature; at constant temperature, the solvating power increases with an increase in pressure. Therefore, the solvating power of a supercritical fluid may be maximized by appropriate manipulations of both pressure and temperature. Hence the density of a fluid can be adjusted to solubilize certain types of compounds in a selective way (Schneider *et al.*, 1980; Stahl *et al.*, 1988; McHugh and Krukonis, 1994; Rizvi, 1994; Clifford, 1999; Kiran *et al.*, 2000; Mukhopadhyay, 2000). These properties of supercritical fluids make them an ideal solvent because of their high mass transfer properties as well as their selective extraction capabilities. They exhibit higher diffusivities than liquid solvents (see Fig. 10.2), lower viscosities and very low surface tensions. Table 10.1 shows the different properties of commonly used supercritical fluids. Apart from high diffusivities and low viscosities, supercritical solvents like CO_2 also offer gentle treatment of heat-sensitive materials, and preserve natural fragrances and aromas of agricultural and biological products such as nutraceuticals and traditional medicines.

Figure 10.3 shows a typical flow diagram of SFE apparatus used for extraction experimentation.

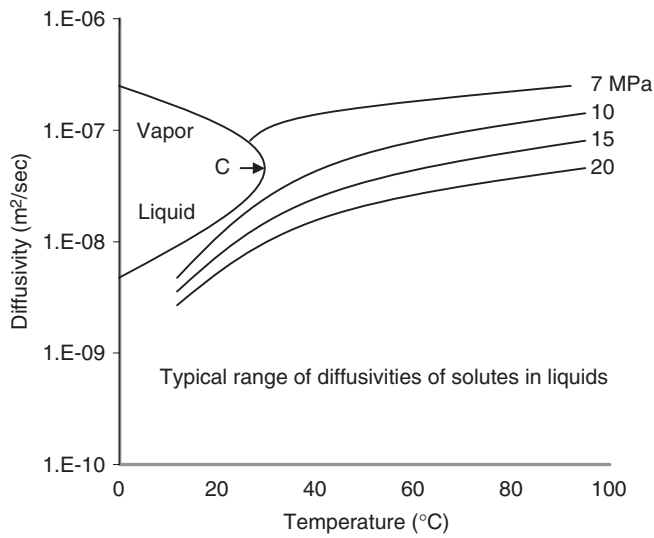


Fig. 10.2 Self-diffusivity behavior of CO_2. C = critical point.

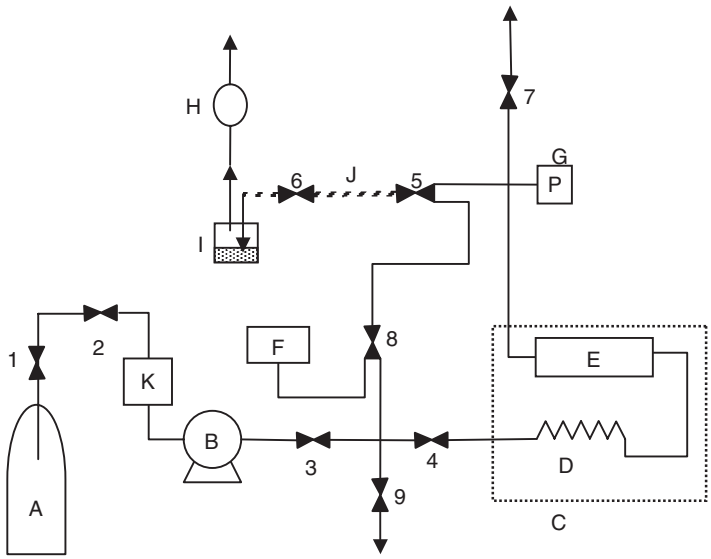
Table 10.1 Physical properties of common supercritical solvents (from Klesper, 1980)

Fluid	Normal boiling point (°C)	Critical constants		
		Pressure (MPa)	Temperature (°C)	Density (g/cm^3)
Carbon dioxide	–78.5	7.38	31.1	0.468
Ethane	88.0	4.88	32.2	0.203
Ethylene	–103.7	5.04	9.3	0.20
Propane	–44.5	4.25	96.7	0.220
Propylene	–47.7	4.62	91.9	0.23
Benzene	80.1	4.89	289.0	0.302
Toluene	110.0	4.11	318.6	0.29
Chlorotrifluoromethane	–81.4	3.92	28.9	0.58
Trichlorofluoromethane	23.7	4.41	196.6	0.554
Nitrous oxide	–89.0	7.10	36.5	0.457
Ammonia	–33.4	11.28	132.5	0.240
Water	100.0	22.05	374.2	0.272

10.3.1 Lipids from natural products

Prospective applications for the SFE of lipids or oils, apart from common vegetable oils (soy oils, corn oil, rice bran oil, sunflower oil, olive oil, etc.), also include animal fats, fish oil, oil from seaweeds and oil from microorganisms like fungi (Walker *et al.*, 1999; Mukhopadhyay, 2000; Shen *et al.*, 1996). Major components of lipids include monoglycerides, diglycerides, triglycerides and free fatty acids (FFAs); minor constituents include sterols, tocopherols, gums, alkaloids, flavonoids, wax and volatile compounds, which provide taste and odor. Most studies concerning SFE of lipids are focused on the optimization of extraction conditions to increase the yield of extractable materials (Hu, 1995). Several components of lipids have significant health and nutritional implications for the food and pharmaceutical industries. PUFAs have important therapeutic value (Shahidi and Wanasundara, 1998; Galli and Butrum, 1991). Unsaturated fatty acids and saturated fatty acids have different health effects. Sterols, antioxidants, wax and volatile compounds are also significantly important for health. Major SFE applications include separation of FFAs from vegetable oils, separation of PUFA from animal fats, refining and deodorization of vegetable oils, fractionation of glycerides, recovery of oil from biological materials, de-oiling of lecithin, and de-cholesterolization and de-lipidation of food products (Mukhopadhyay, 2000).

King and List (1991) reviewed the SFE of fat and observed that the solubility of fats in supercritical CO_2 generally increases with pressure and temperature. At very low pressures, the solubility of fats is slightly higher at lower temperatures. However, they found that SFE of fat worked best at high temperatures above 80 °C and pressure above 55.16 MPa or 8000 psi. Smaller particles yielded more oil as did dryer material. They described SFE



	Instrumentation		Valves
A	CO_2 cylinder	1	CO_2 cylinder supply (from A)
B	CO_2 syringe pump	2	CO_2 pump supply
C	Water bath	3	CO_2 syringe pump isolation
D	Preheating coil for CO_2	4	Vessel isolation
E	Explosion/extraction vessel	5a	CO_2 flow control
F	Hexane reservoir	5b	Hexane rinse control
G	Vessel pressure transducer	6	CO_2 micrometering
H	Gas flow meter	7	Explosion high pressure release
I	Hexane cold trap	8a	CO_2 by-pass
J	Flow control section heating tape	8b	Hexane reservoir outlet
K	CO_2 feed ice bath	9	CO_2 pressure release

Fig. 10.3 Schematic of the supercritical CO_2 extraction apparatus.

as a promising technology for the extraction of small-scale, high-value products from fat-bearing materials. Bjergegaard *et al.* (1999) compared SFE and conventional solvent extraction for the extraction of volatiles and hydrophilic compounds from rapeseed, sunflower and soybean. SFE was also useful for analytical purposes to ascertain lipid content. Montanari *et al.* (1996) used supercritical CO_2 and a co-solvent (ethanol) for the selective extraction of phospholipids from soybean flakes. Taylor and King (2000) used analytical-scale SFE and supercritical fluid chromatography (SFC) for the optimization and fractionation of corn bran oil to achieve a high concentration of ferulate phytosterol esters (FPE). They extracted a maximum 1.25% FPE from corn bran over the different combinations of temperatures (40, 60 and 80 °C) and pressures (13.8, 34.5 and 69 MPa) tried during the experiments.

Eggers and Sievers (1989) studied the SFE of rapeseed with different pretreatments and observed that flaked rapeseed cake and higher pressures

were beneficial. Fattori *et al.* (1988) studied supercritical extraction of canola seed oil (25–90 °C, 10–36 MPa) and found that oil solubility in supercritical CO_2 was strongly dependent on pressure, but was not significantly dependent on temperature. The total oil recovery was also significantly dependent on the pretreatment of the seed (flaking, cooking, pressure rupture, chopping, crushing). Greater amounts of oil were recovered from flaked and cooked seed compared with whole seed.

Brown seaweed extraction with supercritical CO_2 (24.1–37.9 MPa, 40–50 °C) was compared with Soxhlet extraction using chloroform : methanol (2 : 1, v/v) (Cheung *et al.*, 1998). Oil yields of SFE at 37.9 MPa (40–50 °C) were comparable to Soxhlet extraction, but ω-3-fatty acid concentrations were higher (31.4%) in supercritical extract compared with Soxhlet extraction (23.5%). For constant pressure (24.1 MPa), SFE yielded more lipids at 40 °C than at 50 °C. The concentration of total PUFAs in the oil decreased significantly, and that of total saturated fatty acids increased significantly with increased pressure and solvent density.

Studies on the extraction of spearmint oil (essential oil of *Mentha spicata*) from Turkish mint plant leaves with supercritical CO_2 indicated that the concentration of the monoterpenes fraction in the oil and oil yields were inversely related. Compared with conventional methods of hydrodistillation (HD), SFE produced lower concentrations of the monoterpenes in the oil at a low temperature that was safe for heat-sensitive essential oils (Ozer *et al.*, 1996). Lavender essential oil and wax extraction with supercritical CO_2 resulted in higher linalyl acetate content in the oil (34.7%) compared with conventional hydro-distillation (12.1%) (Reverchon and Porta, 1995).

Other recent lipid extraction studies using SFE include canola oil (Bulley and Fattori, 1984; Temelli, 1992), citrus oil (Sato *et al.*, 1998), menhaden oil (Rizvi *et al.*, 1988; Nilsson *et al.*, 1988), rapeseed oil (Eggers and Sievers, 1989), evening primrose oil (Favati *et al.*, 1991), soybean oil (List *et al.*, 1993), soya, canola and corn germ oils (Taylor *et al.*, 1993), peppermint oil (Motonobu *et al.*, 1993), caraway essential oil (Sovova *et al.*, 1994a), soybean oil (Reverchon and Osseo, 1994), ginger oil (Roy *et al.*, 1996), cloudberry seed oil (Manninen *et al.*, 1997), sunflower oil (Perrut *et al.*, 1997), pistachio nut lipids (Palazoglu and Balaban, 1998), almond oil (Marrone *et al.*, 1998), lavender essential oils and waxes (Akgun *et al.*, 2000), grape seed oil (Lee *et al.*, 2000; Murga *et al.*, 2002), hiprose seed oil (Reverchon *et al.*, 2000) and Romanian mentha hybrids oil (Eugenia and Danielle, 2001).

King *et al.* (1996) used combined SFE (25 MPa and 80 °C) and SFC (1.7 cm diameter and 20 cm long columns charged with 60–200 mesh silica gel, 16 g, in a preparative mode) to fractionate and enrich tocopherol components of the oil from soybean flakes and rice bran. Total tocopherol recovery and enrichments were observed as a function of the mass ratio of CO_2/seed charge. Also, tocopherol recovery differed from one seed type to another. Garcia *et al.* (1996) found that at 28 MPa and 70 °C (highest allowable pressure and temperature in their system) they obtained 16–60% of

solvent-extractable oil yield from rice bran. Oil obtained by SFE was lighter in color, high in waxes and had greater long-chain fatty acids (C_{20}–C_{34}) compared with hexane-extracted oil.

Kuk and Dowd (1998) carried out SFE of rice bran (6% moisture, below 0.297 mm particle size) at 48.26 and 62.05 MPa for 1.5 h and reported 19.2–20.4% rice bran oil yield, compared with 20.5% extraction yield using hexane in 4 h. They also found increases in rice bran oil yield with increasing temperatures at constant pressure. Sterol extraction was found to increase with increasing pressure and temperature. Kim *et al.* (1999) compared essential fatty acids (EFAs) in rice bran oil extracted under different conditions (40, 50 and 70 °C; 20.68, 27.58, 34.47 and 41.37 MPa). They found yields to be dependent on reduced density of supercritical CO_2. Up to 70–80% of rice bran oil may be extracted in 4 h. Xu and Godber (2000) compared solvent extraction (50% hexane and 50% isopropanol v/v) of rice bran with supercritical CO_2 extraction at 50 °C and 68.9 MPa pressure for extraction of γ-oryzanol, an important antioxidant component (Xu and Godber, 1999; Xu *et al.*, 2001). Their studies indicated that SFE extraction may extract up to four times more γ-oryzanol (5.39 mg/g of rice bran) in less time compared with solvent extraction. Table 10.2 shows the major sterols co-extracted with various natural oils. These sterols have great potential as precursors to pharmaceutical compounds.

Lipids from fungal and algal biomass

The beneficial health effects of consuming PUFAs, which include (C20:5; ω-3) eicosapentaenoic acid (EPA) and (C22:6; ω-3) docosahexaenoic acid (DHA), have been well documented over the years (Gilli and Valivety, 1997; Li *et al.*, 2003). These fatty acids have been linked to visual and mental health as well as regulating critical biological functions (Simopoulos *et al.*, 1991; Bajpai and Bajpai, 1993; Barclay, 1997). PUFAs are associated with the prevention and treatment of coronary heart disease and abnormal cholesterol levels, in addition to alleviating inflammatory conditions (Babcock

Table 10.2 Comparison of sterols and triterpenes in different oils (% in oil) (from Rukmini and Raghuram, 1991)

Oil	Campesterol	Stigmasterol	β-Sitosterol	Cycloartanol	Cycloartenol	24-Methylene cyloartanol
Rice bran	0.506	0.271	0.885	0.106	0.482	0.494
Safflower	0.045	0.031	0.181	0.001	0.034	0.007
Corn	0.410	0.110	1.180	0.004	0.008	0.011
Sunflower	0.031	0.031	0.235	–	0.029	0.016
Cottonseed	0.017	0.004	0.400	–	0.010	0.017
Sesame	0.117	0.062	0.382	0.004	0.062	0.107
Soybean	0.072	0.072	0.191	–	0.168	0.008
Groundnut	0.036	0.021	0.153	0.001	0.011	0.016

Source: Rukmani and Raghuram (1991)

et al., 2000) and even retarding growth of tumor cells (Youdim *et al.*, 2000; Tapiero *et al.*, 2002; Voet and Voet, 2004). Filamentous fungi, like *Pythium irregulare* (Cheng *et al.*, 1999; Singh and Ward, 1997; Stredansky *et al.*, 2000), and algae like *Crypthecodinium cohnii* (DeSwaaf *et al.*, 2002), are identified as microorganisms that can produce health beneficial, valuable PUFA rich oils (Walker *et al.*, 1999) and can produce EPA and DHA at 10–50% of the total intracellular lipids (De Swaaf *et al.*, 2002). The downstream recovery and purification of this PUFA-rich oil impose certain restraints since these compounds are thermally labile, fragile and easily oxidized. SFE techniques have been applied to the extraction of said oils; however, the intracellular oils can be difficult to extract from the biomass since most of the cells remain intact during the extraction process.

Fish oil supplements dominate the current PUFA market; however, fish oil possesses objectionable tastes and odors along with cholesterol and small amounts of pollutants that may include mercury (Simopoulos, 2004). Microorganisms are promising producers of PUFA-rich oils that can serve as an alternative to the oils obtained from agricultural and animal sources. The technology to produce EPA and DHA from microalgae and fungi has proven to be commercially feasible (Kyle, 2001); these organisms are capable of year-round oil production on a variety of cheap substrates (Bajpai and Bajpai, 1993; Nettleton, 1995; Kyle, 1996; Uauy *et al.*, 2001; Simopoulos, 2004; Voet and Voet, 2004; Ward and Singh, 2005). The filamentous fungi *Pythium irregulare* is a well-researched organism capable of producing EPA and other long-chain fatty acids such as linoleic (C18:2 ω-6) and arachidonic (C20:4 ω-6) acids (Certik and Shimizu, 1999; Belarbi Medina *et al.*, 2000; Robles Medina *et al.*, 1998; Wen and Chen, 2003).

Badal (2002) studied the effects of particle size (16–48 mesh and >48 mesh) and biotreatment with *Pythium irregulare* fungi, on the yield and the quality of rice bran oil extracted with supercritical CO_2 (40 °C, 27.57 MPa, 200 standard cm^3 per min). The extraction yield was approximately 50% of the total ether Soxhlet extractable oil (in 2 h) from the smaller-particle rice bran. Eicosapentaenoic acid and arachidonic acid produced during the treatment by *Pythium irregulare* were extracted by SFE.

Enzyme reactions in supercritical fluids

Enzymes show significant stability when immobilized in CO_2. The stability is partially attributed to the absence of oxidation reactions (Shishikura *et al.*, 1994). Fungal enzymes are typically more stable in non-aqueous environments than bacteria-derived enzymes due to the functionality of fungi at lower water activities (Svensson *et al.*, 1994). Fungal lipases are particularly well suited to catalyze reactions in non-aqueous environ-

ments. This is not only due to this enzyme's stability, but the reacting substrates and products are usually soluble in the non-aqueous phase. Therefore, the equilibrium of the enzyme-catalyzed reaction may be improved by the facilitated removal of byproducts with CO_2 (Shishikura *et al.*, 1994).

An important group of lipases include the 1,3 regiospecific enzymes (e.g. from *Mucor miehei* and *Rhizopus arrhizus*) that produce diacylglycerols, 2-monoacylglycerols and FFAs. This leads to a number of important reactions that include transesterification and interesterification of lipids. For instance, Shishikura *et al.* (1994) found that lipase-catalyzed interesterification – using the immobilized *Mucor miehei* lipase, lipozyme – between medium-chain length triglycerides and free long-chain fatty acids may be accomplished, while the byproducts of the reaction are simultaneously extracted with CO_2.

Enzymes, however, require an aqueous phase directly adjacent to the enzyme to remain active. Therefore, water activity is an important parameter for enzyme functionality in non-aqueous environments and should be controlled to optimize the reaction kinetics (Svensson *et al.*, 1994). Aqueous phases are required for enzyme reactions, but also cause unwanted hydrolysis reactions during interesterification with the lipase enzyme (Shishikura *et al.*, 1994). Therefore it is advantageous to bring the reaction mixture into contact with CO_2 to extract fatty acid moieties produced during the interesterification to improve equilibrium conditions.

Mucor miehei lipase has been shown to be very effective in the interesterification reaction that incorporates long-chain fatty acids into triglycerides to produce a product of greater value (Mukherjee and Kiewitt, 1991; Shishikura *et al.*, 1994; Nagesha *et al.*, 2004). Shishikura *et al.* (1994) noted that glycerol was required at 1–2% to carry out the incorporation of long-chain fatty acids successfully. They noted that 1,3 diglycerides are also formed with glycerol addition. The goal is to improve enzymatic catalysis by increasing the contact of supercritical CO_2 with lipases attached to the silica adsorption phase (from rice ash) for simultaneous reaction and byproduct extraction in the presence of small amounts of water contained in humidified CO_2. Blattner *et al.* (2006) first attempted encapsulation of lipases from *Mucor miehei* and *Candida antarctica* in stable lecithin-based water-in-oil microemulsion organogels for immobilized-enzyme supercritical-CO_2 technology to apply sustainable 'green' chemistry to commercial processes.

Supercritical fluid fractionation of lipids

Concentration and fractionation of the PUFAs and important minor constituents – including antioxidants e.g. oryzanols – may be accomplished during the extraction process (Nilsson *et al.*, 1988; Xu and Godber, 1999;

Xu and Godber, 2000). Friedrich and Pryde (1984) applied SFE to soybean, cotton seed, corn germ, wheat germ and bran, and observed that supercritical extracted oil was light colored compared with hexane-extracted oil. Moreover, they observed some fractionation during the extraction where more polar and higher-molecular-weight compounds were found to increase during later stages of the extraction process. They noted that more polar and high-molecular-weight compounds tended to appear at higher concentrations in the later fractions. Zosel (1978) separated up to 50 fractions of triglycerides from cod liver oil based on increasing molecular weight and degree of unsaturation. McHugh and Krukonis (1994) and Eisenbach (1984) noted that by first transesterifying the triglycerides to EPA ethyl esters, fractionation of individual fatty acids would be possible during the SFE process. Transesterification also increases volatility which would correspond to an increase in solubility of the fatty acids in supercritical CO_2 (Harrison *et al.*, 1994; Smith *et al.*, 1998; Fleck *et al.*, 1998; Riha and Brunner, 2000).

Polar lipid fractions may be significantly solubilized in CO_2 with the addition of small amounts of polar entrainers such as ethanol (Temelli, 1992). Hardardottir and Kinsella (1988) reported that SFE could remove up to 97% of fish lipids with the addition of 10% ethanol compared with 78% of lipids without ethanol. Also cholesterol removal was about 99.5%. Mendes *et al.* (1994) extracted hydrocarbons from a slightly crushed, freeze-dried alga and discovered that the polar phospholipids were not extracted (verified by thin-layer chromatography). Cygnarowicz-Provost *et al.* (1992), however, noted that supercritical CO_2 extraction with 10% ethanol gave a recovery of 89% lipids from the filamentous fungi *Saprolegnia parasitica*, which contains 25% polar lipids. This compared with only 49% lipids extracted with pure CO_2.

Dunford and King (2000) studied extraction of rice bran oil by supercritical CO_2 fractionation for reducing FFAs and minimizing losses of phytosterols. From their experiments at pressures of 20.5–32.0 MPa and temperatures ranging from 45 to 80 °C, they found that lower pressures and higher temperatures reduced the loss of triglycerides and phytosterols during removal of FFAs from crude rice bran oil. Rice bran oil containing less than 1% FFA, up to 95% triglycerides, 0.35% free sterols and 1.8% oryzanol, may be obtained by SFE extraction. During olive oil de-acidification with supercritical CO_2 at different pressures (20 and 30 MPa) and temperatures (35–60 °C), CO_2 extracted fatty acids more selectively than triglycerides (at 60 °C and 20 MPa). Moreover the physical state of the solute significantly affected solubility trends as a function of temperature and pressure. Supercritical fluid de-acidification of olive oil was found suitable, especially for oils with relatively high FFA content (<10%) due to a higher selectivity factor for FFA (Brunetti *et al.*, 1989).

Taylor and King (2000) used SFE (13.8, 34.5 and 69 MPa; 40, 60 and 80 °C) to extract high-value ferulate phytosterol esters from corn bran; highest

yields (1.25%) were obtained in the extract at 69 MPa and 80 °C as well as at 34.5 MPa and 40 °C. Furthermore, SFE (34.5 MPa, 40 °C) extracted corn bran oil with subsequent fractionation with SFC (amino propyl sorbent, commenced at 69 MPa at 80 °C and subsequently lowered to 34.5 MPa at 40 °C with addition of ethanol modifier at the lower pressure) that produced up to a 14.5% FPE enrichment level. Dunford *et al.* (2003) used continuous counter-current supercritical fluid processing (CO_2 flow rate of 2 l/min and oil flow rate of 0.7 l/min) for de-acidification of rice bran oil at isobaric and isothermal conditions (at a pressure range of 13.8–27.5 MPa and a temperature range of 45–80 °C) and observed that fractionation at 13.8 MPa and 80 °C was effective in de-acidification without loss of oryzanol.

In the past few years, attempts have been made to extract lipids from stabilized rice bran using SFE. Kim *et al.* (1999) extracted and separated rice bran oil rich in essential fatty acids (EFAs) using the SFE process. Zhao *et al.* (1987) conducted the fractional extraction of rice bran oil with SFE at pressures of 14.7–34.3 MPa, and at a fixed temperature of 40 °C. They found differences in oil yield (18.6–22.0%) extracted at different pressures. Qualitative differences indicated that the fractions obtained at high pressures contained less FFA and waxes or unsaponifiables in the oil. Grinding of bran was also found to be effective in reducing the required CO_2 and extraction time. Ramsay *et al.* (1991) compared different rice bran oil extraction processes including solvent extraction (hexane), SFE, and SFE with 5% ethanol co-solvent. The oil yield was 20.2% for solvent extraction, 18.0% for SFE extraction and 18.2% for SFE with modifier. For SFE and SFE co-solvent extractions, they used 35 °C for 5 h at a flow rate of 20.5 g/min in a 1 liter vessel at 30.0 MPa. They also compared concentrations of sterol components in the extracts: 9.4, 7.3 and 8.3 mg of sterol per gram of rice bran oil for hexane, SFE and SFE co-solvent extractions, respectively. Entrainers (ethanol and chloroform) and separation columns were used by Saito *et al.* (1993) for SFE of rice bran oil with CO_2 at 40–100 °C and at 8.2–19.8 MPa. A separation column (silica gel-supported nitric acid column) was effective in the fractionation of fatty acids whereas the ethanol entrainer increased extraction efficiency up to 1.6 times. There was not much difference in FFA composition with or without entrainers. For example, $C_{16:0}$, $C_{18:1}$, $C_{18:2}$ were 18.6, 42.5 and 35.1% of total FFA for SFE extraction, respectively whereas their concentrations in SFE with ethanol extraction were 18.2, 43.1 and 35.4%, respectively. Higher temperatures increased the fractionation of fatty acid esters.

10.3.2 Pretreatments to extraction

Intracellular oils can be difficult to extract from biomass due to the presence of intact cells. Cellular disruption greatly enhances the bioavailability of compounds and has been found to be necessary for recovering intracellular products from microalgae (Yongmanitchai and Ward, 1989;

Wen and Chen, 2003; Ward and Singh, 2005). A number of physical techniques have been studied and reviewed, including the use of ultrasonification, high-pressure homogenizers and agitation of biomass in the presence of glass beads (Goto *et al.*, 1993; Mendes-Pinto *et al.*, 2001; Martinez *et al.*, 2003). Most of these techniques are effective in improving product recovery.

The concept of broken and intact cells is introduced to possibly explain the falling extraction rate (FER) period (Barclay, 1997; Belarbi *et al.*, 2000; Kyle, 2001; Molina Grima *et al.*, 2003). This concept takes into account the structure of biological materials where the solute is found in various parts of the cell, especially within the cell walls where the resistance to mass transfer is the largest. In looking at the bulk material being subjected to SFE, a greater number of ruptured cells are seen at the surface since these are vulnerable to the mechanical preparations necessary for successful extractions of the material. The constant extraction rate (CER) period is influenced by these open cells and a fast extraction occurs. This initial high-rate extraction decreases (FER) and is followed by the much slower extraction of the oil diffusing from the intact cells diffusion controlled (DC) regime.

Cellular disruption greatly enhances the bioavailability of compounds and has been found to be necessary for recovering intracellular products from microalgae (Kyle, 1996; Robles Medina *et al.*, 1998). High-pressure homogenizers, agitation of biomass with glass beads and even ultrasonification have been studied (O'Brien *et al.*, 1993; Cheng *et al.*, 1999). Different cellular disruption techniques were assessed for acetone recovery of the carotenoid astaxanthin from cells of *Haematococcus pluvialis*, a microalga used in the aquaculture industry (Robles Medina *et al.*, 1998) (Fig. 10.4). Biomass that underwent more physical pretreatments, autoclaving and homogenizing, produced three times as much astaxanthin as the other chemical treatments. These physical alterations increased the total amount of carotenoids produced by up to four times compared with the intact cells. The chemical treatments with acid, alkali and enzymes only slightly increased carotenoid extraction from 4 mg/g dry weight of biomass to 6 and 8 mg/g dry biomass. Therefore, mechanical disruptions are effective treatments, resulting in a high yield with no detrimental effects observed during processing.

Steam explosion

Steam explosion is an extensively studied technique for the pretreatment of lignocellulosic materials, such as hard woods and agricultural residues, for ethanol production (Zheng *et al.*, 1998; Sun and Cheng, 2002). In this method, chipped biomass is exposed to high-pressure steam and the pressure is then swiftly reduced, which allows the water molecules that penetrated the substrate structure to escape in an explosive fashion. In this process the lignocellulosic structures are disrupted to increase the

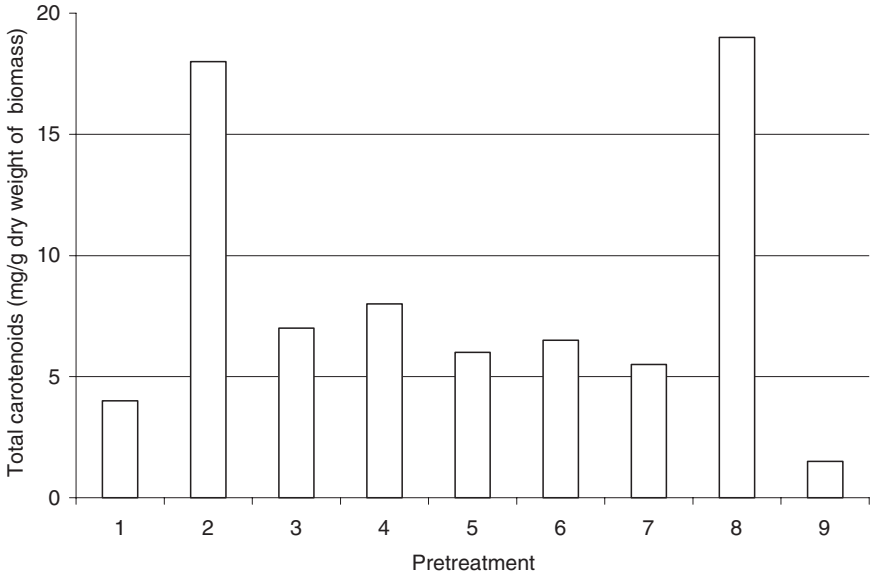


Fig. 10.4 Effects of cellular disruption techniques on the extraction of total carotenoids into acetone from *Haematococcus* biomass. Key: 1, control (intact cells); 2, autoclave; 3, hydrochloric acid, 15 min; 4, hydrochloric acid, 30 min; 5, sodium hydroxide, 15 min; 6, sodium hydroxide, 30 min; 7, enzyme; 8, high-pressure homogenizer; 9, spray drying (from Robles Medina *et al.*, 1998).

accessibility of cellulose to enzymes and cause hemicellulose degradation. Steam explosion has enhanced the cellulose hydrolysis rate and increased glucose yield from 40 to 80% (Gregg and Saddler, 1996). The effectiveness of steam explosion is influenced by the saturation time, temperature, substrate size and substrate moisture content, with a lower temperature and longer residence time being more favorable (Duff and Murray, 1996; Wright, 1998). The advantages of steam explosion include the low energy requirement and the fact that there are no recycling or environmental costs (Sun and Cheng, 2002). The disadvantages associated with steam explosion when applied to microorganisms would be the high temperatures necessary for implementation, between 160 and 260 °C. This temperature range would alter PUFAs and other thermally labile substances.

CO_2 explosion

One novel approach for enhancing CO_2 SFE of fungal lipids is through the application of a CO_2-explosion pretreatment. This process is analogous to the steam explosion and ammonia fiber explosion (AFEX) processes originally designed to improve the digestibility of lignocellulosic products such as wood and corn stover (Chisti and Mooyoung, 1986; Bermejo Roman *et al.*, 2002). CO_2 explosion involves saturation of a substrate with high-pressure CO_2 for a given time. This extended contact time allows the CO_2

molecules to penetrate the cellular structure. Then, the pressure is instantaneously released, causing the CO_2 to flash violently and break the cells apart, theoretically increasing the amount of surface oil for CO_2 SFE. Steam explosion requires high temperatures associated with the high pressure required for steam (Dale and Moreira, 1982; Dale *et al.*, 1984; Holtzapple *et al.*, 1991, 1992). AFEX has a lower operation temperature; however, the ammonia can have chemical effects on the substrate and ammonia recycling is necessary (Sun and Cheng, 2002). CO_2 explosion can operate at the same low-temperature, high-pressure conditions as SFE and use the same non-toxic, non-reactive solvent, making it more attractive than steam and ammonia solvents.

A CO_2-explosion process is similar to steam and ammonia explosion in that there is a saturation phase and a violent release of pressure resulting in a ruptured substrate structure. CO_2 explosion uses supercritical CO_2 as the saturating solvent. Even though a supercritical fluid possesses a liquid-like density over much of the range of interest, it exhibits gas-like transport properties of diffusivity and viscosity (McHugh and Krukonis, 1994; Brunner, 2005). Figure 10.2 shows the self-diffusivity behavior of CO_2 over a range of pressures and temperatures. A characteristic diffusivity value for an SCF at its critical temperature and pressure is $0.7 \times 10^{-3}\,cm^2/s$, and a four-fold increase in pressure would result in a decrease of diffusivity to approximately $0.2 \times 10^{-3}\,cm^2/s$ (Brunner, 1994, 2005). Thereby, an increase in density results in a decrease in diffusivity.

So far, CO_2-explosion experiments have been limited to lignocellulosic substrates for potential ethanol production. The effectiveness of this pretreatment was quantified via enzymatic hydrolysis of the cellulose to yield sugars. CO_2-explosion pretreatment on pure cellulose and industrially processed materials improved glucose yield by as much as 50% (Zheng *et al.*, 1995, 1998). With these documented improvements in an extremely structured substrate, there is the potential for microbial cell disruption with the CO_2-explosion process.

CO_2-explosion effects on pine and aspen and found glucose yield 12 to 14% beyond untreated materials (Kim and Hong, 2001). A major advantage of CO_2 explosion is the fact that this inexpensive solvent is already employed in the SFE process in food, pharmaceutical and nutraceutical industries. CO_2 explosion can operate at the same low-temperature, high-pressure conditions as SFE.

10.4 Modeling of solubility and mass transfer

Mathematical expression of the kinetics of supercritical extraction phenomena can be of great significance for further studies, as well as in understanding general behavior of extraction phenomena for given components.

Knowledge of phase equilibrium behavior and generation of phase equilibrium data – such as solubility, distribution coefficients and selectivity of separation of extractables – in supercritical fluids is of great importance for improved understanding of the process. All applications of SFE for food, flavor, fragrances and pharmaceuticals involve a basic understanding of high-pressure, fluid-phase and equilibrium behavior. Most SFE applications involve multi-component systems. The common approaches for modeling have been to treat the supercritical fluid phase as a dense gas and may be represented using equations of state to calculate fugacity coefficients, or to treat the supercritical fluid phase as an expanded liquid apart from other approaches involving semi-empirical correlations or molecular models based on computer simulations. Although supercritical fluid phase behavior indicates some interesting trends, the practical tasks of modeling and predicting such behavior for qualitative and quantitative understanding pose serious challenges due to the molecular complexities of solutes and uncertainties in specific interactions in dilute supercritical solutions at higher pressure and high compressibility of the supercritical fluid solvents. Agricultural and biological materials add to these challenges by their complex biological structures, which present many undefined variables for the technologist attempting to use supercritical fluids in their processing (Clifford, 1999; King, 2000; Mukhopadhyay, 2000).

10.4.1 Thermodynamic modeling

An equation of state (EOS) is widely used to represent the relationship of temperature, pressure and composition of compounds in supercritical fluids. Solubility may be predicted as a function of the temperature and pressure along with solute and solvent properties. Some of the most commonly used EOS models are the Peng–Robinson and Soave–Redlich–Kwong equations. Both produce similar results; however, the Peng–Robinson equation of state (PR-EOS) will be discussed here as it is more widely used. The PR-EOS equation is

$$P = \frac{RT}{v-b} = \frac{a(T)}{v(v+b)+b(v-b)} \qquad [10.1]$$

where v is the molar volume, a accounts for intermolecular interactions between species of the mixture and b accounts for size differences between the species of the mixture (Peng and Robinson, 1976; McHugh and Krukonis, 1994).

An exact thermodynamic relationship exists for compounds in all phases at equilibrium. This relationship states that the fugacity, which is directly related to the chemical potential of a component, in one phase is equal to the fugacity of the same species in any other phase at equilibrium. The fugacity can be thought of in terms of 'corrected pressure' of a pure com-

ponent or 'corrected partial pressure' of a component in a mixture. The equilibrium relationship is a function of system temperature, T, and pressure, P, for the component having the concentration, y, in the supercritical CO_2 phase and a concentration, c, in the condensed phase. The relation is

$$f_i^{\mathrm{c}}(T,P,c) = f_i^{\mathrm{f}}(T,P,y) \qquad [10.2]$$

where the fugacity follows the relation (Prausnitz *et al.*, 1999),

$$f_i^{\mathrm{c}} = p_i^{\mathrm{sat}}\Phi_i^{\mathrm{sat}} \exp\int_{p_i^{\mathrm{sat}}}^{P} \frac{v_i^{\mathrm{c}}\mathrm{d}P}{RT} \qquad [10.3]$$

where Φ_i^{sat} is the fugacity coefficient of component i at p_i^{sat}, v_i^{c} is the molar volume of solute in the condensed phase and R is the gas constant. Assuming the condensed phase is incompressible and the fugacity coefficient for the component at the saturation pressure is approximately unity (because the vapor pressures are typically low enough to assume an ideal gas), the solubility may be defined as

$$y_i = \frac{p_i^{\mathrm{sat}}}{P}\frac{1}{\Phi_i^{f}} \exp\left[\frac{v_i^{\mathrm{c}}(P - p_i^{\mathrm{sat}})}{RT}\right] \qquad [10.4]$$

Thermodynamic and phase equilibrium properties dictate the feasibility of the SFE process and conditions for maximum possible separations, whereas knowledge of transport properties of supercritical fluids and resistances to the transport processes are required for calculating time required for the extraction and the sizes of the critical components of the plant (Espinosa *et al.*, 2002; Mukhopadhyaya, 2000). Because of the rapid changes in the properties such as viscosity, μ, and diffusivity, D, etc., with small changes in the conditions of the supercritical solvent around the critical point, predictions of these properties are difficult and require sound theoretical considerations and understanding of the process.

10.4.2 Mass transfer modeling

The SFE process from natural materials generally involves the releasing of solutes from a porous biological matrix into the supercritical solvent via internal and external mass transfer mechanisms. The first part of the extraction, the CER period, is governed by the solubility equilibrium between the CO_2 solvent and extract. Once the easily accessible surface extracts are depleted, molecular DC mass transfer occurs and an FER period is seen. Eventually, diffusion of the extracts through the bulk material becomes a more integral part of the extraction process and the accumulation product over time approaches zero (Perakis *et al.*, 2005; Esquivel *et al.*, 1999; Reverchon *et al.*, 2002). Different mathematical aspects related to SFE have

resulted in multiple variables and complex equations being derived to model the SFE process. Many models have been proposed; yet, no single model has been universally accepted (Reis-Vasco *et al.*, 2000; Revererchon *et al.*, 2000; Sousa *et al.*, 2005; Sovova, 2005).

Molecular diffusion (Fick's law)
Fick's second law of diffusion defines the molecular diffusion flux of a component with respect to the concentration gradient for a binary mixture. It states that flux is proportional to the concentration gradient and that diffusion of a compound occurs in the direction of decreasing concentration (Mukhopadhyaya, 2000). Fick's second law for a spherical geometry is given as

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \qquad [10.5]$$

with initial condition $C = C_0$ at $t = 0$ and boundary condition $\mathrm{d}C/\mathrm{d}r = 0$ when $r = 0$ and $C = C_\infty$ when $r = R$ and $t = \infty$
where

C = the concentration of solute in the sphere at time t

C_∞ = the final concentration of solute at the surface

D = the diffusion coefficient

r = distance from the center of the sphere

R = the radius of the sphere

The analytical solution takes the form of an infinite series with 'n' terms, for the total amount of a species diffusing from the sphere (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp^{-(Dn^2\pi^2 t)/a^2} \qquad [10.6]$$

where M_t and M_∞ represent the total amount of solute leaving the sphere at time t and total amount of solute extracted over infinite time, respectively. D represents the effective diffusivity for porous media with void volume, and tortuosity factor (Walker, 1997).

The mass transfer rate for SFE for lipids from natural materials involving high initial concentration of extract (such as in oil seeds) in a fixed bed typically remains constant and then declines. SFE involves the control of solubility by manipulating temperature and pressure. Natural materials contain multiple components whose solubility and extractabilities are difficult to predict (Lira, 1996). Mathematical aspects related to SFE of lipids were discussed by King and List (1996); topics covered included solubility (Maxwell, 1996), phase equilibria (King and List, 1996), mass transfer

(Eagger, 1996), fractionation (Peter, 1996) and modeling of SFE of lipids (Goodrum *et al.*, 1996).

Reverchon model

Reverchon (1996) modeled supercritical extraction of sage oil from leaves at 9 MPa and 50 °C, for four different particle sizes. A model was proposed based on a mass balance along the extraction bed. Diffusivity of solute was the only adjustable parameter in the model. In the model a mass balance over an element of the extractor of height 'dh' was written as:

$$uV\frac{\partial C}{\partial h}+\varepsilon V\frac{\partial C}{\partial t}+(1-\varepsilon)V\frac{\partial \bar{C}}{\partial t}=0 \qquad [10.7]$$

$$\frac{\partial \bar{C}}{\partial t}=-\frac{1}{t_i}(\bar{C}-\bar{C}^*) \qquad [10.8]$$

where

$$t_i=\mu\frac{l^2}{D_i}$$

with initial conditions $t=0$, $C=0$ and $\bar{C}=\bar{C}_0$ and boundary conditions $h=0$, $C(0,t)=0$ where ε is the bed porosity, V is the extractor volume, C is the extract concentration in fluid phase, $\bar{C}$ is the extract concentration in solid phase, $\bar{C}^*$ is the concentration at the solid–fluid interface, u is the superficial solvent velocity, μ is the coefficient dependent on particle geometry, D_i is diffusion coefficient, h is bed height and $l=V_p/A_p$ (particle volume/particle surface) is a characteristic dimension. Testing of the model with experimental results suggested internal mass transfer to be the controlling step for the extraction process. The particle's shape was important for modeling experimental data, with a spherical shape giving a good fit.

Reverchon *et al.* (1999) modeled the fractional extraction of fennel seed oil and essential oil in two stages. In the first step, conducted at 9 MPa and 50 °C, essential oils were selectively extracted and then, at 20 MPa and 40 °C, the remaining vegetable oil was extracted. The flow rates tested were 8.33, 16.67 and 25 g/min. The model described vegetable oil extraction and was based on differential mass balances around the concept of broken and intact cells, with the internal mass transfer coefficient as an adjustable parameter. Essential oil extraction was modeled as desorption from vegetable matter with a low resistance to mass transfer, having the same internal mass transfer coefficient value as that of seed oil extraction. Both models represented good fits to experimental data.

An extraction model for pennyroyal essential oil by Vasco *et al.* (2000), with extraction at 10 MPa and 50 °C for different particle sizes (0.3, 05 and 0.7 mm) and different CO_2 flow rates (18.6, 25.8 and 37.2 g/min), utilized

axial dispersion effects based on the desorption of oil near the leaf surface and mass transfer resistance in the internal part of the vegetable structure. They divided the extraction process into two parts for the purpose of modeling; the first part of the extraction described adsorption equilibrium with superimposed axial dispersion, whereas in the second part of the extraction process internal mass transfer was assumed to be the controlling factor. Yield curves for all particle sizes and flow rates of CO_2 fitted fairly well with an internal mass transfer coefficient K_i as an adjustable parameter. Akgun *et al.* (2000) described the extraction and modeling of lavender flower essential oil with supercritical CO_2 in a semi-continuous system at 8–14 MPa pressure, 35–50 °C temperature and 1.092–2.184 g/min flow rate ranges of CO_2. They used a quasi-steady-state model as a function of extraction time, flow rate, pressure and temperature, with inter-particle diffusion coefficient as an adjustable parameter. The model was satisfactorily correlated with experimental data with best fitted value of effective diffusivity (1.2×10^{-11} m^2/s).

Reverchon *et al.* (2000) conducted experiments for SFE extraction of hiprose seed oil at different pressures (10.34, 20.68, 41.37 and 68.94 MPa), temperatures (40, 50 and 70 °C) and flow rates (1, 2, 4 and 6 g CO_2/min) with different particle sizes of seeds (0.42, 0.79 and 1.03 mm); experiments were validated with a mathematical model based on the structure of hiprose seed particles. For modeling purposes they assumed oil to be a single pseudo-component, the extraction bed was assumed to be continuous, and the pressure and temperature gradients along the column were neglected. The volume of the solid was assumed constant and the solute concentration in the fluid phase was assumed to be dependent only on time t and axial coordinates. Axial dispersion was neglected. Their model was given as

$$\frac{\partial C}{\partial t} + \frac{u}{\varepsilon}\frac{\partial C}{\partial z} + \frac{1-\varepsilon}{\varepsilon}\frac{\rho_s}{\rho_f}\frac{\partial q}{\partial t} = 0 \qquad [10.9]$$

$$\frac{\partial q}{\partial t} = K_i(Y)a(q - q^*) \qquad [10.10]$$

where $q^* = K_{eq}C$, $C = C_0$ at time $t = 0$ for each z, $q = q_0$ at time $t = 0$ for each z, $C = 0$ at $z = 0$ for each t. Here u is superficial velocity of supercritical CO_2, ε is void fraction of extractor, ρ_s is density of hiprose seed, ρ_f is density of supercritical CO_2, Y is the yield of oil seed, a is the specific surface of the vegetable matter, K_{eq} is the linear equilibrium constant, C and q are the concentrations expressed as a mass ratio of oil in fluid phase and solid phase, respectively, and q^* is the concentration of oil in solid phase at the solid–fluid interface. z is the axial coordinate and K_i is the internal mass transfer coefficient. The model assumed internal mass transfer coefficient as linearly variable and fitted well to the experimental data.

Goto model

Goto *et al.* (1993) extracted peppermint oil with supercritical CO_2 at varying conditions (313–353 °K, 8.83–19.6 MPa) and studied extraction curves and extraction rates of major components (L-menthol and menthone). A mathematical model was also developed based on local adsorption equilibrium of essential oil lipid in leaves as well as mass transfer (Goto *et al.*, 1993, 1998). Their model was based on the following assumptions: (1) leaves are porous solids with essential oil and lipid; (2) essential oils are extracted from leaves as if desorbed from solid biological tissue where lipids are associated with essential oils; (3) essential oils dissolved in supercritical fluid diffuse to the external surface and through the external film to be carried away by bulk flow. The adsorption equilibrium constant, determined by fitting the theoretical extraction curve to experimental data, increased with temperature and decreased with pressure.

Canela *et al.* (2002) applied the Goto model to supercritical fluid extraction of fatty acids and carotenoids from microalgae (*Spirulina maxima*) to describe the extraction process. In their study to determine the kinetic parameters, extraction experiments were conducted at varying pressures (15, 16.5 and 18 MPa) and temperatures (20, 25 and 30 °C), and the yield and composition were determined at a constant solvent flow rate (3.33×10^{-5} kg/s). They applied the Goto *et al.* (1993) model assuming the substrate to be a porous matrix with diffusion occurring through the inside of the particle pores and with mass transfer resistance being offered by the film around the particle.

The Goto model (Goto *et al.*, 1993) treats the solid particle as a solid substrate with a porous matrix. The solute diffuses within the pores and through a film surrounding the particles, then is extracted into the supercritical fluid and carried away by the bulk flow. At equilibrium, the total initial solute concentration existing in both the solid phase and at the surface is given by a linear relationship

$$C_0 = \left[\frac{\varepsilon_P}{K} + (1-\varepsilon_P)\right] x_0 \rho_S \qquad [10.11]$$

where C_0 is the total solute concentration in the particle (kg/m^3), the particle porosity is represented by ε_P, K is the partition coefficient of the solute in the solvent, x_0 is the initial solute mass ratio in the particle (kg solute/kg particle), and the real solid density ρ_S is measured as kg particle/m^3 particle volume.

The solute mass balance equations within the interstitial space and the porous particle can be written in terms of dimensionless variables. The use of dimensionless variables requires the introduction of dimensionless time, θ, which is the real time t divided by the CO_2 residence time, τ (in seconds). The parameter τ is the total bed volume divided by the volumetric flow rate of supercritical fluid at the extractor vessel conditions:

$$\frac{dx}{d\theta} + \frac{x}{\varepsilon} = -\frac{\phi(1-\varepsilon)}{\varepsilon}\left(x - \frac{x_s}{K}\right) \quad [10.12]$$

$$\frac{dx_s}{d\theta} = \frac{\phi\left(x - \frac{x_s}{K}\right)}{\varepsilon_P / K + (1-\varepsilon_P)} \quad [10.13]$$

where: x is equal to C/C_0 (dimensionless solute concentration in the fluid phase); dimensionless time, θ, is found as t/τ; ε is the void fraction of the bed; the dimensionless mass transfer coefficient, ϕ, is equal to $k_p a_p \tau$ with $k_p a_p$ being the combined mass transfer coefficient; lastly, x_s (C_s/C_0) is the dimensionless solute concentration in the solid. The initial conditions are:

$$x(\theta = 0) = 0 \quad [10.14]$$

$$x_s(\theta = 0) = \frac{K}{\varepsilon_P + (1-\varepsilon_P)K} \quad [10.15]$$

This model has been successful in describing the CO_2 extraction of fatty acids and carotenoids from the microalgae *Spirulina maxima* (Canela *et al.*, 2002) and volatile oils from various plant materials: *Croton zehntneri* Pax et Hoff (Sousa *et al.*, 2005), rosemary (*Rosmarimus officinalis*) (Carvalho *et al.*, 2006) and fennel (*Foeniculum vulgare*) (Moura *et al.*, 2005). This model was applied to the extraction of lipids from *P. irregulare* because of the similarity of experimental materials, extraction procedures and experimental variables (Cantrell, 2006).

Logistic model

The logistic model (LM) may be applied to the solute transfer to the fluid phase while neglecting accumulation and dispersion of the solute in the fluid phase since these phenomena have no significant influence on the process when compared with the effect of convection (Martinez *et al.*, 2003; Campos *et al.*, 2005). This model observes that when the extraction time approaches infinity, the mass of the extracted material tends to a fixed value asymptotically. This value can be considered the total extractable mass at the given process conditions. The OEC can be represented by the following equation with only two adjustable parameters (b, t_m)

$$m_{ext}(h = H, t) = \frac{m_t}{\exp(bt_m)}\left\{\frac{1 + \exp(bt_m)}{1 + \exp(b(t_m - t))} - 1\right\} \quad [10.16]$$

with m_{ext} as the mass of the extract (kg), bed length, H (m), extraction time, t (s), m_t as the total initial mass of extract in the solid (kg), and the adjustable parameters b (s^{-1}) and t_m (s).

The physical meaning of the adjusted parameter t_m corresponds to the instant in which the extraction rate of the solute reaches its maximum. The parameter b is more than likely a function of mass flow rate and density of

the supercritical fluid (Martinez *et al.*, 2003). This model can be applied to OECs that consider the solute as a single pseudo-compound or group of compounds. The fungal oil in this study is considered to be a single pseudo-compound despite the variety of glycerides and fatty acids the oil contains.

The first part of the extraction process is limited by the solubility equilibrium between the oil and CO_2 solvent. This CER period is linear with the partition coefficient usually being a part of the constant of proportionality. Once the easily accessible surface oil diffuses into the flowing supercritical CO_2, it is necessary for the lipids to diffuse through the biomass and appear on the substrate surface. The oil extraction rate starts to decrease since diffusion is slower than the convective mass transfer and a FER period is seen. Once diffusion completely controls the mass transfer process, the extraction is said to be in a DC regime (Reverchon *et al.*, 2000; Ferreira *et al.*, 2002; Sovova, 2005).

OECs during the SFE process are plots of the cumulated extract yield versus the extraction time. Figure 10.5 shows an example OEC fitted with the Goto and logistic models for extraction of fungal oil. The first part of the OEC is governed by the solubility equilibrium between the solute and fluid phase. Once the easily accessible solute is depleted, then DC mass transfer occurs (Reverchon *et al.*, 2000; Ferreira *et al.*, 2002; Sovova, 2005). Mathematical models have been proposed to correlate OECs during the SFE process (Goto *et al.*, 1993; Reis-Vasco *et al.*, 2000; Reverchon *et al.*, 2000; Martinez *et al.*, 2003; Sovova, 2005).

Other models

Marrone *et al.* (1998) modeled supercritical extraction of almond oil from crushed almond seeds of three different sizes at 35 MPa and 40 °C. The following assumptions were made: the oil was considered a single pseudo-component; the solute concentration was dependent only on time and the axial coordinates; uniform temperature, pressure, flow conditions existed along the extraction vessel; negligible axial dispersion and constant solid mass existed in the vessel during the extraction process.

Their model was based on physical evidence of broken and intact oil cells and considered two different phases of the extraction process. The initial phase contained freely available oil and was contained within the broken cavities on the surface of the crushed particles and an oil phase was contained inside the particles or internal surfaces. A good fit was observed for experimental data with an internal mass transfer coefficient of 7.5×10^{-9} m/s.

Sovova (1994) and Sovova *et al.* (1994b) modeled grape oil extraction at 28 MPa and 40 °C with grape seed of different particle size, flow rates and flow directions. Plug flow was observed for downward flow of compressed

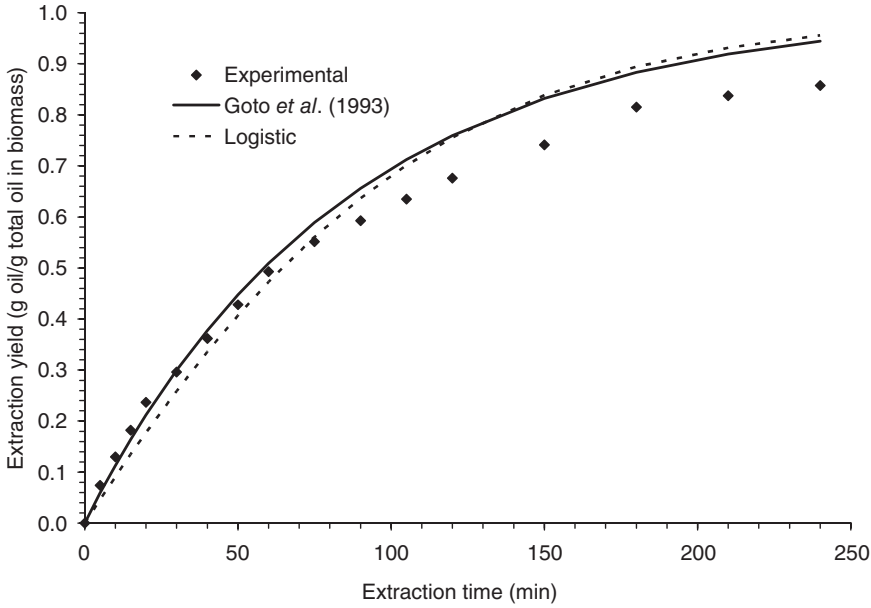


Fig. 10.5 Experimental and modeled OEC for SFE of *Pythium irregulare* oil at 20.7 MPa, 40 °C and a CO_2 flow rate of 3.94×10^{-6} kg/s. Total oil in biomass is from SFE and Hexane: isopropanol (3:2) extractions (0.168 g) (Cantrell, 2006).

gas, whereas extraction was retarded by natural convection in the case of up-flow. The up-flow model with parallel plug flow more closely represented the extraction process. Roy *et al.* (1996) modeled oil extraction from freeze-dried ginger root as a function of flow rate of CO_2, pressure, temperature and particle size. The extraction process was controlled by intra-particle diffusion within the root. The rate of extraction increased with small particle size due to a decrease in the diffusion path. A crossover effect was observed with temperature and pressure. High temperatures increased extraction rates at 24.5 MPa, but low temperatures increased extraction at 10.8 MPa. When applied to experimental results, a shrinking-core model with effective diffusivity and solubility as fitting parameters fitted the data for large particle sizes. Goodarznia and Eikani (1998) developed a two-phase model composed of solid and supercritical phases, which when tested for essential oil extraction showed a dependence on particle size and shape.

Most of the mathematical models presented in the literature describe the OECs based on the mass balance for the extraction bed and the trans-

port phenomena that occur inside it. The extraction bed can be divided into two phases: (1) a solid phase that contains the solute and (2) a fluid phase, composed of the supercritical solvent with the extract dissolved in it. Many of these models assume: constant temperature and pressure; solute-free entering solvent; a linear equilibrium relation between solid and fluid phases – and the intra-particle transport is described by a diffusional process (Goto *et al.*, 1993; Martinez *et al.*, 2003; Campos *et al.*, 2005; Sovova, 2005).

The concept of broken and intact cells has been introduced to account for the sudden change between the two phases of extraction: the linear constant equilibrium phase and the DC extraction phase. This concept takes into account the easily accessible solute from broken cells and the more difficult to extract part of the intact cells located at the particle core. Many studies have successfully applied these types of models to the extraction of essential oils from seeds and plant material (Goto *et al.*, 1993; Reverchon *et al.*, 2000; Ferreira *et al.*, 2002; Martinez *et al.*, 2003; Campos *et al.*, 2005; Sovova, 2005).

10.5 Other technologies

Traditional extraction technologies including Soxhlet solvent extraction, steam distillation and hydro-distillation have been employed successfully for many applications in the food and pharmaceutical industry. These technologies have drawbacks in that they use toxic, flammable solvents that are in many cases being banned from food use. The techniques typically are time and energy consuming and often require further separation or clean-up. Many other innovative extraction technologies now exist in addition to novel SFE techniques. Some of the notable achievements in extraction technologies include microwave-assisted solvent extraction (MASE) (Priego-Capote *et al.*, 2004; Assad *et al.*, 2004; Hogendoorn *et al.*, 2002; Raman and Gaikar, 2002; Luque and Luque, 2004), ultrasound-assisted extraction (Ruiz-Jimenez and Luque de Castro, 2004), pressurized liquid extraction (PLE) (Boselli *et al.*, 2001), accelerated solvent extraction (ASE) (Richter *et al.*, 1995), microwave-assisted hydro-distillation (MAHD) (Stashenko *et al.*, 2004) and solid-phase microextraction (SPME). SPME is an attractive technique that is solvent-free, and extraction and concentration are combined in a single step (Arthur and Pawliszyn, 1990).

Extraction of specialty biochemical components from biological systems presents a vast array of possible extraction systems; users should consider the appropriate variables necessary for optimization. The first step to narrow down the best possible extraction system is to consider the thermodynamic properties of temperature, pressure, volume and composition relations to determine the solubility of the desired compound in the solvent or mixture of solvents based on polarity, molecular weight and

volatility of the solvents and solutes. The solubility may be predicted through engineering analysis using classic EOS or statistical mechanics approaches combined with fugacity relationships (Prausnitz *et al.*, 1999). From the equilibrium analysis set as the reference point, operational curves may be constructed (Treybal, 1981). The next step is to characterize the system in terms of fluidity: whether the system will be gas–liquid, liquid–liquid, solid–liquid or solid–fluid; and whether porosity and diffusivity of the solvent into the extraction matrix becomes a rate-limiting factor. With these primary extraction variables defined for the particular system, the final important factors of rapidity, energy savings, cleanliness (purity), environmental impact and ultimately product quality must be considered in the design of the extraction system. As the saying goes, 'time is money', and both short-term and long-term analysis based on the previous design steps to determine both operational and capital cost, with greater emphasis on environmental impact, will ultimately determine which extraction system would yield the greatest return on investment. Table 10.3 shows some general ranges of important system properties such as operating temperature (for labile components) and component volatility on the specific extraction mechanism for component separation. Cleanliness is the extraction purity of the components after separation relative to conventional solvent extraction techniques; this is important in the consideration of further purification steps.

10.5.1 Microwave-assisted solvent extraction (MASE)

MASE has several distinct advantages over most extraction technologies, primarily in reduced extraction times and reduced solvent use. The microwave process disrupts hydrogen bonds from microwave-induced dipole rotation of the molecules and movement of ions which tends to enhance solvent penetration (Hubaib *et al.*, 2003). Hubaib *et al.* (2003) showed that MASE of lipophilic compounds from Echinacea with methanol–water combinations, when compared with conventional Soxhlet extraction and ultrasonic extraction techniques, resulted in better recovery and reproducibility. MASE has been successfully coupled to steam distillation (MASD) for extraction of essential oils from lavender and resulted in a nearly ten-fold decrease in extraction time with higher recovery, lower energy requirement (ten-fold less), lower solvent use and lower CO_2 emissions released during distillation (Chemat *et al.*, 2005). Disadvantages include the high temperatures associated with MASE that affect thermally labile components and also the required presence of water. However, if extraction rates may be accelerated for lipophilic compounds in high-moisture systems (most biological systems), this could work as an advantage to prevent the high energy cost of removal of water before lipid extraction.

Table 10.3 Potential ranges of the physical properties of extraction systems

	Extraction method						
Property	MASE/MAHD	SFE-water	SFE-CO_2/Ethanol	SFE-CO_2	SPME	ASE	HD
Temperature	M–H	M	L–M	L–M	L–H	M–H	M–H
Pressure	L	H	H	H	L–H	M	L
Polarity	L–H	M	L–M	L	L–H	L–H	L–H
Molecular weight	L–M	L–M	L–M	L–M	L–H	L–H	L–H
Volatility	L–H	L–M	L–M	L–M	L–H	L–H	L–H
Fluidity	G Li S	G Li S	G Li S	G Li S	G Li	G Li S	G Li S
Rapidity	M–H	M	M	M–H	M	M–H	L–M
Cleanliness	M–H	M	M	M–H	H	M	L
Solvent use	L	L–M	L–M	L–M	L–M	M	H
Environmental impact	Good	Excellent	Excellent*	Excellent*	Good	Good	Poor
Energy	L–M	M	M	M	L–M	M	M–H
Capital cost	M–H	H	H	H	M	M–H	M

Abbreviations: G, gas; Li, liquid; S, solid; L, low; M, mid-range; H, high. * Efficient recycling of CO_2 is necessary.

10.5.2 Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) coupled to gas chromatography has also become a powerful solvent-free technique for analysis of essential oils (Field *et al*., 1996; Lagalante and Montgomery, 2003; Deng *et al*., 2006). Deng *et al*. (2006) introduced a combined technology of microwave distillation and SPME for rapid analysis of essential volatile oils in *Artemisia selengensis* Turcz.

10.6 Future trends

With the advent of synthetic biology on the horizon, many advanced technological tools for enabling new techniques for synthesis will determine the success rate of bringing novel bioproducts to the market, including manufacturing biomaterials, bioenergy and biopharmaceuticals from synthesized biomimetic components (Sarikaya *et al.*, 2003). The basic tools of genetics integrated with the new tools of tunable solvents such as supercritical fluids, superheated fluid extraction (Morales-Munoz *et al*., 2005) and a pH-tunable medium (Yao and Chmielewski, 1999) and physical medium such as biologically controlled microfluidic and microarray devices where synthetic biological reactions may rapidly occur in a controllable order. Bridging these novel technologies, through collaborations of biological engineers and scientists, will enable the next step in the evolution of synthetic biology to tackle the challenge of using these smaller biological and physical tools to create larger integrated biosystems with increasing functionality. Tunable solvents will play a significant role in meeting the challenges of controllable tools to function in biosynthesis *in vitro* as well as *in vivo*, as in the case of dispersing targeted biocompounds into specific tissue layers of biological systems (Turk *et al*., 2002).

With increasing interest in biofuels as a replacement for non-renewable sources of oil, coal, uranium, etc., supercritical extraction and synthesis techniques will emerge in the near future – as in supercritical methanol or ethanol synthesis of methyl or ethyl esters in biodiesel (Madras *et al*., 2004; Cao *et al*., 2005) and intermediates for surfactant manufacture. Removal of heavy metals from water streams and contaminated soils has been of major interest in environmental clean-up technologies where a wealth of information on the potential supercritical solvent technologies with the aid of fluorinated solvents, including supercritical water as well as CO_2, has been reported (Walker *et al*., 1999). The concept of pH switching with supercritical CO_2 was described by Hanrahan *et al*. (2003). As with all emerging technologies, their future depends on unlimited imagination and creativity at the interface of the science, art and engineering from which they emerge.

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11

Membrane and filtration technologies and the separation and recovery of food processing waste

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11.1 Introduction

In 1990, the prognosis for the annual marked growth of membrane technology was 12–15%. A particularly high growth potential was predicted for newly developed membrane processes such as gas separation and pervaporation.[1] However, these high expectations were fulfilled only by seawater desalination using reverse osmosis. This application field has still the highest annual growth together with hemodialysis, at 12%. All other membrane processes have shown less than 10% growth, far below all expectations. The annual turnover for membranes and membrane modules for the food industry is shown in Table 11.1.

The reasons for this unexpected development are diverse: (1) the rate of development of membrane materials has not been as rapid as expected; (2) the established membranes do not fulfill the needs of industry; (3) the membranes in use are generally sensitive to organic solvents and to traces of chlorine; (4) membrane fouling is still an unsolved problem that leads to a decrease in membrane efficiency and durability. In fact, due to the loss of yield caused by microbial growth and related fouling issues, membrane processes are usually unprofitable for low-value products. Regarding high-value products the selectivity of current membrane material is not sufficient to fulfill the high demands concerning product quality and cleanliness. For these reasons, membrane technology has been successfully implemented in relatively few application areas on a large scale. These areas are mainly:

- reverse osmosis for seawater and brackish water desalination;
- microfiltration for sterile filtration;
- dialysis and ultrafiltration for hemodialysis and hemofiltration.

Table 11.1 Annual turnover of membrane and membrane modules, after Strathmann[1,2]

Application	Turnover 1990 (Million US$)	Turnover 1996 (Million US$)	Turnover 2000 (Million US$)	Predominant applied membrane process
Haemodialysis, haemofiltration	1020	1400	2250	Dialysis, ultrafiltration
Water desalination	90	250	430	Reverse osmosis
Reprocessing of industrial process water	75	250	400	Micro-, ultrafiltration
Biotechnology	20	150	250	Micro-, ultrafiltration
Food and beverage industry	169	150	250	Micro-, ultrafiltration
Purification of surface water	n/a	100	160	Micro-, ultrafiltration
Chemical industry	115	160	300	Gas separation, electro-dialysis
Diagnostic, analytical applications	n/a	90	130	Micro-, ultrafiltration
Other applications	n/a	230	380	

In spite of these limitations, membrane users predict a further growth potential for this industry. Due to the development of new membranes and especially new membrane processes such as perstraction and vapor permeation the membrane market is set to expand. Additionally the inclusion of membrane processes in hybrid processes is expected to stimulate further growth.[3,4] It is considered that membrane processes should not replace established technologies, but augment them.

Current research in membrane technology is therefore focused on the following areas:[5,6]

- new application fields such as electro-chemistry and biotechnology;
- development of new membrane techniques such as perstraction;
- development and characterization of membrane materials;
- the combination of a number of processes to create hybrid processes;
- modeling and simulation of membrane processes.

In order to address these points, this chapter will describe established and sophisticated membrane processes and their applications. Established processes include ultra- and microfiltration for the purification of proteins, and sterile filtration as well as reverse osmosis for seawater desalination. New processes to be described include organophilic pervaporation and perstraction.

11.2 Established membrane technologies

11.2.1 Micro- and ultrafiltration

Description of the process

Micro- and ultrafiltration (MF and UF, respectively) are some of the most important processes in food technology and biotechnology due to their easy handling and universal application. MF and UF are simple membrane filtration processes, i.e. the separation principle is a form of sieving.[7] The only difference between membrane filtration and sieving is the cut-off point, which is determined by the pore size of the membrane. Using MF it is possible to separate particles with diameters between 200 nm and 20 µm. In contrast, UF can separate macromolecules with diameters between 5 and 200 nm. Hence, there is a functional overlap between the two processes.

A range of different polymeric materials such as polyethylene, polypropylene, polycarbonate, and cellulose acetate are used for MF and UF. In the food and biotechnology sectors, very stable (mechanically, chemically, and thermo-stable) membranes made of polysulfon or polyether sulfon (Fig. 11.1) are preferred since they can be easily cleaned and disinfected regularly to prevent fouling.

When choosing a membrane for processing a solution by MF/UF it is also important to consider the additional parameters of selectivity, Ψ, and molecular cut-off point. Notation for this chapter can be found in Section 11.5.

Selectivity

The selectivity, Ψ, describes the separation efficiency. This relates to the ability of a membrane to hold back a specific component under defined conditions and is defined as:

$$\Psi = \left(1 - \frac{c_{iP}}{c_{iF}}\right) \times 100\%$$

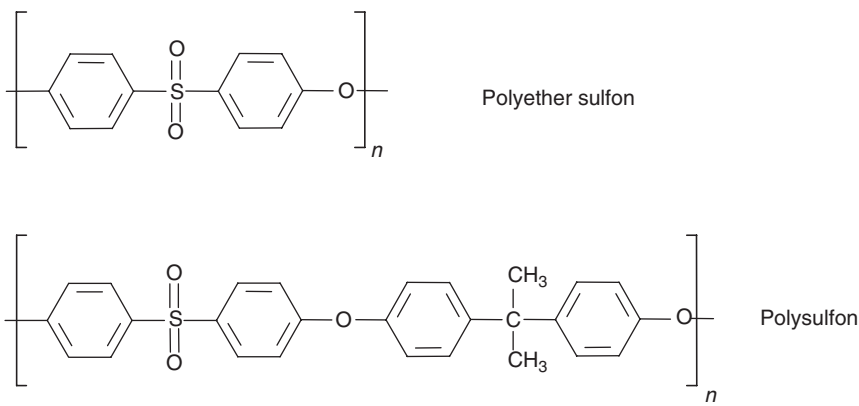

Fig. 11.1 Chemical structure of membrane materials.

where Ψ is selectivity, c_{iP} is the concentration of i in the permeate solution and c_{iF} is the concentration of i in the feed solution. The selectivity Ψ can be determined in the same way as the retentate, R, of the reverse osmosis (see below).

Molecular cut-off

This is defined as the molecular mass at which a dissolved globular protein (or another defined macromolecule) is nearly completely held back by the membrane at a given transmembrane pressure difference. Usual cut-off points are between 1000 and 100 000 Da for UF and between 10 000 and 300 000 Da for MF. When determining the molecular cut-off point it is important to avoid the formation of sediment layers which would influence the cut-off value.

The molecular cut-off point cannot be regarded as the absolute measure for the separation properties of a membrane, because these properties are also influenced by the molecular structure and the interaction between the membrane and the component that is held back (Fig. 11.2). Nevertheless, the cut-off point is still helpful when choosing the right membrane for a concrete separation problem.

As mentioned above, the separation efficiency of a UF/MF membrane is reduced by sediment layers and fouling. The formation of a sediment layer involves a concentration polarization (Fig. 11.3). An insufficient rediffusion of the component that is held back in the feed solution results in a higher concentration adjacent to the membrane. In the steady state, the convective transport towards the membrane is just compensated by the rediffusion in the laminar boundary layer. However, if the solubility limit of one component is reached, precipitation takes place, which results in the formation of a covering layer.

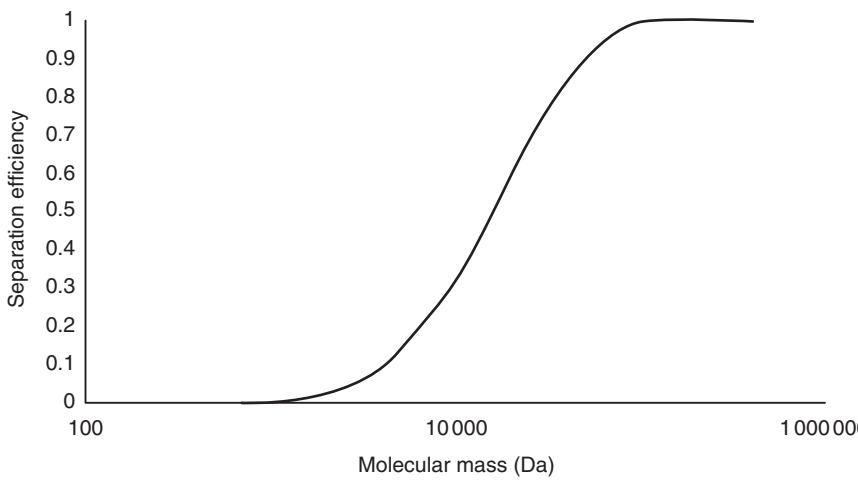

Fig. 11.2 Separation curve for a UF membrane.

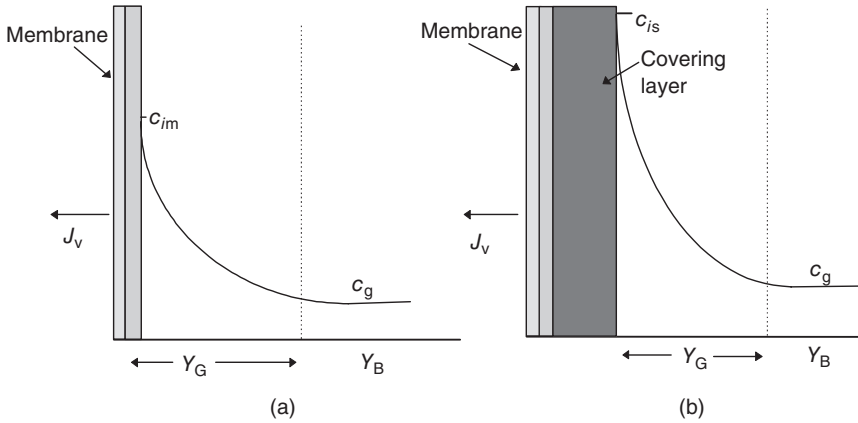

Fig. 11.3 Concentration polarization, schematic of membrane without a covering layer (a) and with a covering layer (b). Y_G, thickness of the laminar boundary layer; Y_B, solution (turbulently mixed); c_g, concentration of dissolved component in the solution; c_{im}, concentration of substance i at the membrane surface; c_{is}, saturation concentration of substance i; J_v, permeate flow rate.

To decrease the thickness of the covering layer, the rediffusion of the molecules from the membrane into the solution has to be ensured through better mixing. This is usually achieved through the control of the direction and velocity of the flow. A cross-flow is the most widely used process mode. This is depicted schematically in Fig. 11.4. Here, the suspension or solution constantly flows over the membrane. There are two main flows cross-wise to each other: the filtrate flow (J_F through the filter material) and the overflow (parallel to the filter medium). Hence cross-flow is the most commonly used process mode (see Fig. 11.4).

Membrane fouling is mainly caused by adsorption layers of bound proteins. Such protein adsorption depends on the local protein concentration. It will therefore exist, to some degree, at the beginning of the concentrating process of any dissolved protein(s) (for example in the process of whey concentrating) and is then strengthened by concentration polarization. The protein layer is a good food source for microorganisms which can reinforce the fouling and even result in the destruction of membrane material. Only by cleaning the whole plant with anti-fouling reagents such as acids, bases, or inorganic salts (K_2CO_3, Na_2CO_3) can such fouling be reduced.

Possible applications of MF/UF

Table 11.2 provides an overview of the types of applications to which MF and UF have been applied. Wastewater treatment is a very interesting field for MF and UF, as current research shows. In the food industry many aqueous effluents occur and need to be reprocessed. For example, Drouiche

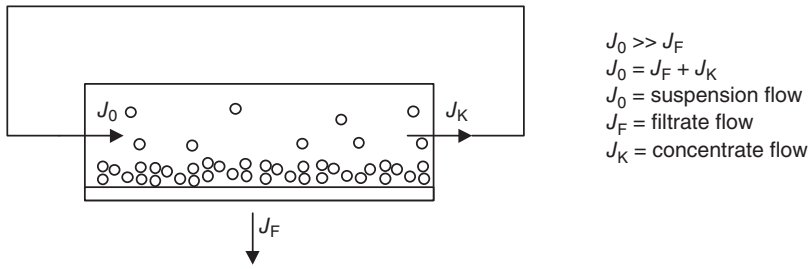

Fig. 11.4 Principle of cross-flow filtration.

Table 11.2 Application fields of microfiltration (MF) and ultrafiltration (UF)

Pharmaceutical industry/biotechnology
Sterile filtration: diagnostics, antibiotics, blood products, culture mediums, solvents, acids
Food industry
Sterile filtration: wine, beer, whisky, cooking oil, vinegar, amino acids, sugar solutions
Clarifying whey, degreasing and sterilizing milk
Clarifying wine, beer, fruit juice
Chemical industry
Sterile filtration: solvents, reagents, inorganic solutions, fatty acids, waxes, polymers
Treatment of process water and other waste water
Recovery of catalysts and solvents
Filtration of strong acids, process chemicals
Separation of heavy metals as hydroxides, and lignin

et al.[8] developed a process for the treatment of olive mill waste water by combining UF and advanced oxidation processes. A process combining UF and clay support is used to purify water effluents from a milk factory.[9] The recovery of proteins from effluents is a particularly interesting field of application. Lo *et al.*,[10] for example, developed a process for the recovery of proteins from poultry processing. Membrane filtration of Mozzarella whey can be used to recover high-value proteins, this process is described in Rektor and Vatai.[11] Even valuable enzymes can be recovered by UF and MF. Daufin *et al.*[13] give a good overview of current developments in the use of membrane technology in the food industry.

11.2.2 Reverse osmosis

Description of the process

Principle of reverse osmosis

The phenomenon of osmosis occurs when a semi-permeable membrane separates a solution from its solvent or a similar solution with a lower concentration. The difference in concentration results in a difference in the osmotic pressures which leads to a volume flux towards the solution with the higher concentration. The phenomenon of osmosis is described in detail in basic literature for physical chemistry (e.g. Atkins[14]).

However, the direction of the volume flow can be reversed. This can be achieved by applying hydrostatic pressure to the solution. This pressure has to be higher than the osmotic pressure difference across the membrane. The result is the active concentration of the solution. The process described is also known as reverse osmosis.

If, in the case of systems with more components, the couplings can be excluded, the driving force for the permeated component of a mixture i is exclusively the chemical potential difference, $\Delta\mu_i$, on both sides of the membrane. The chemical potential is a parameter that accumulates all effects that may influence the behavior of a system, in our case it includes the following parameters: pressure, temperature, and the concentration of the solution of one side of the membrane. This chemical potential is defined as an infinitesimally small change in the free molar (Gibbs) enthalpy, G, at an infinitesimal change of the concentration x_i of those components in the case of an isobar–isothermal process.

$$\mu_i = \left(\frac{\partial \bar{G}}{\partial x_i} \right)_{p,T,x_i \neq x_k}$$

where μ_i is the chemical potential of i, G is Gibbs enthalpy, p is hydrostatic pressure, T is temperature and x_i is the mole fraction of i. Therewith, it follows that the work, W, needed to change the concentration x of a system beginning with a concentration 1 and ending with a concentration 2 is:

$$W_{1,2} = \int_1^2 \mu_i(T, p, x_i) dx_i$$

where $W_{1,2}$ is the work needed to change the concentration x from x_1 to x_2.

For the thermodynamic principles behind these equations please refer to Atkins.[14] The chemical potential of the components in liquids is:

$$\mu_i(T, p, x_i) = \mu_i^0(T, p^0) + \Re T \ln a_i(T, p^0, x_i) = \tilde{V}_i(p - p^0)$$

where $\Re$ is the universal gas coefficient, a_i is the activity of component i, p^0 is standard pressure and $\tilde{V}$ is the molar volume of i.

The chemical potential of the components in an ideal gas mixture is:

$$\mu_i(T) = \mu_i^{\,0}(T) + \Re T \ln \frac{p_i}{p_i^{\,0}}$$

The osmotic pressure definition equation is:

$$\pi = -\frac{\Re T}{\tilde{V}_i} \ln a_i$$

and the definition equation of transport of the component i in the case of reverse osmosis is:

$$\Delta\mu_{i|\mathrm{RO}} = \tilde{V}_i[p_\mathrm{F} - p_\mathrm{p} - (\pi_{i,\mathrm{F}} - \pi_{i,\mathrm{P}})] = \tilde{V}_i(\Delta p - \Delta\pi_i)$$

where p_F is the feed pressure and p_P is the permeate pressure.

The notation demonstrates why reverse osmosis is so named. If the pressure difference on the transmembrane pressure (Δp) is higher than the difference of the osmotic pressures π, then it results in a change in flow direction (see Fig. 11.5).

By letting water flow through a selective membrane out of the more concentrated solution it would be possible to produce (provided that $\Delta p > \pi_\mathrm{W}$) pure water out of brine. Based on above-mentioned notations that describe the membrane separation behavior, it is possible to project separation efficiency (and also the manufacturing yield). Figure 11.6 shows a schematic diagram of a reverse osmosis unit depicting the necessary parameters required for the calculation of the separation efficiency. The separation performance of a reverse osmosis system can be described in terms of the medial permeate flux $\bar{v}_\mathrm{P}$ and the medial permeate mass fraction $\bar{w}_\mathrm{P}$, provided that entry mass fraction w_F and operation pressure p are given.[16]

- Medial permeate flux, $\bar{v}_\mathrm{P} = \dfrac{\dot{v}_\mathrm{P}}{A_\mathrm{mem}}$
- Medial permeate mass fraction, $\bar{w}_\mathrm{P} = \dfrac{\bar{m}_i}{m_\mathrm{ges}}$
- Retention, $R = 1 - \dfrac{c_\mathrm{P}}{c_\mathrm{R}}$
- Yield, $\Phi = \dfrac{\dot{m}_\mathrm{P}}{\dot{m}_\mathrm{F}}$

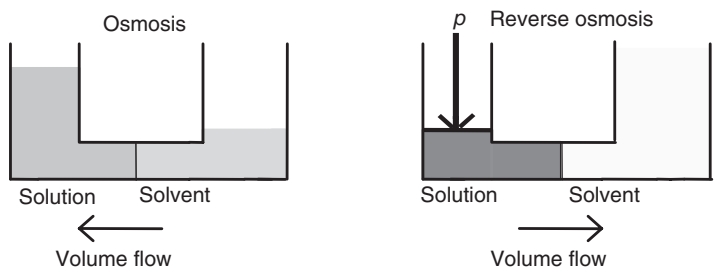

Fig. 11.5 Principle of reverse osmosis.

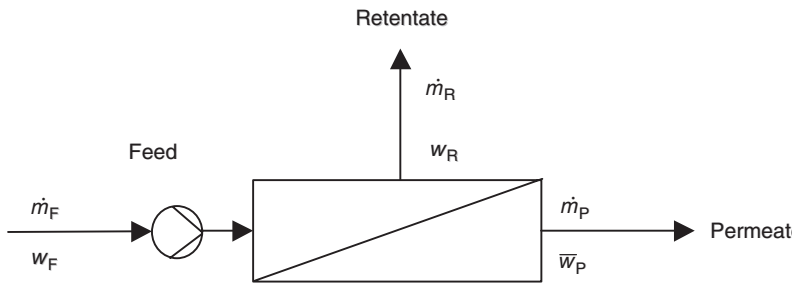

Fig. 11.6 Reverse osmosis installation scheme.[15]

where $\dot{v}$ is volume flux; A_{mem} is membrane area; m is mass; subscripts ges and R indicate overall and retentate, respectively; $\dot{m}$ is mass flux; subscripts P and F indicate permeate and feed, respectively.

Modeling of the mass transport through the membrane

Mathematical models are necessary for dimensioning and optimizing modules and processes. They are also important for an economic evaluation and comparison of membrane processes with conventional alternatives. The basis for each plant dimensioning and process simulation is the preservation of mass/impulse/energy and matter equations. The differential balancing of the conserved quantities in each segment of the process (membrane) leads to a coupled differential equation system. The solution of such an equation system is in most cases only numerically possible. It is very important that these models contain relations that describe the mass transfer in the membrane not only qualitatively but also quantitatively. Such relations describe the mass transfer of the components through the membrane as a function of the operating conditions. This means that it is described as function of (along the module) changing internal state variables (pressure, temperature, concentration) and external state variables (e.g. feed flux) of the system. Unfortunately it is very difficult to model the mass transfer through the membrane because there are numerous interactions between all the components involved in the process. These, again, lead to non-idealities and cross-effects. However, there are different ways to get quantitatively reliable dimensioning equations. Figure 11.7 shows an overview of the different ways in which modeling of mass transfer could be conducted.

For engineering interpretations, the half-empirical models have been useful. They have enabled qualitatively and quantitatively precise characterization of the separation performance. They are based on real-system permeation experiments in conjunction with idealized physico-chemical models. The essence of half-empirical modeling is that the genuine, elementary physical and thermodynamic processes of molecular-level, descriptive mass values are combined into meaningful 'parameter groups'. The values

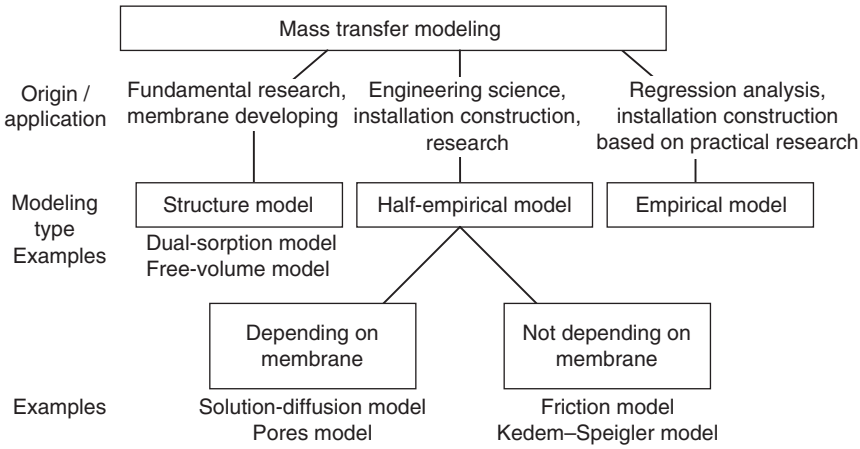

Fig. 11.7 Overview of possibilities for modeling the mass transfer.[16]

of those parameter groups are determined by conducting permeation experiments on real-system membrane–mass systems. Mass transport models of this kind cannot and should not be used to make any predictions about the transport coefficient and separation power of a membrane. They should rather be used to create qualitatively and quantitatively precise descriptions of the membrane separation characteristics. In practice, in order to have reliable and manageable notations, it is important that only a few readily determined free model parameters are introduced into the models.

In cases where modeling of the mass transport in the membrane is not possible, for example because few free model parameters are available, it is necessary to use (in order to determine the dimensions or a membrane unit) a regression analysis that is based on experiments in the field of interest.

Industrial application of reverse osmosis
Traditionally, reverse osmosis is used predominantly in the seawater and brackish water desalting industry. However, because of the increasing availability of more proficient membranes, it is frequently being applied to process organic/watery systems in many industries. Table 11.3 gives an overview of the applicability of reverse osmosis in industrial separation.

11.3 New fields of application

11.3.1 Pervaporation

Description of the process
The term ‘pervaporation’ comprises two Latin words: ‘per’ (through) and ‘vapor’ (vapor, steam). Pervaporation is a separation process in which one

Table 11.3 Some applications of reverse osmosis

Dairy industry
Concentrating of milk[17]
Concentrating and dematerializing of whey[11,17]
Other processes with animal proteins
Concentrating of egg white[18]
Beverage industry
Concentrating of fruit and vegetable juices such as:
• apple juice[19]
• orange juice[20]
• tomato juice[20]
Reduction of alcohol in beer and wine[21]
Concentrating of wine[22]
Concentrating of tea[23]
Sugar industry
Concentrating of low-viscosity juice[24]
Water production
Ultra-pure water production from river water[25]
Drinking water[26,27]
Wastewater treatment[23,28]
Fine chemicals production
Lactic acid[29]
L-Phenylalanine[30]

or more components (the permeate) are extracted selectively from a liquid mixture (the feed) by use of a membrane. The membrane is nearly impermeable to all the remaining components of the solution (retentate) (Fig. 11.8).

Membranes used for pervaporation are so-called dense (non-porous) composite membranes. Composite membranes consist of at least two materials and are built up in layers. There is a difference between selective layers and supporting (stabilizing) layers (Fig. 11.9). The selective layer is responsible for the separation effect. Supporting layers provide strength to the membrane. In most cases the selective layer is a polymer, e.g. polydimethylsiloxane (PDMS) (Fig. 11.10). The separation effect is caused by a stronger interaction of specific substances with the membrane. These substances are selectively absorbed by the membrane polymer and are transported through the membrane by diffusion.

As found in the process of reverse osmosis, the interactions are electrochemical and/or steric. Those chemicals that are enriched in the permeate 'dissolve' more easily in the membrane polymer than those that remain in

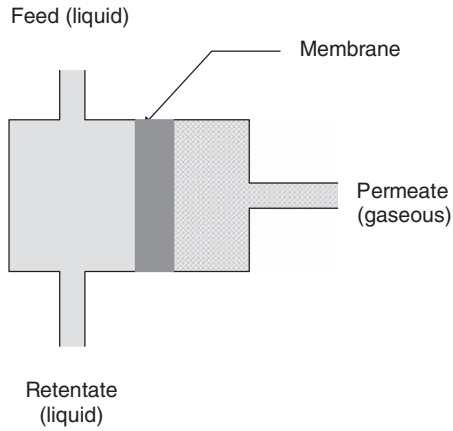

Fig. 11.8 Test cell of a pervaporation unit, schematic.

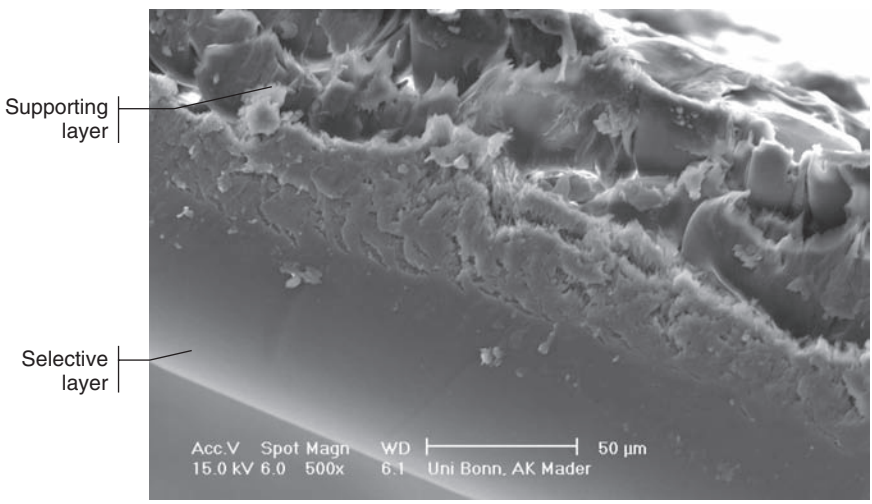

Fig. 11.9 Scanning electron micrograph of a composite membrane.

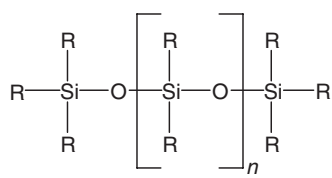

Fig. 11.10 General chemical structure of silicones; for PDMS, R = CH_3.

the retentate. According to the solution–diffusion model,[31] which describes this mechanism, the material transport through the membrane, the so-called 'permeation', is subdivided into five steps:

1 Diffusion of dissolved substance to the membrane.
2 Sorption of the component into the membrane.
3 Diffusion of the component through the membrane.
4 Desorption of the component out of the membrane.
5 Removal of the component into the gaseous phase by evaporation.

The driving force for the transport of molecules through the membrane is a gradient of chemical potential μ_i. This gradient is maintained in the first place by a partial pressure difference and also by a temperature difference. Because of a low pressure of $p < 20$ mbar on the permeate side, the absorbed components evaporate into the gaseous phase and can be transported away (Fig. 11.11).

In a vacuum, ideal gas behavior may be assumed for the permeate side without restricting the precision. Furthermore, the pressure dependence of the chemical potential on the feed side may be ignored since operating systems do not involve a high overpressure.

Hence, chemical potential can be described as:

$$\Delta\mu_i = \Re T \ln \frac{x_{i\mathrm{P}} p_i^{\,0}}{x_{i\mathrm{F}} p_{i\mathrm{P}}}$$

It follows that the driving force can be increased by:

- reducing the permeate pressure, p_{P};
- raising the temperature, T;
- increasing the concentration x_i of the component in the feed.

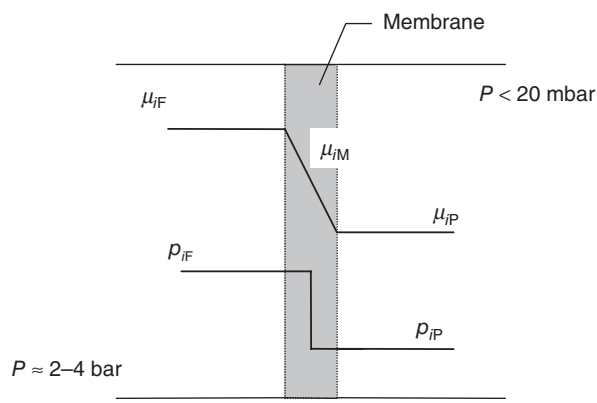

Fig. 11.11 Gradient of chemical potential over the membrane.

In the process of pervaporation, the separation of the components is connected with a transition from liquid to gas. The pervaporation can also be interpreted as a distillation that is hindered by a membrane. However, in contrast to a distillation, pervaporation does not depend on vapour–liquid balances and can therefore be used to separate azeotropic mixtures.

A critical factor in the practical application of pervaporation is the selectivity of the process, which depends on the membrane used. The already mentioned PDMS membrane is a hydrophobic (organophilic) polymer for the separation of organics from water. There are also hydrophilic (organophobic) membranes for the separation of water from organics (e.g. polyvinyl alcohol (PVA) membranes). Some examples for the application of pervaporation are given in Table 11.4.

The selectivity of the membrane towards specific components i in the solution is required for the separation activity of the membrane. These components permeate through the membrane and become less concentrated in front of the membrane, while less permeable substances j are enriched in front of the membrane. The weaker the turbulence of the flow and the weaker the convective remixing of the components, the stronger the concentration and de-concentration in the laminar boundary layer on the feed side. If the flow is laminar, a concentration gradient can only be compensated by diffusion (which is slow compared with convection). There is a transport resistance. This phenomenon is called 'concentration polarization' and is shown schematically in Fig. 11.12.[32]

Application of pervaporation

While hydrophilic pervaporation is state of the art for the dehydration of organic solvents, organophilic pervaporation is hardly applied on an indus-

Table 11.4 Examples of the application of pervaporation

Process	Application
Hydrophilic pervaporation	Dehydration of solvents
	Increase in chemical reaction by removing the reaction water
	Removal of methanol and ethanol from hydrocarbons
Hydrophobic (organophilic) pervaporation	Dealcoholization of beer
	Removal of ethanol, butanol, acetone, etc. from fermentation broths
	Treatment of waste water containing organic material
	Production of aromatizing agents
	Treatment of laboratory waste water
Target organophilic pervaporation	Separation of organic-organic azeotrop
	Separation of isomers

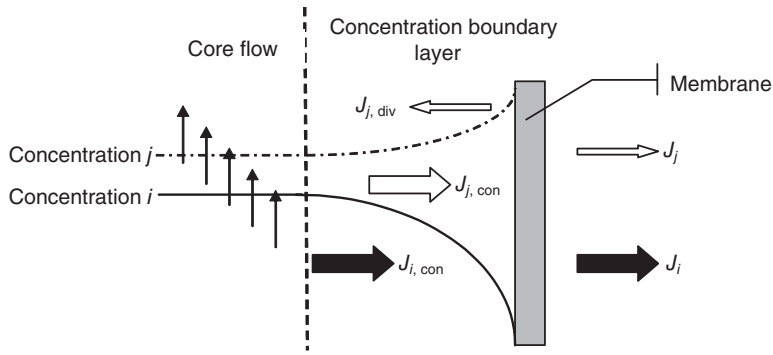

Fig. 11.12 Concentration polarization, schematic.[32] J_j and J_i are the flows of components j and i, respectively.

trial scale, even though there are many interesting application fields for organophilic pervaporation. One of these applications involves the removal of alcohol from wine and beer. Studies of this topic show that the problem with current techniques, such as distillation, is that many flavor compounds are removed in addition to the ethanol and water, leading to a negative impact on the taste. This problem can be solved by using an organophilic membrane with high selectivity or by using a hydrophilic membrane. Hydrophilic membranes can be used for the reduction of polar substances and can therefore separate short-chain alcohols as well. To reduce the water flow, the difference in partial pressure of water could be reduced by applying a water-vapor-rich carrier gas stream on the permeate side. With this technology the ethanol concentration of chardonnay wine could be decreased to 0.5% without any losses of taste and flavor.[35]

The depletion of flavor components from aqueous solutions exemplifies a further application of organophilic pervaporation in food technology on which there are many studies. In general there are two main areas: the separation of flavors in fruit juice production and the separation of flavors from fermentation broths. The driving force of the application of pervaporation in fruit juice production is the high loss of flavors during juice concentration. This is especially the case when using vaporization. The main target of process optimization must be the minimization of these losses by separating flavor and product stream. After the concentration of the beverage, flavors can be added to the product (see Fig. 11.13).

Using pervaporation in a bioreactor hybrid process

One example of an industrial possibility for the application of organophilic pervaporation is the combination of a fermentation process with a pervaporation bioreactor hybrid process. In a fermentation process the target product is usually part of a very complex mixture consisting of metabolites, proteins, sugars and inorganic salts, as well as cells and cell fragments.

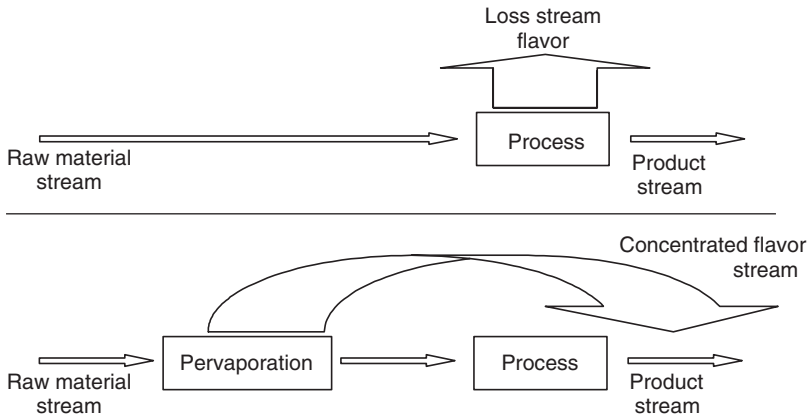

Fig. 11.13 Separation of flavor and product stream in beverage processing.[6]

Another problem is the product inhibition caused by other metabolites. By using a coupling of fermentation and organophilic pervaporation the products can be separated and the concentration of inhibiting substances could be reduced, which enables a continuous fermentation. An increase of productivity of from 80 to 500% is possible compared with a non-integrated batch process.[37] Table 11.5 shows an overview of possible products and microorganisms used.

The pervaporation bioreactor hybrid process can be realized by integrating a membrane in a reactor or by using an external process unit as shown in Fig. 11.14. The application of membrane processes in downstream processing of fermentation solutions is usually inhibited by biofouling. Hence, an integration of a microfiltration between the reactor and the pervaporation unit is necessary to avoid fouling on the pervaporation membrane. Additionally, by immobilization of the cells, the exploitation of suitable modules or a semi-continuous cleaning of the pervaporation module, fouling can be avoided. An additional processing of the permeate can be carried out by distillation or by other membrane processes.

11.3.2 Membrane extraction

Perstraction or membrane-supported extraction is an efficient method of separating process solutions and is an interesting alternative to established extraction processes. In this process, non-miscible phases are separated by a porous membrane. Hydrophilic and organophilic membranes can be employed. By using a hydrophilic membrane the pores are filled by the aqueous phase, by using an organophilic membrane they are filled by the organic phase. The wetting phase is floating within the membrane pores, so that on the surface of the pores an immobilized phase surface is generated. A breakthrough through the pores is avoided by applying a pressure

Table 11.5 Overview of pervaporation–bioreactor hybrid processes described in the literature

Application	Microorganisms
Separation of acetoin and butandiol	*Bacillus subtilis*
Separation of acetone–butanol–ethanol	*Clostridium saccharoperbutylacetonicum* *C. beyerinckii* *C. acetobutylicum*
Separation of benzaldehyde	*Bjerkandera adusta*
Separation of butanol	*C. saccharoperbutylacetonicum* *C. acetobutylicum*
Separation of butanol and isopropanol	*C. beyerinckii* *Saccharomyces cerevisiae* *Candida pseudotropicalis*
Separation of ethanol	*Candida thermohydrosulfuricum* *S. carlsbergensis*

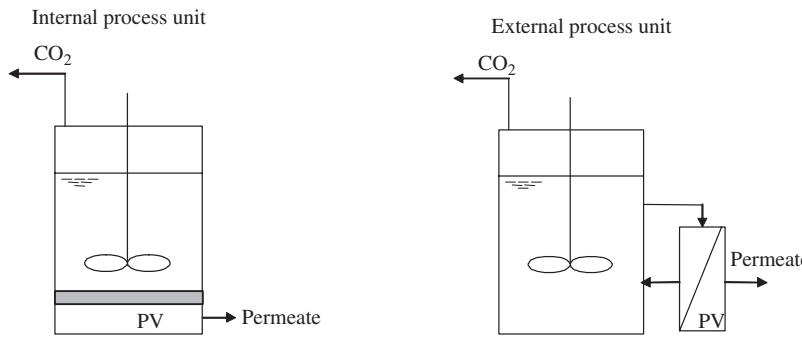

Fig. 11.14 Comparison of internal and external process unit in a pervaporation (PV) bioreactor hybrid process.

on the side of the wetting liquid. In this way a mass transfer without dispersion can be enabled. The principle of perstraction is depicted in Fig. 11.15.[38,39]

The main advantage of this process compared with an established extraction process is the enlarged surface and hence an enlarged exchange area. The exchange area using a hollow-fiber membrane contactor is 200 times larger compared with a packed column.[38] Other advantages compared with a conventional separation process are shown in Table 11.6. The process is limited by the costs of the membrane. As in all membrane processes the limited stability and potential fouling can complicate the application. Due to agglomeration, the wetting properties of the membrane can change and can hence endanger the process. Compared with conventional extraction the additional resistance of the membrane must be taken into account; this can limit the efficiency of the process.

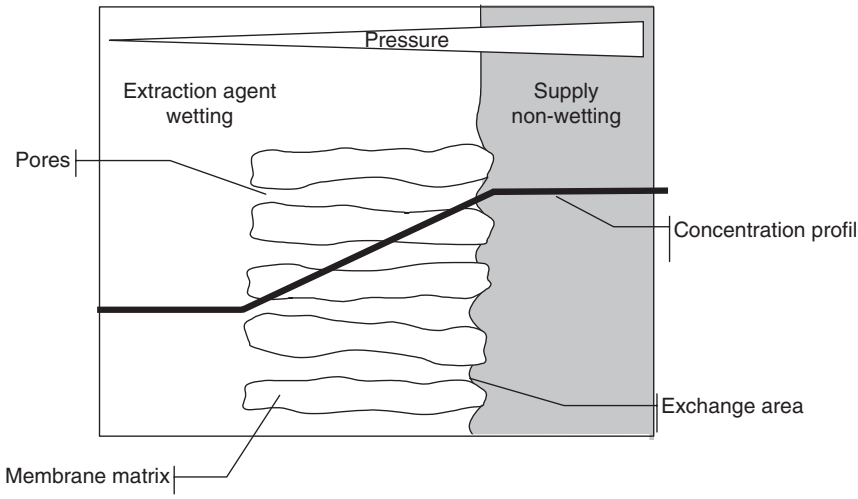

Fig. 11.15 Principle of perstraction.

Table 11.6 Advantage of perstraction compared with conventional processes

Advantages compared with conventional extraction	Advantages compared with thermal separation processes
• Immobilization of the phase surface, no mix of the phases • No difference in density necessary for separation of phases • High flow rate due to the fixing of the surfaces	• Separation of aceotropes possible • Low energy costs • No temperature stress of feed and permeate

Of particular interest is perstraction in the food industry, especially for the separation of fermentation products. One example involves the continuous separation of flavors from fermentation broths. By immobilization, the mixing of the phases and hence the stress for the microorganisms can be reduced and a continuous extraction can be enabled. Additionally, temperature stress of microorganisms and substrate can be avoided. In the example below, the extraction of the flavors 1-octene-3-ol, 2-heptanone, isoamyl acetate and ethyl butyrate is examined. The extraction agent was hexane. The hollow fiber was Celgard® hollow fibers made of hydrophobic polypropylene (see Fig. 11.16). With this experimental set-up, high extraction rates can be achieved. After 18 min, 98% of the flavors could be extracted. Figure 11.17 shows the high efficiency of the process.[41] It can be seen that extraction using hollow-fiber membrane contactors is a most

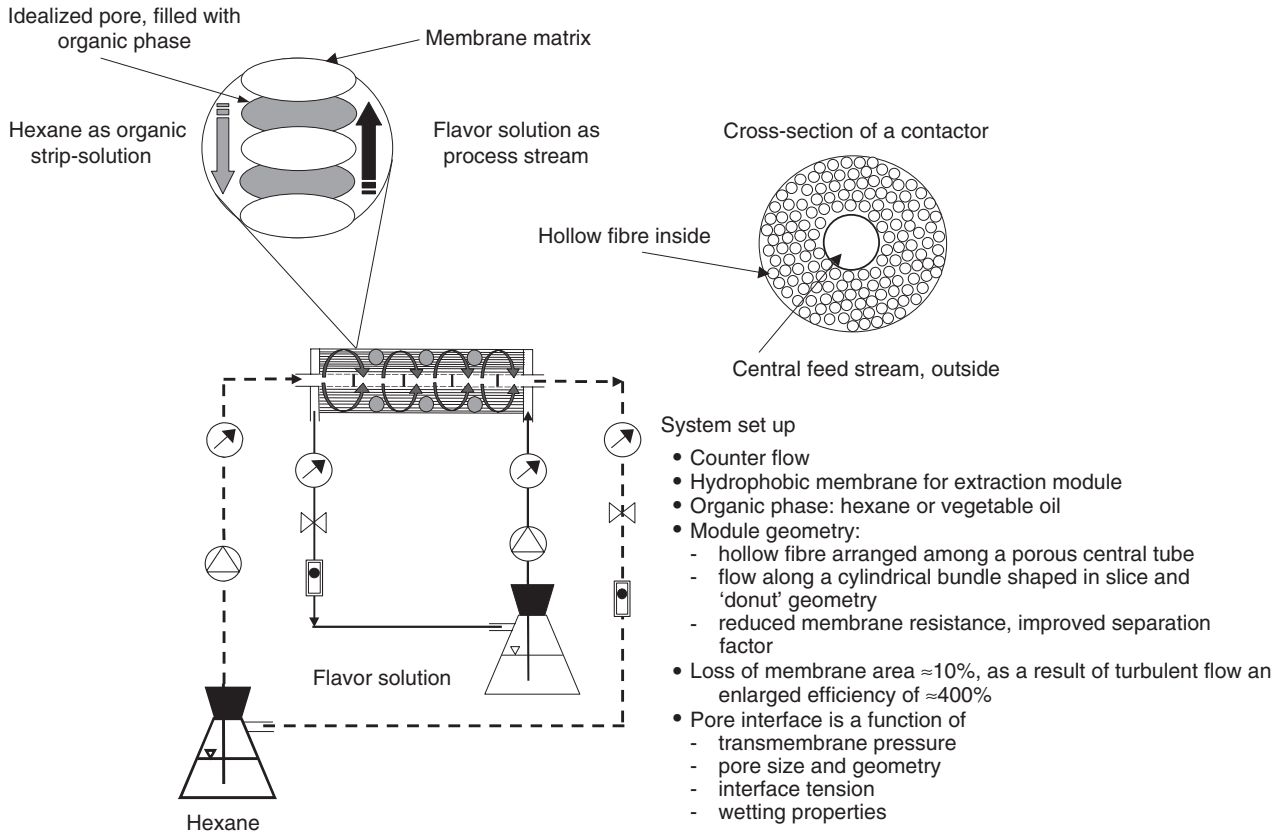

Fig. 11.16 Extraction of an aqueous solution of flavors using a hollow-fiber membrane contactor (after Wickramasinghe *et al.*,[36,40] and Laufenberg and Cussler[41]).

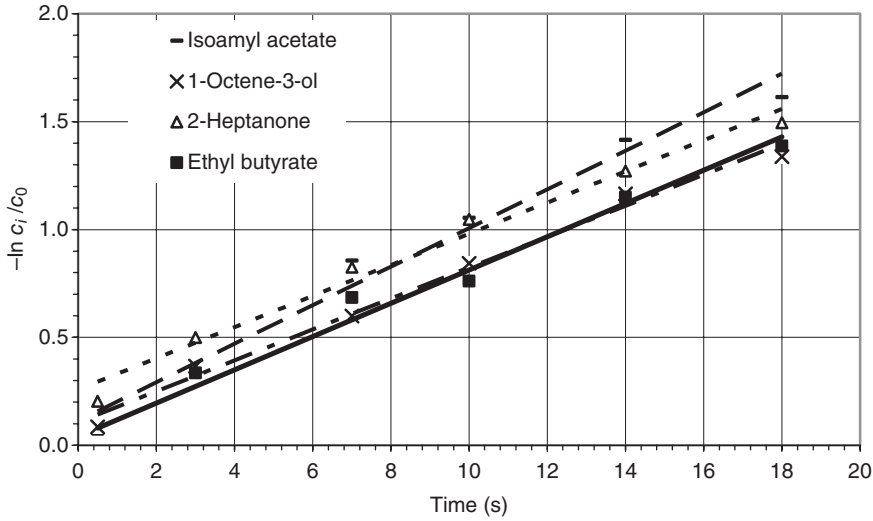

Fig. 11.17 Membrane extraction, $-\ln c_i/c_0$ versus time, all flavours in aqueous solution, feed flow 4.8 ml s^{-1}; extraction agent, hexane.[39]

efficient method of extracting organic substances from aqueous organic solutions. The extraction agent, hexane, is of considerable concern in German food legislation, and an easy separation enables a quality product. An interesting alternative is seen in the application of vegetable oil as an extraction agent. Here no additional reprocessing is necessary, which reduces costs. The product could be flavored oil which can be applied in food without any further purification.

11.4 Conclusions

Nowadays in the food industry, membrane technology is reduced to a few main applications, such as water treatment and milk processing, although membranes can be used for many processes in almost every branch of the industry. New technologies, in particular membrane extraction or pervaporation, have great potential in the separation and purification of high-value products. The reasons for this lack of application are high membrane costs and slow operation speeds. Membrane processes are still too expensive for low-price products. Hence, membrane technology is hardly adequate, especially for those processes where alternative technologies like distillation are established. Niche applications for high-cost products are apparent, especially in biotechnology and the food industry. Here the price ranges are high enough to make an investment in new technologies lucrative.

11.5 Notation

π	bar	osmotic pressure
ρ	$kg\,m^{-3}$	density
Ψ	—	selectivity
$\Re$	$J\,mol^{-1}\,K^{-1}$	universal gas coefficient
A	m^2	membrane area
c	$g\,mol^{-1}$	concentration
c_g	$g\,mol^{-1}$	concentration of dissolved component in the solution
c_{im}	$g\,mol^{-1}$	concentration of substance i at the membrane surface
c_{is}	$g\,mol^{-1}$	saturation concentration of substance i
D	$m\,s^{-2}$	diffusion coefficient
G	$J\,mol^{-1}$	Gibbs enthalpy
μ_i	$J\,mol^{-1}$	chemical potential of i
J_0	$m^3\,s^{-1}$	suspension flow
J_F	$m^3\,s^{-1}$	filtrate flow
J_K	$m^3\,s^{-1}$	concentrate flow
J_s	$kg\,s^{-1}$	diffusive and convective mass flow
J_v	$m^3\,s^{-1}$	permeate flow rate
l	m	membrane thickness
M	$kg\,kmol^{-1}$	molecular weight
m	kg	mass
$\dot{m}$	$kg\,s^{-1}$	mass flux
p	bar	hydrostatic pressure
p^0	bar	standard pressure (1 bar)
p_1	bar	partial pressure of i
p_F	bar	feed pressure
p_P	bar	permeate pressure
R	%	absolute retention
t	s	time
T	°C	temperature
$\tilde{V}$	m^3	molar volume of i
v	$m\,s^{-1}$	speed
$\dot{v}$	$m^3\,s^{-1}$	volume flux
w	–	mass fraction
x	–	mole fraction

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12

Separation technologies for food wastewater treatment and product recovery

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12.1 Introduction

Proper treatment of food processing wastes, including the recovery of valuable products, is important both environmentally and economically to the food processing industry. For this industry, as for many others, environmental regulations and disposal costs for waste/wastewater are two major forces driving the use of alternative treatment technologies that will recover valuable products from waste streams and recycle water effectively.

The quantity and quality of wastes and wastewaters generated in the food industry vary widely. Depending on the operations and the products, wastes can amount to over 50% of the raw materials on a weight basis (Zaror, 1992). The characteristics of a specific waste depend mainly on the product being processed, the processing methods and the amount of water used to wash raw materials and clean equipment. Segregating waste streams at the source will facilitate resource recovery and reduce the amount of waste that has to be treated (Zaror, 1992). This often results in simpler operations, less energy consumption and lower treatment costs. One simple but effective segregation practice is to separate liquid streams from solid streams.

This chapter reviews both the theoretical basics and practical applications of different separation technologies. The suitability of each for specific food processing wastes and wastewaters is discussed, and the quality and final uses of the products recovered through separation are presented.

12.2 Principles for separation

A separation process may be defined as a unit operation that divides multi-component or multi-phase material into two or more components and/or phases, using physical, thermal, chemical or biological principles. It may involve changing the phase of the matter. Some separation technologies combine two or more of these principles. The basic process for separating three phases of matter, with each phase containing three components, is shown in Fig. 12.1. The commonly applied separation technologies for treatment of the food processing wastes are shown in Fig. 12.2.

Separation can be achieved in a single- or multiple-step operation. Adding steps may increase the quality of the final product but may also increase capital and operation costs. If the separation involves m steps and each step has a similar recovery efficiency of ε, overall recovery efficiency can be calculated as follows (Liddell, 1994):

$$\text{Overall recovery} = \varepsilon^m$$

With different recovery efficiencies for each of m steps, the overall efficiency can be calculated as follows:

$$\text{Overall recovery} = \varepsilon_1 \times \varepsilon_2 \times \cdots \times \varepsilon_m$$

12.3 Separation and recovery technologies

This section presents the theories of various physical and chemical separation technologies and the application of each for treatment of food processing waste/wastewater.

12.3.1 Physical and thermal processes

According to Kentish and Stevens (2001), physico-chemical processes are usually effective in reducing organic pollutants in the wastewater to levels

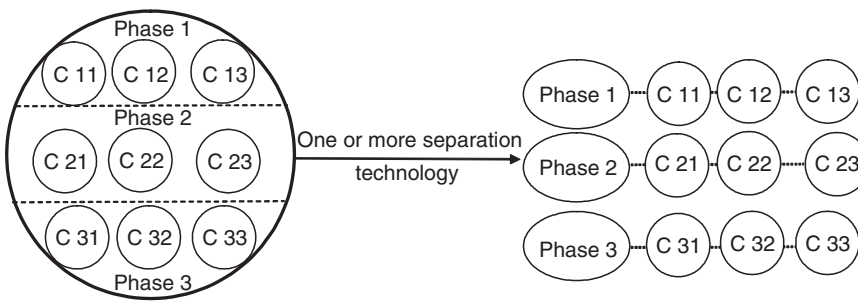

Fig. 12.1 Definition of a separation process.

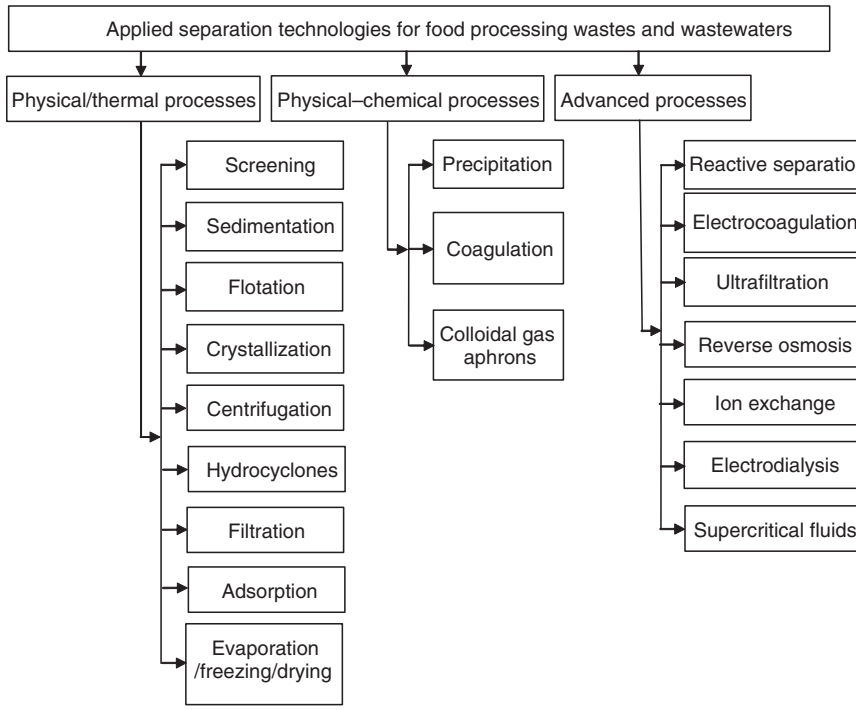

Fig. 12.2 Common separation technologies for food processing wastes/ wastewaters.

suitable for discharge into public sewer systems or natural water sources, depending on the initial concentrations of the pollutants. The processes may be capable of recovering some of the nutrients either for recycling or to be marketed as byproducts, but they cannot significantly reduce heavy metals. Nor do they usually provide the selectivity necessary for producing pure products suitable for recycling or reuse. In the following sections, eight different separation processes and their example applications are described.

Screening

Screening is commonly used as a primary separation process to remove solid materials from food processing wastes and other waste streams. The separated solids can be converted into valuable products by other technologies such as drying. Screening is widely used in food processing operations, including seafood, vegetable, meat, poultry, winery, distillery and canning plants (Zaror, 1992). Many screen designs are appropriate for food processing wastewater, including static or inclined screens, rotating cylindrical screens and vibrating screens (Dearborn Environmental Consulting

Services, 1979). The choice among these designs depends on financial and technical considerations. According to Zhang and Westerman (1997), the performance of the screen separator depends on screen-opening size and characteristics of waste to be separated, such as total solids content and particle size distribution. Another important parameter is the ratio between the flow rate of waste and the available surface area for screening. Knowledge of the waste flow rate relative to the screen openings and area is required to achieve effective solid–liquid separation.

Sedimentation (also called gravity settling or clarification)
Sedimentation is the separation of solids from a liquid by means of gravity. It depends on the differences in specific gravities between the suspended matter and the solution. Gravity clarifiers are often equipped with skimming mechanisms for removing floating materials such as grease and fibers. They are commonly used to treat waste streams from sugar beet, meat, fish and poultry processing operations. Sedimentation can sometimes be done naturally without adding chemicals. However, for many applications, use of chemical coagulants can be very helpful in enhancing the removal efficiency of suspended colloids (Soderquist and Montgomery, 1975). Theoretically, sedimentation can be modeled by the laws of relative motion between particles and the enclosing liquid (Pinheiro and Cabral, 1993).

Flotation
Flotation involves the separation of suspended matter from an aqueous solution due to the differences in their specific gravity. Some applications use gas bubbles to enhance the separation by attaching the bubbles to the solid particles, causing them to rise to the surface through the buoyant effect. Depending on the methods used to generate the gas bubbles, flotation technologies can be divided into dispersed-air, dissolved-air and electrolytic flotation (Svarovsky, 1990). Dissolved-air flotation is the most commonly used method (Rubio *et al.*, 2002). For example, dissolved-air flotation is used to remove soluble proteins in soybean processing wastewater after precipitation and flocculation (Schneider *et al.*, 1995). The size of a protein flotation unit depends on the protein concentration and the volume of wastewater to be treated (Zaror, 1992). Generally, the main factors affecting the design of a flotation process are the specific gas flow per unit volume of the liquid, the concentration of suspended matter and, in the case of protein separation, the pH of the liquid. The latter is due to the fact that the pH affects protein solubility.

Crystallization
Crystallization is the formation of solid particles within a homogeneous phase (McCabe *et al.*, 1985). It may include the formation of solid particles in a vapor (e.g. in snow) or from an aqueous phase. It is a powerful technique for isolating pure substances from mixtures (Kennedy *et al.*, 1993;

Prazybycien *et al.*, 2004). Crystallization processes are currently used to isolate and purify a wide range of inorganic and organic constituents from food products and waste streams (Adler *et al.*, 2000). One important application is protein lactose recovery. Prazybycien *et al.* (2004) mentioned that at least four factors define crystal morphology and the yield of the protein crystallization process: protein concentration, precipitant concentration, pH and temperature.

Impurities such as minerals and protein are believed to hinder crystallization of lactose and should be removed beforehand. For example, in separating lactose from whey, Guru and Zall (1991) found that K^+, Ca^{2+} and PO_4^{3-} had significant effects on lactose recovery. Their results also showed that seeding with fine lactose crystals increased recovery efficiency, whereas extending the aging period made little difference.

Mukhopadhyay *et al.* (2003) studied the removal of interfering substances – such as protein from whey, using chitosan as a coagulant. They found that first treating crude and heat-deproteinized whey with this material brought reductions in protein, ash and fat of 62–85%, 50–75% and 70–80% of the original content, respectively. The chitosan-treated whey was subjected to lactose separation. Lactose was precipitated using ethanol, or crystallized using crystalline lactose as seed. Lactose precipitated by ethanol had lower ash content (1.2 g/100 ml for crude whey and 0.1 g/100 ml for heat-deproteinized whey) than the lactose separated by crystallization (2.25 g/100 ml for crude whey and 1.57 g/100 ml for heat deproteinized whey). Lactose prepared from the heat-deproteinized whey had a purity of 99.89% and met the standard for pharmaceutical grade.

The most important parameters affecting the design and performance of a system for crystallization are temperature and pH of the liquid, concentration and solubility of the intended component, and the presence of nucleation seed and its origin. For crystallization of a specific protein, Berry (1995) mentioned that seed solutions from the same protein, but from different species, may activate crystallization of that specific protein.

Centrifugation

Centrifugation is an effective pre-treatment process for food processing wastewaters. It separates suspended solids by increasing their gravitational forces. Stokes' law may be modified to apply to centrifuges as follows (MacConnell *et al.*, 1990):

$$V_c = \frac{[(\rho_s - \rho_l)d^2\omega^2 r]}{18\mu}$$

where: V_c is the settling velocity of the particle due to equivalent gravitational force from the centrifuge; ρ_s and ρ_l are the densities of the solids and liquid, respectively; d is the diameter of the particle; ω is the speed of rotation; r is the radius of rotation; and μ is the viscosity of liquid.

Centrifugation is widely used in fishmeal plants to separate fish oil from processing wastes (Archer *et al.*, 2001). Several different types of centrifuge

are available – including basket, solid-bowl, countercurrent-flow and concurrent-flow systems (Philips, 1997). Centrifugation can be used as a pre-treatment before membrane separations to reduce membrane fouling and increase overall separation efficiencies. Turano *et al.* (2002) used centrifugation in combination with ultrafiltration in the treatment of wastewater from olive oil production, and noted that centrifugation was considered economic since the centrifuge was already used during the production of olive oil. Another application is the use of high-speed centrifuges for separating plasma (60–80%) and red cells (20–40%) from whole animal blood (Liu, 2002). The red cells contained 34–38% protein while the plasma contained 7–8% protein.

Centrifugation has also been used for recovering protein precipitates. Stavrinides *et al.* (1993) stated that operating the centrifuges at their maximum efficiencies, minimizing the losses of protein into the supernatant and minimizing the supernatant in the precipitate, were important considerations in keeping processing costs reasonable. Gómez-Juárez *et al.* (1999) used centrifugation as a unit operation prior to breakdown of red blood cells (hemolysis), enzymatic hydrolysis and ultrafiltration during the recovery of white protein concentrate from bovine-waste blood. Sachindra and Mahendrakar (2005) reported that centrifugation was an important step during the recovery, from shrimp wastes, of carotenoids, a group of oil-soluble pigments. Toyoshima *et al.* (2004) used centrifugation to separate sardine oil from surimi wastewater without heating or chemical refining. Their results showed that more than 70% of the oil could be recovered from surimi wastewater by using continuous centrifugation. Bough *et al.* (1976) successfully used centrifugation to separate solids that had been coagulated with chitosan in an effort to reduce suspended solids in wastewater produced in vegetable, poultry, meat, cheese, seafood and egg-breaking plants. The separated solids were used as animal feed additives.

Hydrocyclones

Hydrocyclone technology, which takes advantage of centrifugation forces, has been suggested as a practical technology in solid–liquid separation of biological materials. Ortega-Rivas (2004) explained the theory and essential parts of hydrocyclones. A hydrocyclone consists of a cono-cylindrical body that creates a vortex when a liquid is pumped through it. The vortex creates a centrifugal force which hurls the coarse particles from the cyclone center toward the walls, where they fall out through an underflow orifice. The fine particles remain around the central axis and exit the hydrocyclone with the upflow stream. As with centrifugation devices, the efficiencies of hydrocyclones depend on the difference in density of the solid particles and the liquid, plus liquid viscosity, diameter, rotation speed and radius of the particles. Compared with centrifuges, hydrocyclones are easier to manufacture, install, maintain and operate, but they have lower separation efficiencies. According to Curtis (1996), they can be very efficient and cost-effective ways of separating some food wastewaters, such as oily wastewater.

Adsorption

Adsorption is considered to be an effective method for treatment of dilute effluents (Laufenberg *et al.*, 2003). The mechanism here is the attachment of molecules from an aqueous solution or a gaseous phase onto a solid surface (the adsorbent) because of intermolecular attractive forces. Adsorbents attach atoms, molecules, ions and/or radicals from their surrounding gaseous or liquid phase onto their surfaces. The amount of the trace chemicals to be absorbed is proportional to the available surface area of the adsorbent. Therefore, commercial adsorbents are extremely porous, giving high surface areas per unit of mass. Adsorbents are divided into three classes: hydrocarbon materials, inorganic materials and synthetic polymers. A number of low-cost adsorbents have been used for wastewater treatment – including peanut and walnut shells, orange peel, wool fibers and corncobs (Laufenberg *et al.*, 2003).

Much research has been done on the use of adsorption for recovery of various chemical substances (e.g. polyphenolics) from food processing wastes. Polyphenolics are highly valuable compounds that may be used as functional food ingredients and as natural antioxidants. Schieber *et al.* (2003) investigated the recovery of pectin and phenolic compounds from apple pomace. The recovery process included extraction of dried apple pomace with diluted mineral acid and adsorption of phenolic constituents by a hydrophobic styrene–divinylbenzene copolymerizate, which is a resin used for reducing the bitterness of citrus juices. Edris *et al.* (2003) studied the recovery of aromatic components by adsorption from wastewaters produced from aromatic plant distillations, using granular activated carbon as adsorbent. Recovery ranged from 44 to 90%, depending on the selectivity of compounds. The authors found that adsorption did not affect the structure of the recovered aromatic compounds. Moreover, the adsorbent had no effect on the chemical nature of the aromatic components. The important factors affecting the rate of adsorption are shown in Table 12.1 (Vasanth Kumar *et al.*, 2004).

Table 12.1 Factors affecting the adsorption rate (Vasanth Kumar *et al.*, 2004)

Factors related to the adsorbent:
1 Dimensions of the adsorbent (i.e. diameter or surface area).
2 Structure of the adsorbent.
Factors related to the nature of the adsorbate (i.e. solute) and the solvent:
1 pH of the liquid.
2 Solubility of the solute.
3 Molecular size of the solute.
4 Molecule geometry.
5 Degree of ionization.
6 The presence of materials for surface tension modification.

Thermal processes: freezing, evaporation and drying

Three thermal processes can be used as solid–liquid separation technologies. Freezing is one: as it occurs, ice crystals are formed from pure water molecules, while other molecules are rejected to crystal boundaries where they can be recovered. The process is energy intensive if freezing is manmade, but natural freezing can be economic in cold regions (Martel, 1990).

Evaporation, another thermal process, is used to concentrate a solution consisting of a nonvolatile solute and a volatile solvent (McCabe *et al.*, 1985). A third thermal process, drying, is normally used to remove moisture from the waste. Like artificial freezing, evaporation and drying are energy-intensive processes only used in applications that recover products of high value. Göğüs and Maskan (2006) studied the air drying of olive pomace, which could be used as an animal feed or as a raw material for the production of glycolipids, at low temperatures (60–80 °C) and found that an increase of both drying temperature and particle size decreased drying time.

Generally, the major factors that affect the choice of thermal technologies are the energy consumption and the required characteristics of the recovered product. The most important design parameters are: (1) operational temperature and pressure; (2) surface area for heat transfer; (3) type of media used for heating or cooling; (4) relative flow rates between the waste and the heat-transfer media and (5) design of the equipment used.

12.3.2 Chemical processes

Chemical separation processes use chemicals to bring about reactions that change the surface characteristics of solid particles, and change compounds from soluble to insoluble forms to enhance the separation. The chemical treatment processes are usually used in conjunction with physical processes. Three chemical treatment processes – precipitation, coagulation and colloidal gas aphrons – and examples of their application are described in the following sections.

Precipitation

Precipitation involves converting a soluble compound into an insoluble form by adding a chemical to an aqueous medium (Dearborn Environmental Consulting Services, 1979). Boychyn *et al.* (2000) reviewed the factors that affect the physical properties of the aggregates formed by precipitation. These are: type of precipitation reactor, precipitating reagent type, concentration and rate of precipitating reagent addition; method and extent of mixing; and residence time in the reactor. The choice of precipitating reagent depends on precipitation yield, selectivity, denaturation, viscosity and density of the suspension, and the end use of the final product (Stavrinides *et al.*, 1993). Precipitation processes can be carried out either

in batch or continuous operations. Garcia (1993) pointed out that two steps should be considered in designing a precipitation process: (1) selection of the precipitation method and (2) the dynamics of precipitation. Selecting the method includes selecting the precipitation agent and dosage, operation costs, product yield, purity of final product and evaluation of possible damage that could be caused to biomolecules by the precipitation agent.

Precipitation has various applications in treating food processing wastewater, including separation of soluble phosphorus, and recovery of sugar and protein from wastewater. Precipitation can easily be adapted to a large scale using simple equipment (Singh and Singh, 1996). In the Steffen process of the sugar beet industry, calcium is used to precipitate and recover residual sugar (as calcium sucrate) from molasses (Dearborn Environmental Consulting Services, 1979). In the case of protein recovery, precipitation results in a lower degree of protein purity than ion exchange, but its cost is relatively low too (Zaror, 1992). Proteins are brought to an insoluble state either by heat or by adjusting the composition of the solution (pH, ions, polyelectrolytes, solvents) and then removed by solid–liquid separation techniques such as sedimentation or dissolved-air flotation (Hearn and Anspach, 1990).

Fernández and Fox (1997) studied the use of chitosan for the selective precipitation of proteins and peptides in cheese wastewater. They found that chitosan gave good fractionation of water-soluble extracts at pH 2, 3 and 4. At pH 5, 6 and 7 most of the nitrogen in the water-soluble extract remained soluble. Effective fractionation was obtained at pH 4.0. This approach could also be used to recover protein and peptides from cheese and other dairy industry wastewater. Thus, from an environmental point of view, the use of chitosan is preferred to chemical precipitation agents, because chitosan is a biologically based byproduct.

Protein can be precipitated by using one of the following methods (Zaror, 1992): isoelectric precipitation, salting out and heat precipitation. More details about the isoelectric and salting out methods are given below.

Isoelectric precipitation

Isoelectric precipitation refers to the separation of a protein from a solution when the protein loses its charge at its isoelectric point (pI), reducing its solubility. The surface charge of the protein is largely affected by the pH of the solution. Protein has a net positive charge at low pH levels and a negative charge at high pH levels. At the pI, it has no net charge. This leads to reduced solubility because the protein is unable to interact with the medium and will then fall out of solution (Righetti, 2004). In addition to using pH as a variable to control precipitation, zeta potential is used. The zeta potential is a measure of the electrostatic charge on the surface of particles suspended in a liquid (Singh and Singh, 1996).

Salting out

Adding large amounts of neutral salts (e.g. ammonium sulfate) and organic solvents (e.g. ethanol and acetone) can also lead to protein precipitation. The choice of a precipitating salt depends largely on its cost and solubility, and the stability of the protein (Zaror, 1992), while the choice of an organic solvent depends on its cost, its miscibility with water, and the effect on protein stability and solubility. According to Singh and Singh (1996), the major concerns with precipitating proteins using organic solvents are miscibility, the safety of solvent handling and the prevention of protein denaturation. Non-ionic polymers and polyelectrolytes are also used for protein precipitation and stabilization in solution. Carboxymethylcellulose solutions have been used to recover proteins from whey, egg and muscle.

In addition to isoelectric and salting out precipitation, heat can also be an effective means of changing the physical properties of protein and bringing about coagulation/precipitation. The heat-enhanced separation method can be used when the biological properties of the protein are not important (Zaror, 1992).

Chemical coagulation

Coagulation generally is a process that causes destabilization and aggregation of colloidal particles in a solution. In chemical coagulation, insoluble colloidal particles are agglomerated by the action of a chemical additive to produce flocculant materials, which can then be removed by sedimentation or flotation (Dearborn Environmental Consulting Services, 1979). Flocculation is a method of aggregating the suspended fine particles into large flocs (Moudgil and Shah, 1986). Floc properties in a given application are determined by other steps such as sedimentation, filtration or floc flotation. The desired floc characteristics for specific applications are presented in Table 12.2 (Moudgil and Shah, 1986). Flocculation promotes contact between native or coagulated particles, thus increasing aggregate size (Pinheiro and Cabral, 1993). Although some coagulation can occur naturally by particle contact and agglomeration, chemicals increase the rate of agglomeration. The chemicals have charged ions that neutralize the surface charges on the surface of colloidal particles. Metal ions (e.g. ferric chloride, ferric sulfate) and polyelectrolytes are examples of the chemicals applied

Table 12.2 Floc characteristics for specific applications (Moudgil and Shah, 1986)

Separation technique	Desired characteristics of floc
Filtration	Porous, strong, permeable flocs
Sedimentation	Dense, strong, large, regular in shape
Centrifugation	Strong, dense, large flocs
Floc flotation	Low-density, strong, narrow size distribution

in wastewater treatment. Organic materials, such as chitosan, may also be used as coagulant agents. The choice of suitable chemicals depends on cost, effectiveness of coagulation, and the quality and final usage of the recovered byproduct.

Many factors influence the effectiveness of coagulation, including the characteristics and dosage of the coagulation chemical and the pH of the wastewater. In a study conducted by Genovese and González (1998), maximum solid removal of 31% and 27% from fish-filleting wastewater was achieved at pH 5.5 using $FeCl_3$ and chitosan, respectively, both at 60 mg/l. A maximum solids removal of 31% was achieved using 60 mg/l $Al_2(SO_4)_3$ at pH 7.2. Using fish scales as a coagulant, a maximum removal of 27% was achieved at pH 7.2 and a dosage of 40 mg/l. The researchers concluded that not only classic inorganic coagulants but also ground fish scales could be used as coagulants. Wibowo *et al.* (2005) mentioned that chitosan appeared to work by mechanical entrapment and electrostatic interaction of chitosan amino groups with anions on the proteins. They found that the effectiveness of chitosan for recovering soluble proteins from surimi wash water (SWW) was increased by adding alginate complex (Chi–Alg) and by adjusting treatment time. Flocculation of SWW protein using Chi–Alg at a concentration of 100 mg/l for 1 h achieved high protein adsorption and reduced turbidity.

Good clarification (up to 96%) of suspended solids in slaughterhouse wastewater was achieved using aluminum sulfate and a commercial polymer (Al-Mutairi *et al.*, 2004). Selmer-Olsen *et al.* (1996) investigated chitosan as a coagulant for the treatment of dairy wastewater and reported that use of chitosan at pH 5.3 achieved 60% phosphorous removal and 90% chemical oxygen demand (COD) removal. These removals were similar to those achieved with carboxymethylcellulose, which is commonly used at pH 4.2. Thus using chitosan could reduce treatment costs by reducing the need for acid to lower the initial pH of dairy wastewater (i.e. pH > 9). Moreover, the sludge recovered when using chitosan can be used as an animal feed.

Meyssami and Kasaeian (2005) studied the optimum conditions for using air flotation to separate coagulated oil droplets from a model olive oil–water emulsion. Their results showed that a decrease of 90% in turbidity of the olive oil–water suspension could be achieved at an optimum pH of 6 and an optimum chitosan dosage of 50 mg/l. They found that neither starch nor ferric chloride proved to be an effective coagulant in reducing the turbidity of the emulsion samples. They also mentioned that at 20 °C, 1 min aeration time and chitosan concentration of 50 mg/l, the coagulated oil droplets were fragile. An optimum aeration rate of 3 l/min was established for the chitosan concentration of 100 mg/l, which corresponds to a reduction of more than 90% in the initial turbidity. Their results also indicated that temperature (10, 20, 30 and 40 °C) had little effect on the flotation process at an aeration flow rate, residence time and chitosan concentration of 3 l/min, 45 s and 100 mg/l, respectively.

Xu *et al.* (2001) evaluated a coagulation process using the coagulants lignosulfonate, carboxymethylcellulose, ferric chloride and bentonite to treat egg processing plant wastewater. Protein and fat recoveries were over 95% for all of these coagulants. Optimal pH values for achieving maximum removal efficiencies were 3.5 with lignosulfonate, 3.0 with carboxymethylcellulose, 8.0 with ferric chloride and 4.0 with bentonite. The optimal coagulant concentration for maximum byproduct recovery depended on the initial wastewater concentrations of protein, total solid and fat. The dried products contained high concentrations of protein (36–50%) and fat (32–42%). The recovered byproducts could be used safely as livestock feed ingredients, especially when carboxymethylcellulose, lignosulfonate, chitosan and bentonite were used. These coagulants did not adversely affect the growth rate of animals.

Coagulation, like precipitation, is a practical process for reducing pollution and recovering byproducts from food processing wastewaters. The characteristics of the wastewater affect the choice and the optimum dosage of coagulants as well as the operational parameters. Although inorganic coagulants are widely used, organic coagulants such as chitosan and carboxymethylcellulose are more attractive due to the fact that the recovered products can be safely used as an animal feed. Many factors affect the design of a chemical coagulation process, including wastewater characteristics (e.g. pH, protein content) and the type and dosage of the coagulant.

Colloidal gas aphrons

Colloidal gas aphrons (CGAs) are defined as microbubbles created by intense stirring (5000–10 000 rpm) of a surfactant solution (Sebba, 1987). These microbubbles differ from conventional foams. According to Sebba (1987), CGAs consist of a gaseous inner core surrounded by a thin aqueous surfactant film or shell composed of two surfactant layers and a third surfactant layer that stabilizes this structure. Figure 12.3 shows the structure of CGAs. They have high surface areas for adsorption of molecules by means of electrostatic and/or hydrophobic interactions. Their surface properties can be modified by changing the type of applied surfactant. Furthermore, CGAs show relatively high stability and short separation time from the bulk phase. CGAs can also be easily transferred by pumping and have significant cost advantages compared with membrane and chromatographic separation methods (Fuda *et al.*, 2004). Jauregi and Varley (1999) summarized the properties of CGAs as follows:

- They expose a large interfacial area per unit volume for the adsorption of molecules.
- They exhibit relatively high stability, measured in terms of the time required for their collapse.
- They have flow properties similar to those of water.
- The aphron phase separates easily from the bulk liquid phase because of its buoyancy.

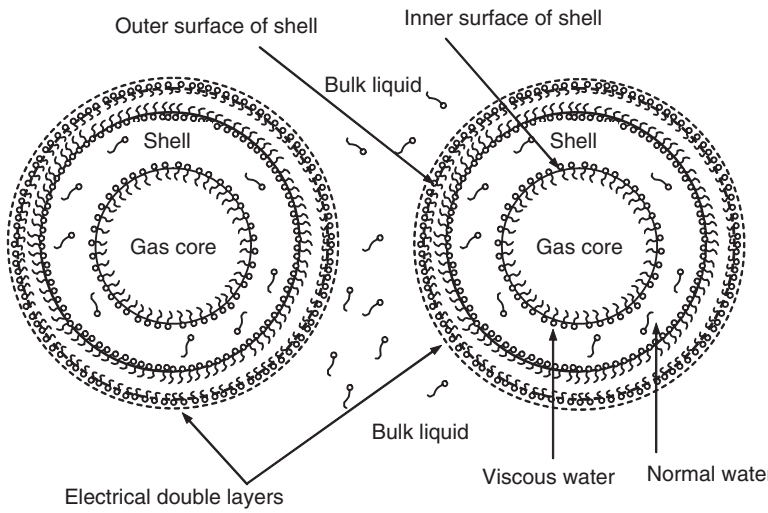

Fig. 12.3 Proposed structure of colloidal gas aphrons (adapted from Sebba (1987) and Jauregi and Varley (1999)).

Jauregi and Varley (1999) mentioned four main areas for CGA application: (1) flotation for the removal of biological and nonbiological products; (2) protein recovery; (3) enhancement of oxygen mass transfer; (4) bioremediation. In the application of CGAs for protein recovery, four subsequent steps are included (Jauregi and Varley, 1998): (1) generation of CGAs from a surfactant solution; (2) addition of CGAs to a protein solution and gentle mixing to allow protein adsorption at the aphron–liquid interface; (3) separation of the aphron phase from the bulk-liquid phase; (4) collapse of the aphron phase. The protein should be concentrated in the aphron phase. Removal of oil droplets from wastewater is another important application of CGAs. According to Sebba (1987), limonene can be recovered from citrus processing wastewater using CGAs. He considered the process a profitable one because of the low cost of CGA production. The cost of surfactant, needed to form CGAs, was the main cost of the process. However, costs can be reduced substantially by using cheap surfactants.

According to Amiri and Valsaraj (2004), CGAs offer particular advantages in the removal of ultra-fine particles. They postulated that it was because of the small molecular size of whey protein (less than a micron) that conventional flotation was not successful. They used sodium lauryl sulfate as a surfactant and found that the separation efficiency was increased by adding aphrons in different pulses. They attributed this to the high concentrations of surfactant remaining around the spinning disk in the aphron generator. Such high concentrations of surfactant decreased surface tension and enhanced mass transfer into solution, thus smaller aphron bubbles were

produced and the CGA dispersion became more stable. Fuda *et al.* (2004) demonstrated that under certain conditions, CGAs could be applied for recovery and separation of lactoferrin (Lf) and lactoperoxidase (Lp) from sweet whey. Their results showed that the amount of total protein in the starting whey and the pH of the separation mixture are the main factors influencing the partitioning of the Lf and Lp fractions into the aphron phase. The best separation performance was achieved with conditions favoring electrostatic interactions (pH < pI and low ionic strength (IS)), whereas conditions favoring hydrophobic interactions (pH > pI and high IS) led to lower performance. Protein adsorption in the aphron phase mainly occurred via electrostatic interactions. Barnett and Lin (1981) used microgas dispersions (MGDs) to remove proteins from seafood processing waste. They showed that without addition of a synthetic surfactant to clam processing wastewater, a foam was produced that could be used after freeze-drying to reform an MGD. The protein produced would be used as an animal feed.

It is clear that the use of CGAs is an efficient and economic separation technique for protein recovery from food wastewater and for recovery of oily products from citrus and seafood processing wastewaters. CGAs may also be used for oil recovery from wastewaters produced during vegetable oil processing.

12.3.3 Other separation technologies

Other advanced technologies are being applied for separation purposes (Fig. 12.1). This section discusses reactive separation and alternating current electrocoagulation.

Reactive separation

Reactive separation refers to the process that combines reaction with separation in a single operation. This integrated design is intended to solve particularly difficult separation problems, such as thermally unstable systems, by means of selective and reversible reactions (Gaikar and Sharma, 1987). Another purpose is to improve a given reaction by overcoming chemical equilibrium limitations through selective separation and removal of reaction products, or by suppressing undesired side reactions (Gilles *et al.*, 1996). Adler *et al.* (2000) noted that separative reactors are devices that can achieve chemical reaction and separation simultaneously. They may include adsorption and membrane reactors, reactive distillation, and biological reactor systems. Simultaneous reaction and separation offer certain advantages that cannot be matched by conventional processes. For one, they can both reduce capital investment and overcome reaction equilibrium limitations (Samant and Ng, 1998).

According to Blaschek (1992), microbial fermentation and cell-free enzyme-based systems are good options for converting food processing

byproducts and wastes into valuable products. Reactor separators convert the substrate to products and simultaneously remove them by stripping them from the fermentation broth into the gas phase. Bioreactor separators should increase reaction rates and cell viabilities, leading to reductions in plant size and cost (BFPE, 1988). Dale *et al*. (1985) investigated the application of the immobilized cell reactor–separator (ICRS) to produce ethanol from whey lactose. Their main objective was to remove inhibitory compounds formed during the reaction so that reaction rates and microbial activities could be maintained. The ICRS consisted of two separate columns in which the fermenting broth was contacted by both the immobilized cells and a 'stripping' gas phase. The inlet substrate and the gas moved cocurrently in the first column, which was called the enriching column. In this column some substrate was converted to a volatile product. Part of the product moved into the gas phase and later to a condenser, while the liquid phase moved to the second column, called the stripping column. Here the remaining substrate was converted to product while the product was stripped into the gas phase, resulting in a final exhausted liquid effluent containing ideally no substrate or product. Thus, the ICRS both converts the substrate to product and removes the product from the fermentation broth. High reaction rates were obtained due to high-density cell loading in the reactor. A separation efficiency of 98% was obtained in this system.

Reactive separation could be a promising technology for the recovery of gases and liquids such as hydrogen, methane and ethanol from food processing wastes. Separation of intermediate products, for example volatile fatty acids during anaerobic digestion, could be an important help in preventing reactor failures.

Electrocoagulation (EC)

EC is a rather complicated process. It involves a number of chemical and physical phenomena that use consumable electrodes to supply ions into the wastewater stream (Mollah *et al*., 2004). Three successive steps are involved: (1) formation of coagulants by electrolytic oxidation of the 'sacrificial electrode'; (2) destabilization of the contaminants, suspension of particulates and breakdown of emulsions; (3) aggregation of the destabilized phases to form flocs. Ryan *et al*. (1990) mentioned that alternating current electrocoagulation (AC/EC) could be used as an alternative to chemical flocculation for liquid–liquid separation. It can be applied for phase separation of wastewater containing suspended and emulsified oils. The main components of an AC/EC system are shown in Fig. 12.4 (Ryan *et al*., 1990).

To the authors' knowledge, this technology is not commonly used for food processing wastewaters. Adhoum and Monser (2004) studied the application of EC using aluminium electrodes for the treatment of olive mill wastewater. They found that increasing the current increased the

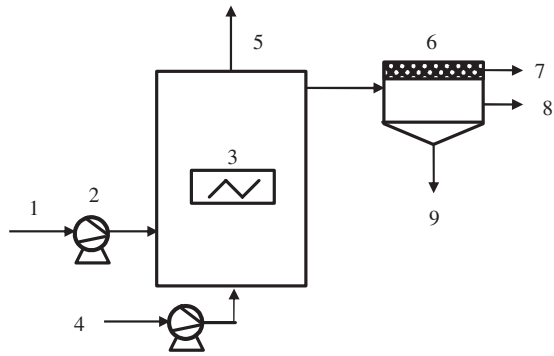

Fig. 12.4 Schematic of a continuous AC/EC system: 1, wastewater; 2, pump; 3, AC/EC coagulator; 4, air for mixing; 5, gas vent; 6, separator; 7, oil; 8, liquid; 9, solids (Ryan *et al*., 1990).

efficiency at an optimal pH of 4–6. After 5 min of application, the removal efficiencies for COD, polyphenol and dark color were 79, 91 and 95%, respectively. Chen *et al*. (2000) studied the application of EC for the treatment of restaurant wastewater and found that the removal efficiency of oil and grease exceeded 94% while the removal efficiency of COD was in the range 84.1–99.0%. EC can be used to neutralize wastewater pH as well. The important design parameters for AC/EC include – but are not limited to – electrode spacing, the material of the electrode, retention time, and current strength and frequency.

12.4 Future trends

Research is needed to examine whether CGAs can be used to separate proteins from complex mixtures (Jauregi and Varley, 1998). Assessing the use of CGAs for recovery of valuable products from cheese whey and vegetable-oil-containing wastewater should be carried out. Continuous operation of this technology should also be evaluated. Further research is needed to optimize the yield of gases and ethanol from different kinds of food processing wastes using reactive-separation technology. Further research is also required to examine the effectiveness of AC/EC to remove organic contaminants from diluted wastewaters. Optimization of many of the aforementioned separation processes has not yet been accomplished. Finally, detailed studies need to be made of the elemental composition of the byproducts recovered from different food processing wastes using the different separation technologies.

12.5 Conclusions

Many separation technologies are being used to treat wastes and wastewaters from the food processing industry. This chapter reviews the theories behind, and the applications of, many of the different technologies. The most important design parameters for each are presented.

The major purpose of using the various separation technologies is to reduce environmental pollution by the waste or wastewater discharge. The recovery of valuable products for human, animal or industrial uses is, in many cases, a secondary purpose. In some cases, the product recovery is necessary in order to make the treatment process an economically viable option. The separation technologies use one or more of the physical, thermal, chemical or electrical processes or a combination of them. The choice among different technologies depends mainly on the characteristics of the waste stream, the technical and financial considerations, and the final use of the recovered products. Increasing the number of separation steps increases the purity of the final product, but sometimes at an unacceptably high cost.

Some physico-chemical processes do not usually provide the selectivity necessary for producing valuable products for reuse. Screening and sedimentation are applied as a primary treatment of wastewater. Crystallization can be applied for protein recovery from milk processors. Chemical coagulation is applied for removal of insoluble colloidal particles. Many inorganic or organic coagulants of different origin (natural or synthetic) are currently used. The most preferred ones are those produced from biological origins, such as chitosan, because the recovered product can be safely used as animal/human feed additives. Another physico-chemical process uses CGAs; it can be a cost-effective technology for the recovery of protein and oil products from wastewaters. Some other technologies, such as reactive separation and EC, can also be used for treatment of food processing wastewater. Reactive separation could be a promising technology for the recovery of hydrogen, methane, ethanol and volatile fatty acids from food processing wastes. EC was found to be effective for the removal of oil and grease from food processing and olive mill wastewaters.

12.6 Sources of further information and advice

Muralidhara (1986) edited a useful book concerning the different theories of solid–liquid separation. It contains useful information on different theories and principles of solid–liquid separation such as sedimentation, centrifugation, flocculation, membrane filtration and electrodewatering. Another good book regarding foams and colloidal gas aphrons has been edited by Sebba (1987). Guidance and recommendations on the application of different separation technologies can be obtained from the research

group of Professor Ruihong Zhang, Department of Biological and Agricultural Engineering, University of California, Davis, California. Advice on the recovery of valuable products from food processing wastes can be obtained from the research group of Drs Tara H. McHugh and Zhongli Pan, United States Department of Agriculture (USDA Agricultural Research Service (ARS), Western Regional Research Center (WRRC), Albany, California).

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Part IV

Waste management in particular food industry sectors and recovery of specific co-products

13

Waste management and co-product recovery in red and white meat processing

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13.1 Introduction

Global meat production is approaching 2×10^8 tonnes/year with pork (40%), poultry (30%) and beef (26%) making up the vast majority of global production. China and the USA are the world's largest producers of beef and pork; while Brazil, Mexico, the Russian Federation and a number of Western European countries are also large producers (UNEP, 2000). Although information on volumes of generated waste is variable, and dependent on the type of meat being processed, a report from the World Bank Group has suggested that meat processing waste is generally about 35% of the animal weight (World Bank Group, 1998). Thus, as a guide to the reader, at least 7×10^7 tonnes of meat-derived waste is generated annually through processing.

This chapter addresses key issues associated with the overall management of waste resulting from the processing of red (beef, sheep) and white (chicken, pig) meat. The chapter covers various aspects of best practice in the management of such waste, including its minimization, its 'disposal' in an environmentally responsible manner and, perhaps of most importance, approaches to value addition to the waste – including the manufacture of key co-products for application in the food, biotechnology, medical and related industries. Most attention has been paid to this area of co-product recovery and application. This section highlights several key co-products, derived from blood and non-blood sources, that have been successfully isolated, characterized and commercialized. Key references have also been highlighted, together with sources for further information, notably via the internet.

13.2 Waste minimization and processing efficiency

This section outlines the drivers and modern approaches to minimization of waste material and maximization of efficiencies in the processing of red and white meat. Process optimization and good housekeeping practice have been highlighted, and approaches to overall minimization of end waste material and effluent have also been addressed. Enhancements in processing efficiency and minimization of waste can lead to a range of positive benefits for the meat processor, including: a reduction in manufacturing costs and resultant increase in profitability; cost savings on raw materials and energy; reduced environmental pollution; reduced waste disposal and treatment costs; improved worker health and safety; diminished need for 'end-of-pipe' solutions; and an enhanced image for the company and industry.

While meat cuts represent the most significant primary product from an abattoir, by- and co-products such as hides, bone, blood, fat and offal are also generated via the animal slaughtering process. The profitability of an abattoir can often depend on the extent to which these materials are minimized and/or utilized in a value-added manner. For example, edible co-products are further processed into saleable products, and inedible by-products are converted into animal feeds or feed supplements, often through the rendering process. Utilization of, and value addition to, meat processing wastes are covered in later sections of this chapter.

As with most food processing operations, the key waste and efficiency issues associated with abattoir operations include: (1) the high consumption of clean potable water; (2) the generation of high-strength/biochemical oxygen demand (BOD) effluent streams and by-/co-products; and (3) the consumption of energy. For some locations, odour and noise may also be problematic. Techniques for minimizing waste and maximizing operational efficiency include improved housekeeping practices by staff, process optimization, application of new technology and/or new product design. Indeed, through effective application of these techniques aimed at reducing inefficient use of resources and avoiding unnecessary generation of waste, the processor can benefit from reduced operating costs, reduced waste treatment/disposal costs and reduced liability.

13.2.1 Water

In the abattoir setting, large quantities of water are used for the watering and washing of livestock, washing of transport infrastructure (e.g. trucks), washing of carcasses and by-/co-products, and cleaning and sterilizing equipment and processing areas. Strategies for reducing water consumption can involve technical solutions or equipment upgrades. However, revision of operator practice and cleaning procedures can lead to some of the most

significant gains in efficiency and waste reduction. Suggested approaches are summarized in Table 13.1.

13.2.2 Effluent

Most water consumed at abattoirs ultimately becomes effluent. Abattoir effluent contains high levels of organic matter due to the presence of manure, blood, fat and other animal remains. The effluent can also contain high levels of sodium, phosphates and nitrates. The most significant contributors to the organic load include blood and fat-containing material, and blood represents the major contributor to the nitrogen content of the effluent stream. High contents of sodium and phosphorus can also be present in effluent, and these usually originate from manure and stomach contents. At those plants where rendering of animal remains occurs, the effluent typically represents the single most significant source of pollutant load from the abattoir. Usual strategies for reducing the pollutant load of abattoir effluent focus on excluding blood, fat, manure and animal remains from the

Table 13.1 Suggested approaches to minimization of waste and maximization of efficiency in modern meat processing operations

Area of operation	Suggested approaches to operational efficiency
Water use	• Dry cleaning prior to final wash/rinse with water. • High-pressure/low-volume systems. • Automatically operated equipment and shut-off valves. • Dry dumping techniques. • Reuse of 'grey' and cooling water for some non-critical cleaning operations (e.g. washing livestock).
Effluent streams	• Segregate organic material from the slaughtering operation. • Collect solid material (by-/co-products) for waste value addition. • Install screens to eliminate solid material from drains. • Limit fat in water effluent through use of low-pressure washing of carcass. • Segregate high-BOD streams from others and manage them separately.
Energy consumption	• Implement 'switch-off' programmes and install power-down/off equipment where appropriate. • Enhance insulation on heating/cooling systems. • Recover waste heat from various unit processing steps. • Install more-energy-efficient equipment. • Implement a preventative maintenance programme to ensure maximum efficiency of equipment at all times. • Eliminate leak points for hot water and steam.

effluent stream. Implementation of such a strategy means isolating materials from the effluent stream before they enter drains and using alternatives, where available, to wet cleaning methods. Approaches to the minimization of effluent are summarized in Table 13.1.

13.2.3 Energy

In an abattoir, thermal energy is used to heat water for cleaning, scalding for hair removal, rendering, blood coagulation and blood drying. Approximately 80% of total energy consumed by abattoirs is provided by thermal energy from the combustion of fuels in on-site boilers, and the remaining 20% is usually provided by electricity – used for operating equipment in the slaughter and boning areas, for by-/co-product processing, for refrigeration and for compressed air. Typical ranges for energy consumption are 1200–4800 MJ/tonne of hot standard carcass weight. The consumption of energy is an area where considerable savings can be made almost immediately with no capital investment, through simple housekeeping approaches. Additional savings can be made through the use of more-energy-efficient equipment and heat recovery systems, although these require capital investment. Approaches to maximization of energy savings are summarized in Table 13.1.

In summary, waste can be minimized and processing efficiency maximized in a modern abattoir and meat processing operation through a number of worker and process/product improvements. These include (1) housekeeping improvements to work practices and proper equipment maintenance (typically low cost); (2) optimizing existing processes/equipment to reduce resource consumption (typically low/medium cost); (3) adapting and adopting new technologies to reduce resource consumption and minimize waste via enhanced operating efficiencies (typically capital intensive but often with short payback time); and (4) changing product design to reduce waste disposal/treatment, reduce energy consumption and enhance efficiency of production (typically long-term, but often with large paybacks).

13.3 Responsible waste disposal

This section addresses the key modern issues in ‘disposal’ of meat processing waste, including environmental considerations and the issue of transmissible spongiform encephalopathies (TSEs) (e.g. bovine spongiform encephalopathy (BSE)), with regard to destruction of infectivity.

13.3.1 Waste management planning

A range of varied wastes are generated as a result of meat processing. All of these wastes require disposal in one form or another, as dictated by local

legislation, local environmental sensitivity and, in some instances, the requirements of the markets into which products and co-products are sold.

All of these requirements (legislative, environmental, health and safety, market, etc.) are captured, cross-referenced and addressed in a single waste management plan for the operation. While the specifics for each jurisdiction may vary, some typical 'key steps' in developing a waste management plan (EPA Victoria, 1993) are to describe:

- Background: the relevant legislation that governs plant operation.
- Production processes: the activities that generate waste including the types and amounts of waste generated and procedures for dealing with accidents, spills and other incidents that affect waste management.
- Waste assessment: how the waste is disposed and current costings. Procedures for identifying and implementing opportunities to minimize the amount of waste generated.
- Evaluation and feasibility: technical and economic feasibility analyses for each waste minimization opportunity including the best available technology (BAT) and commonly available technology (CAT).
- Selected projects: list of waste minimization projects with expected costs and savings.
- Implementation: a timetable and funds for implementation.
- Review: indicators or other criteria on which the performance of waste management practices will be assessed, and how often performance is assessed.
- Details of staff training.

The four broad categories of waste generated through meat processing are waste odours, wastewater, organic solid waste and specified risk material (SRM). Unless managed well, each of these categories has the potential to significantly undermine the operational viability of a meat processing plant.

13.3.2 Waste odours

The major sources of odour from slaughterhouses emanate from manure, paunch content and rendering. Manure and paunch content odours can be effectively managed through routine collection and composting of these materials.

The biodegradable nature of rendering raw materials has a direct impact on the generation of odours. Storing these raw materials for prolonged periods at ambient temperature, prior to their separation and stabilization via the rendering process, both increases the production of noxious odours and diminishes the value of the resultant protein meals and fat fraction. Rapid processing minimizes noxious odours and maximizes the quality of rendered products. In order to minimize odour emissions, modern plants

totally enclose the entire rendering process line to allow entrapment and treatment of odours. Biological scrubbers operate via microbial decomposition of air pollutants absorbed in the scrubbing medium.

Burning rendering gases in an existing boiler presents a simple and effective method for significantly reducing noxious odour emissions. Steam collected from rendering cookers, driers and evaporators is passed through a cyclone to separate solids, and a heat exchanger to dewater the moist air. The condensate is discharged to wastewater treatment and the remaining odour laden air is burned. This method is particularly applicable to the low-volume, high-concentration odours generally associated with rendering and has been described in the BREF (best available techniques (BAT) reference) document from the European Commission (BREF SA, 2003).

13.3.3 Wastewater

The consumption and discharge of water by food industries, in particular the meat processing industry, could be considerably minimized by means of water re-use (Casani *et al.*, 2005). However, water re-use has not been widely practised due to a number of constraints and barriers. These constraints range from economic and legislative barriers, to the perceived complexity of testing and documentation required, and the negative perception and poor acceptance of water re-use by the consumer.

Previously, meat processors have combined wastewater from all operations and directed them to a single treatment system. More recently, meat processors have seen the benefits of segregating various process water streams so that direct re-use is possible or water treatment can be optimized. An example of direct re-use is slaughter-floor knife and equipment sterilizer water being collected for stock and stockyard washing. Most sterilizer water is only lightly contaminated, and due to the high temperatures, is free from pathogenic bacteria. Other examples of optimized water treatment are re-use of chiller shower water from meat processing (Mavrov and Belieres, 2000), carcass scalding water from swine processing (Miller *et al.*, 1994), and carcass washing water from poultry processing (Chang and Sheldon, 1989).

A significant number of practical suggestions and recommendations on how water utilization can be minimized and waste discharge reduced can be found in McNeil and Husband (1995). In particular, a significant body of knowledge addressing the waste issues facing the meat processing industry has been assembled by the European Commission (BREF SA, 2003). At the time of publication, November 2003, this BREF document on the slaughterhouse and animal by-product industries, represented a compendium of the BAT for meat processing in Europe.

The treatment of wastewater involves the removal of foreign suspended and/or dissolved solids, organic compounds and inorganic salts. A hierarchy

of treatments is commonly used to effect this removal. These treatments are categorized as primary, secondary and tertiary treatments. Primary treatment might involve mechanical screening, fat separation, dissolved air flotation or coagulation/flocculation (BREF SA, 2003). Secondary treatment generally involves a form of biological treatment, which converts soluble and colloidal materials into biosolids. Both aerobic digestion (activated sludge) and anaerobic digestion are used either individually or in combination. Tertiary treatment such as filtration – e.g. using sand filters, reed beds (constructed wetlands), coagulation/flocculation or precipitation – are sometimes used to make the treated effluent suitable for discharge to a watercourse.

Constructed wetlands have found favour in the Czech Republic where over 100 operations have been established (Vymazal, 2002). Construction costs are equivalent to those for conventional treatment systems, but the operational and maintenance costs are significantly lower.

A recent publication on coagulation/flocculation of slaughterhouse wastewater reported improved performance through the use of anionic polyacrylamide as a coagulant aid, in combination with a range of commercially available coagulants (Aguilar *et al.*, 2005). The use of anionic polyacrylamide increased the efficiency of flocculation and the speed of settling, while reducing the overall cost of the process.

13.3.4 Organic solid waste

The major options for the stabilization and re-use or disposal of organic solid waste from meat processing and rendering operations are composting (aerobic digestion) and vermicomposting, anaerobic digestion, disposal to landfill and incineration (Salminen and Rintala, 2002).

Incineration has recently been adopted by the meat industry as a viable option as it is considered among the most effective methods for destroying potentially infectious agents (Ritter and Chinside, 1995).

Composting is an aerobic biological process whereby organic materials are decomposed to form a product that has value as a soil conditioner or fertilizer. Composting is generally conducted in windrows (elongated mounds) or reactors, in order to achieve near-optimal conditions. Composting requires particular conditions with respect to temperature, moisture, aeration, pH and carbon/nitrogen (C/N) ratio to optimize biological activity at the various stages of the process (Sharma *et al.*, 1997). If good quality compost is the desired result, then the moisture content (<65%) and the C/N ratio (30–35%) are of particular importance. From the perspective of abattoir operations, the amount of manure, paunch content or sludge intended for composting may be limited by these requirements. Alternatively, if all available material is to be recycled via composting, additional composting raw materials may be obtained from neighbouring industries to balance the moisture content and C/N ratio.

A useful fact sheet (Natural Rendering: Composting Livestock Mortality and Butcher Waste) offering guidelines for on-farm disposal of fallen or dead stock via composting, is available online from the Cornell Waste Management Institute (2002). However, this reference adds a note of caution, notably that any animals exhibiting signs of neurological disease must be reported to the authorities and disposed of in the recommended manner.

Vermicomposting refers to the use of earthworms to augment or replace conventional composting. This process involves the physical breakdown (comminution) of organic matter by the worms and the action of intestinal microorganisms and the microorganisms that inhabit vermiculture substrates (Pizl and Novakova, 2003). The major attraction of vermicomposting is the improved market value of stabilized organic fertilizer produced, as compared with the soil conditioner/fertilizer generated by standard composting. Several commercial vermicomposting facilities have been reported in North America and Asia (Sharma *et al*., 1997) along with a cautionary mention of issues of increasing production costs and low wholesale price margins while retail profits are high.

Some attention has been focused on the importance of microscopic fungi to the process of vermicomposting cattle manure (Pizl and Novakova, 2003). Earthworm (*Eisenia andrei*) feeding experiments showed that generally the addition of microfungi to vermiculture substrates resulted in an increase in growth rates; however, the only statistically significant increase was related to the addition of *Aspergillus flavus.* This result raises the prospect of seeding commercial vermiculture substrates with particular fungi to enhance the vermicomposting process.

Anaerobic digestion is a biological process where organic material is broken down under anaerobic conditions to yield methane, stabilized sludge and partially treated wastewater. Many variations of anaerobic digestion have been, and continue to be, evaluated in order to improve digester performance and maximize the systems' ability to cope with variation in abattoir solid waste output. Some advantages of this system are the production of methane as a by-product, and the ability to handle the high organic loads associated with abattoir waste. Salminen and Rintala (2002) have published an excellent review of the degradation pathways involved in the conversion of carbohydrates, proteins and lipids (typical of solid slaughterhouse waste) through to methane. In addition, these authors have addressed the two major process-limiting by-products (long-chain fatty acids and ammonia) of anaerobic digestion.

The process-limiting effects of ammonia have again been evaluated and these effects have been overcome in trials using a two-stage anaerobic digester (Wang and Banks, 2003). In this trial, the authors de-coupled the solids and liquids retention time in a hydrolysis/acidification reactor. This promoted the effective stripping of ammonia from the process liquors in the first stage, while allowing the necessary solids retention time. This

system, described as a hydraulic flush reactor (HFR), showed significant improvements in process efficiency (compared with a conventional single-pass reactor) as measured by total solids (TS) reduction, chemical oxygen demand (COD) removal and biogas conversion.

13.3.5 Animal feed bans and specified risk material (SRM)

The appearance of mad cow disease or bovine spongiform encephalopathy (BSE) during the 1980s has had an enormous and far-reaching effect on the processing of meat industry co-product materials. The BSE epidemic, first recognized in the United Kingdom (UK), has now been detected in the livestock of many countries (http://www.oie.int/eng/info/en_esbincidence.htm).

The subsequent appearance of variant Creutzfeldt–Jacob disease (vCJD) in humans is due to the same agent that caused BSE in cattle (http://www.who.int/mediacentre/factsheets/fs113/en). Both of these related diseases, collectively referred to as transmissible spongiform encephalopathies (TSEs), are characterized by a sponge-like degeneration of the brain, and both diseases are fatal. TSEs continue to force radical changes to the way in which meat industry co-products are collected, handled, processed, marketed and regulated.

Initially, large numbers of cattle were culled in an attempt to limit the spread of BSE. As theories on the transmission of the infection were supported by scientific evidence, two primary lines of defence were suggested to curtail the bovine and human diseases. Firstly, the use of ruminant tissues in ruminant feeds must be prohibited to minimize further BSE infection. Secondly, all tissues that are likely to contain the infective agent (designated SRMs) must be excluded from any animal or human food chain. Uptake of these defensive measures into industry regulation was not rapid or uniform from one country to another.

Over time, these two lines of defence have been extended. At times, the changes were driven by new scientific evidence, and at times they were driven by political influences. The original UK ban on feeding ruminant protein to ruminants has extended to a European Union ban on the use of all animal protein in livestock rations.

The original UK ban on specified bovine offals (later SRMs) was altered and adopted by the European Union. This ban removes the tonsils, intestines (from duodenum to rectum) and mesentary from cattle of all ages. In cattle aged over 12 months old, the skull (including brains and eyes), spinal chord and vertebral column are also removed from any food chain (http://www.food.gov.uk/bse/beef/controls). Once removed, this material can be rendered in dedicated rendering plants to generate meat and bone meal (MBM) and tallow. The only approved use of these products in the UK is as a combustible fuel. MBM has an energy content of about two-thirds that of coal, while the calorific value of tallow is approximately 90% of fuel oil

(http://www.ukra.co.uk/Factsheet6.htm). If no market can be found for these fuels, then disposal involves incineration or high-pressure sterilization prior to land-fill dumping.

These changes have had an enormous impact on rendering, which is an integral and vital part of the meat processing industry. Rendering operations have been forced by regulation to commission new plants and upgrade existing plants in order to cope with the fallout from the BSE epidemic, while at the same time, their traditional markets have disappeared. The future direction of BSE policy and the possible relaxation of the TSE Regulation in the European Union have been canvassed by the European Commission (2005).

13.4 Waste value-addition

This section focuses on co-product component recovery in meat waste processing. Important co-products, derived from both blood and non-blood sources, have been highlighted, including protein hydrolysates, bone meal, blood plasma, blood cells, collagen and key biochemicals, noting successful approaches to their recovery, stabilization, characterization and application in the animal feed, biotechnology, nutraceutical/functional food, cosmetics, and medical/veterinary sectors.

13.4.1 Blood-derived co-products

The principal components of meat are water, protein and fat, with a significant level of vitamins and minerals with a high bioavailability (Fernandez-Gines *et al.*, 2005). Blood accounts for ~3–5% of an animal's live weight and contains ~18–19% protein, similar to that found in lean meat, with the protein contents of plasma and red blood cells (RBCs) being ~7–8% and 34–38%, respectively. Blood is the first co-product obtained while processing animals for meat. Blood should be collected in a hygienic manner for food use with or without anticoagulants, and serum can be separated from RBCs with the help of a cream separator and can be preserved under refrigeration. From serum and RBCs, protein isolates can be prepared separately by spray drying, freeze drying, solvent precipitation or by chromatographic techniques (Mandal *et al.*, 1999).

Blood proteins make up the largest part of the meat proteins market by volume. Blood products typically contain ~70% protein, and blood components such as plasma, haemoglobin, immunoglobin and globin are used in a number of food applications. Plasma is often used in sausages to help bind and emulsify meat, haemoglobin is used in pet food and to colour black pudding, immunoglobin can be used as an emulsifier in meat products, while globin is used in mincemeat and hamburgers, where it improves textural properties and to some extent helps bind ingredients together. Blood

proteins such as immunoglobulin are also used in the pharmaceutical industry in the preparation of antibodies and other immunoassay products (Frost & Sullivan, 2005a). The pharmaceutical industry also creates isolates such as creatine from blood proteins, to be used in nutraceutical applications. Although the demand from the pharmaceutical industry is increasing, it only constitutes a small fraction of the blood protein market.

A meat product formulated with mechanically deboned poultry meat and bovine blood has been used as an iron supplement to evaluate its effect on the haematological parameters of school children with ferropenic anaemia. Children with subnormal haematological parameters with a diagnosis of ferropenic anaemia received the meat product over 30 days. The results indicated that the consumption of the meat product increased the haematological parameters in children with ferropenic anaemia, with the authors suggesting that the product could be used in social programmes for school children with this deficiency in order to prevent and recover from ferropenic states (Rangel *et al*., 2003).

Cooked hams have been formulated with bovine plasma and/or red cells with the objective of evaluating the effect on yield, moisture, protein and palatability. The results indicated that products with plasma at 3.6% and 5.4% protein gave a higher yield. Addition of plasma, red cells or a mix of both at 1.8% protein did not affect yield. Final moisture and protein content were unaffected, but sensory evaluation indicated that only products with added red cells were unacceptable. The study concluded that plasma proteins are more suitable than red cell proteins for addition to these products and that it is necessary to add more than 1.8% of plasma proteins to increase yield (Rodas *et al*., 1998).

The blood plasma of calves and cattle has been reported to be a good nutritional supplement to meat products due to the essential amino acids it contains (Belkot, 2001). The pet food industry also uses blood proteins as a protein source as they contribute all the amino acids needed for a carnivorous pet diet. The pet food industry is a fast-growing market in Europe, and demand from the pet food industry for blood proteins is expected to increase in coming years.

Dehydrated blood plasma is also used as a protein ingredient for its gelation properties, especially in meat derivative products. Generally, blood is concentrated by either ultrafiltration or evaporation under reduced pressure. The resulting pH and ash concentration (mineral salts and sodium citrate) allow modification of the gelation properties of the products (Dailloux *et al*., 2002).

Bovine blood has been fractionated into blood plasma protein concentrate, red blood cell concentrate, globin isolate and a carboxymethylcellulose–heme-iron (CMC–heme) complex. The amino acid content in plasma protein concentrate has been shown to be well balanced and produced net protein utilization and a net protein ratio equivalent to 95% that of casein. Globin isolate (similar to 91% protein) is deficient in

isoleucine and S-containing amino acids and was unable to support rat growth at a 10% concentration in the diet. Red blood cell concentrate and the isolated CMC–heme complex were good sources of bioavailable iron. Iron availabilities for CMC–heme and whole blood cell concentrates were 64 and 70% of ferrous sulphate, respectively (Duarte *et al*., 1999).

Globin is an edible protein that is obtained in large quantity from animal blood and can be used as an ingredient in a variety of meat products. However, globin has been reported to have a low solubility at neutral pH and little advantage compared with other proteins used in the food industry. However, globin's functional properties can be improved via hydrolysis with citric acid. These aggregates of globin hydrolysates were composed of the beta-chain, originating from the native globin and beta-1, originating from the beta-chain by cleavage between 99 (Asp) and 100 (Pro) of the beta-chain (Liu *et al*., 1996).

For the preparation of globin protein isolates, decolourization is one of the important steps. These protein isolates have excellent functional properties and nutritive value, which make them suitable for use in meat and bakery products. Furthermore, they can replace the use of chicken egg to some extent in food products without affecting acceptability (Mandal *et al*., 1999).

Response surface methodology has been used to investigate the effects and interactions of blood plasma (0–2%), microbial transglutaminase (0–0.6%) and kappa-carrageenan (0–0.8%) on water binding, textural and colour characteristics of pork gels. An increased addition of both kappa-carrageenan and plasma protein favourably affected thermal stability of pork batters, but the effects were attenuated by increased blood plasma and kappa-carrageenan addition, respectively. The combination of kappa-carrageenan with blood plasma was unable to improve springiness or cohesiveness and led to the formation of less cohesive and more brittle gels. Microbial transglutaminase had little effect on colour and water binding properties although it was found that its addition improved cohesiveness and elastic properties of meat gels processed with blood plasma. Addition of blood plasma produced an increase in lightness and a decrease in redness of pork gels, while the presence of kappa-carrageenan resulted in lower chromametric values (Jarmoluk and Pietrasik, 2003).

Animal blood is a by-product from meat processing and contains a variety of proteins that can be reclaimed. The efficiency of protein precipitation from blood by the use of hydroxyethyl cellulose (HEC) has been reported to be similar to that precipitated by the use of CMC with both methods being superior to precipitatation by trichloroacetic acid. The plasma protein–HEC complex was reported to contain a large amount of essential amino acids and electrophoretic separation of plasma protein resulted in complex albumin forming the major fraction (El-Sayed *et al*., 1998).

The recovery of protein from red cells of bovine blood has been achieved by hydrolysis of red cells with papain and up to 32% of the heme group being released by this process was separated by ultrafiltration. Treating the retentate with sodium hypochlorite produced a white product that was 75% protein, nearly 1% ash and almost tasteless. The results indicated that the protein contained in this fraction had a good amino acid profile for use as an ingredient in formulated foods for human consumption (Gomez-Juarez *et al.*, 1999).

13.4.2 Non-blood derived meat co-products

When meat animals are processed, considerable quantities of trim are produced which is sorted according to the lean meat content and sold to sausage makers or ground for hamburger meat. Surplus fatty trimmings are rendered to recover the fat from beef and lard from pork as tallow (Clark, 2005). Rendering involves cooking, usually by direct steam injection, followed by centrifugation and drying of the fat and protein fraction. The protein from edible rendering can be used in animal feeds, while that from inedible rendering can be used as a fertilizer. Tallow and lard are used in baking and frying, although recent nutritional concerns with respect to saturated fats are affecting their use. The aqueous phase from the above centrifugation step is usually called 'stick water' and may be concentrated as a source of beef flavour. If the 'stick water' is not concentrated, it can represent a significant waste disposal challenge (Clark, 2005).

Body condition score (BCS) of beef cows has been shown to have a significant effect on by-product yield and value. By-product weights – including blood, bone meal and a variety of organs – have been obtained during the slaughter process and by-product yields have been calculated as a percentage of the animal's live weight taken 24 h before slaughter. Total by-product value was quadratically related to BCS. The results from this study indicated that the BCS of cows at the time of slaughter had a profound influence on by-product yields and values of by-products that are credited back against the cost of production to the beef cattle producer (Apple *et al.*, 1999).

Refined meat protein isolates are produced from non-blood sources such as pig rind, which is a source of collagen. Ultrasonic fragmentation of the myofibrils and subsequent solubilization in buffer have been used to isolate intramuscular collagen in high yield and purity, and maintains intact fibres (Avery and Bailey, 1995). Many meat protein isolates contain over 90% protein and are used for their emulsifying properties in hotdogs, replacing carrageenan. The majority of such products are derived from pork, with smaller amounts coming from chickens.

Collagen is a natural constituent of bovine skin and can be converted by suitable procedures to a very thin collagen film which has food-grade

properties and is edible. In the production of net-wrapped raw sausage, such a collagen film may be used both for enclosing the sausage meat and as a separating or release layer between the sausage meat and the net (Bisson and Weisenfels, 1992).

Computer-assisted simulation has been used to study the effect of collagen content on the biological value of meat proteins. It has been shown that an increase in the collagen content from 2.5% to 15–20% of the total amount of proteins contained in minced meat tangibly enhanced protein utilization for tissue synthesis. The above collagen content in meat products heightens their nutritional and biological value and renders them more suited to human metabolism (Rogov *et al*., 1992).

It has also been reported that collagen, in conjunction with soy protein and carrageenan, is able to increase the water holding capacity and improve the texture of deli rolls (Daigle *et al*., 2005). Collagen proteins have also been used as aids for the improvement of technological and sensory properties of meat products and ready meals (Marggrander, 1995).

The effects of pork collagen in emulsified and whole muscle products has been evaluated. Eight frankfurter treatments (0–3.5% pork collagen) and four ham treatments (0–3% pork collagen) were formulated and frankfurters and hams were evaluated for cooked yields, purge, colour, texture and sensory characteristics. Incorporation of pork collagen at 1% and above, significantly increased cooked and chilled yields in frankfurters but did not have any effect in hams. Purge was significantly reduced in both frankfurters and hams after 4 weeks of storage, while sensory difference testing showed no significant difference up to a 2% usage level of pork collagen in both frankfurters and hams (Prabhu *et al*., 2004).

The quantity of collagen has also been used as an index of quality of raw materials employed in the preparation of meat products. It has been reported that a high content of collagen and, as a consequence, of connective tissue found in meat samples, was a fairly good index of poor quality of the raw materials employed in the preparation of meat products (Bonafacci *et al*., 1992), with collagen also being a good predictor of protein quality (El, 1995).

Freeze drying of collagenous material from chicken feet skins and tendons, has been shown to affect physical functionality by increasing solubility, gel strength, emulsion stability and water holding capacity at 60 °C. Such modifications to this co-product of chicken processing would suggest the potential use of these products as functional ingredients in meat products (Alves and Prudencio-Ferreira, 2002).

Gelatin is a protein product commercially made from pork skin, cattle bones, calf skin or fish skin. It is prepared by the thermal denaturation of collagen (the protein in connective tissue and bones) isolated from animal skin and bones with very dilute acid. Gelatin contains a large number of amino acids such as glycine, proline and 4-hydroxyproline residues (Frost & Sullivan, 2005b).

Gelatin is a heterogeneous mixture of water-soluble proteins obtained from collagen material and is a highly digestible food protein that can be added to other foods to increase protein content. However, gelatin is used primarily for its numerous functional properties. Gelatin is probably the most versatile of the animal proteins in terms of functionality. Gelatin has a variety of applications as an ingredient, particularly in the confectionery industry – where it provides mouth-feel sensory attributes – but also in baked goods, dairy products, the meat industry, pet foods and the pharmaceutical industry. In confectionery products, the addition of gelatin results in improved foaming, gelling and final solidification of the candy in such a way that it dissolves slowly once ingested. In certain products such as marshmallows, it lends characteristics such as texture and prevents the sugar from crystallizing. The dairy industry has used gelatin to stabilize and improve the texture of yoghurt and sour cream products and to provide a smooth texture and mouth feel to cheese products (Frost & Sullivan, 2005b).

Gelatin is also used in the photographic film, cosmetics, adhesives and printing industries; it can also be used in microencapsulation processes and as an additive in finishing products for the leather industry (Cabeza *et al.*, 1998). Specifically, gelatin is used in food applications as a gelling agent, a thickener, a film former, a protective colloid, an adhesive agent, a stabilizer, an emulsifier, a foaming and whipping agent, and a beverage-fining agent. The pharmaceutical industry uses gelatin to manufacture hard- and soft-gel capsules, to coat vitamins and tablets, and to manufacture blood plasma substitute. Soft-gel capsules are being used more extensively in over-the-counter pharmaceuticals, vitamins, nutraceuticals and cosmetics. Soft-gel capsules are odour-free and are easier to swallow than hard capsules and also tend to be more aesthetically appealing to consumers and have longer shelf lives. Gelatin is the primary material used in soft-gel capsules (Frost & Sullivan, 2000). In cosmetics, gelatin is used to encapsulate bath oils, while the photography industry uses high technical specification gelatin for silver halide photography and other imaging applications (Frost & Sullivan, 2005a).

Gummy products have been in high demand among American consumers for over a decade and are continuing to drive market growth for gelatins. Gummy candies are popular because of the wide variety of shapes, colours and flavours available for these products. Gelatin gives gummy candies their chewy structure and is the primary gelling agent of American gummy candies (Frost & Sullivan, 2000).

Hydrolyzed gelatin has a protein content greater than 90% and is used as a dietary supplement in sports drinks and protein bars. It is rich in the amino acids glycine and proline, which are suited to support of collagen synthesis. Medical studies indicate that hydrolyzed gelatin may improve joint pain and enhance mobility in people with joint disease. Hydrolyzed gelatin also has direct applications in the treatment of joint pain in strength-training athletes. Studies have also indicated that hydrolyzed

gelatin may have a positive impact on post-menopausal bone maintenance by extending the effect of calcitonin. Hydrolyzed gelatin also has applications in the treatment of periodontal disease and is used in the form of a biodegradable matrix to deliver subgingival sustained-release chlorhexidine for supplemental treatment of periodontal pockets (Frost & Sullivan, 2000).

Prion diseases are a group of mammalian diseases that produce large vacuoles in the cortex and cerebellum of infected subjects. Prion diseases are caused by proteinaceous infectious particles called prions. Examples of prion diseases include BSE or 'mad cow disease', which affects cattle, and CJD, which affects humans. Outbreaks of BSE in the UK and deaths resulting from CJD have created worldwide scepticism about the safety of bovine-derived products originating from BSE-infected countries. Gelatin has been placed under scrutiny since it is produced in some instances from the bones and hides of cattle. There is presently no evidence that BSE can be transmitted to humans through gelatin products. However, uncertainty about the BSE infectivity of gelatin has raised questions about the safety of gelatin and has been a large contributing factor to the rise in popularity of gelatin replacers (Frost & Sullivan, 2000).

Industrial veal hydrolysate has been produced enzymatically for possible use as a gelatin-replacing ingredient for human consumption. Protein digestibility was determined by a pH-stat method and cell dialysis. Amino acid composition including 4-hydroxyproline, allowed determination of connective tissue, amino acid score and protein digestibility-corrected amino acid score. A high correlation was found between true digestibility and the pH-stat method. The meaty flavour and gelling properties of veal hydrolysate could make it useful for high-quality soups, sauces and gravies (Linder *et al*., 1997).

Dehydrated meats are primarily used as flavour enhancers in gravies, soups, stews and other food applications, but are also found in ready-to-eat military rations, survival rations, and outdoor sports and recreation rations. Beef, chicken and pork are the primary sources for dehydrated meats. Dehydrated meats are also referred to as bouillon in many food applications (e.g. beef bouillon, chicken bouillon). Dehydrated meats come in powder, granule, diced or jerky form and have protein contents ranging from 20% to 52% (Frost & Sullivan, 2000).

Meat proteins, in general, have good functionality – such as water binding and emulsification capabilities – and form strong elastic gels, they have good flavouring properties and a high nutritional value (related to the high essential amino acid content and minerals such as calcium, potassium, phosphorus and iron).

The effects of time and temperature on the water binding ability of chicken skin connective tissue (CCT) and its ability to form model gels have been studied, and it has been shown that CCT gels can be used as water binders in reduced-fat bologna. Processing qualities, and textural and

sensory attributes were also analyzed to assess the feasibility of manufacturing a reduced-fat processed poultry product containing a modified poultry by-product. Heating (60 °C) CCT for 0.5 h allowed the formation of model CCT gels with added water decreasing CCT gel fat, protein and collagen content, and also decreasing hardness due to a protein (collagen) dilution. All bologna treatments exhibited acceptable sensory attributes and the data indicated the feasibility of using lower-water CCT gels as texture-modifying agents in reduced-fat comminuted meat products (Osburn and Mandigo, 1998). Another study by the same group has also demonstrated similar properties for pork connective tissue gels (Osburn *et al.*, 1997).

MBM is a high-protein agricultural commodity that currently has few applications other than as an animal feed. Unmodified MBM has poor functional properties, due to its low solubility. A recent study has demonstrated that MBM can be extrusion-processed along with sodium caseinate to produce a useful plastic material which has been developed for use as a dog chew toy. For this application, elastic modulus (stiffness) was a key characteristic. The influence of moisture content on the glass transition temperature and elastic modulus reflected the plasticization of this material by water. On the basis of a comparison with a commercially available dog chew, the range of stiffness achieved, 0.25–2.50 GPa, encompassed the values appropriate for a dog chew. The results showed that a particular desired stiffness can be maintained by applying an edible moisture barrier to the surface of the material (Garcia *et al.*, 2004).

MBM contains appreciable amounts of nitrogen (N), phosphorus and calcium, making it an interesting fertilizer for various crops. The effect of MBM as an N fertilizer has been evaluated in pot and field experiments. The soils used in the pot experiment were peat and a sand–peat mixture, both low in content of plant nutrients. The field experiment was carried out on a silt loam. In the pot experiment, increasing amounts of MBM gave significantly increased yields although there was, in part, N immobilization shortly after seeding the soil based on peat organic matter. In the field experiment there was no period of N immobilization and a good N effect was found for small amounts of MBM (total N, 50 kg/ha). At a total N content of 100 kg/ha there were no significant differences in grain yield of spring wheat between the treatments with MBM, mineral N fertilizer, and a combination of MBM and mineral N fertilizer (N 50 kg/ha from each). The results indicated that the relative N efficiency of MBM compared with mineral fertilizer was 80% or higher, if MBM was applied to cereals in spring (Jeng *et al.*, 2004).

De-fatted meat and bone meal protein concentrate (MBMPC) has also been reported to have significant adhesive properties. Adhesiveness increased linearly as the MBMPC concentration increased up to 20%. The highest adhesiveness was observed in the range of 70–90 °C with improved adhesiveness and water resistance being observed with 0.05% glutaraldehyde treatment (Park *et al.*, 2000).

Data from animal feeding trials have indicated that the protein value of MBM and poultry by-product meal is limited by the amount of metabolizable methionine they contain (Klemesrud *et al*., 1997). The potential for improving the efficiency and rate of dietary nitrogen utilization in Holstein steers, by feeding an amino acid-balanced mixture of animal by-product protein sources, in combination with urea, has also been evaluated. A combination of porcine meat and bone meal, fish meal, hydrolyzed feather meal and blood meal was also formulated as an undegradable intake protein blend to complement amino acids derived from microbial protein synthesis. The results demonstrated that use of an amino acid-balanced blend of animal by-product protein sources did not improve the efficiency of dietary nitrogen usage when added to corn-based diets formulated to meet the nutrient requirements of rapidly growing steers (Knaus *et al*., 2001). MBM has also been evaluated as a potential substitute for fish meal in the diet of various fish species, although the results have not generally been positive with some studies reporting that the final body weight of fish, the weight gain, feed efficiency and protein efficiency ratio of fish fed MBM diets tended to decrease as the proportion of MBM in the diet increased (Kikuchi *et al*., 1997).

Brine-incorporated meats have become major novelty food items in the present fresh meat retail market in the United States. Brines are used to 'enhance' products' moistness and juiciness; however, the physicochemical processes involved in water binding and entrapment in the meat protein matrix have not been completely elucidated. The results of a recent study have shown that the dynamics of brine penetration into muscle fibrils were specific to phosphate types, with pyrophosphate and tripolyphosphate having the highest efficacy. Both phosphate treatments produced a transverse expansion of the myofibrils with a simultaneous extraction of myosin from the ends of the A-band in the sarcomere. These structural/biochemical changes resulted in substantial swelling of muscle fibres, i.e. an enhanced water uptake and immobilization. Furthermore, depending on the type of proteases employed, soy protein hydrolysates or peptides interacted differently with myofibrillar proteins, producing an array of morphologies and rheologies of protein gels that played a major role in water immobilization in salted pork products (Xiong, 2005).

A macromolecular meaty-flavour enhancer has been fractionated from a commercial beef extract. The macromolecular fraction was obtained by dialysis and separated by anion-exchange chromatography, copper-chelate chromatography and gel-filtration chromatography. Two fractions were isolated as active meaty-flavour enhancers. Determinations of the amino acid compositions and amino acid sequences of the isolated fragments showed that the two active fractions consisted of collagen and tropomyosin. The macromolecular material obtained from heated collagen and tropomyosin in the low-molecular-weight fraction of beef soup stock enhanced the meaty flavour. These results suggested that collagen and tropomyosin were

precursors of the macromolecular meaty-flavour enhancer (Kuroda and Harada, 2004).

Functional protein isolates have been separated from coarsely and finely ground mechanically deboned (MDB) turkey using a combined alkaline solubilization and isoelectric precipitation method. Although the two types of MDB turkey were quite different in original composition, the compositions of the resultant protein isolates were very similar. Almost all of the fat, collagen and calcium originally present in the MDB turkey were removed during the processing. Gels made from the resultant protein isolates showed good textural properties and water retention ability with a lighter colour than the original MDB turkey (Liang and Hultin, 2003).

The enzymatic activities of a bovine spleen lysosomal-enriched extract have been characterized for their proteolytic potential. The extract showed a high activity of cathepsins, i.e. a considerable ability to degrade both myofibrillar proteins and collagen, as well as of exoprotease. Proteolytic activity was accompanied by acid lipase and esterase activities, and a minor peroxidase activity. The bovine spleen lysosomal-enriched extract may therefore be a useful tool for the tenderization and ripening of muscle foods such as meat, fish and their products (Melendo *et al*., 1998).

Market growth for meat co-products has been curtailed somewhat due to the farming crisis (BSE, foot and mouth) and due to high production costs (in comparison to plant-derived proteins such as soy proteins). While some of the restrictions (related to the BSE crisis) on the use of animal proteins in the food industry have eased, restrictions in the European Union on the use of meat proteins in animal feed applications are expected to remain until 2007 and therefore, the recovery of such markets is expected to be slow (Frost & Sullivan, 2005a).

13.4.3 Functional ingredients from meat

Dietary proteins are known to possess a variety of nutritional, functional and biological properties. Nutritionally, the proteins are a source of energy and amino acids, which are essential for growth and maintenance. Functionally, the proteins contribute to the physicochemical and sensory properties of various protein-rich foods. Furthermore, many dietary proteins possess specific biological properties that make these components potential ingredients of functional or health-promoting foods. Many of these properties are attributed to physiologically active peptides encrypted in protein molecules. Particularly rich sources of such peptides are milk and eggs, but they are also found in meat and many plants. These peptides are inactive within the sequence of the parent protein but can be released during gastrointestinal digestion or food processing (Korhonen and Pihlanto, 2003).

An angiotensin converting enzyme (ACE) inhibitory peptide has been isolated and purified from the hydrolysates of irradiated bovine blood

plasma protein. Blood plasma protein was irradiated to eliminate microbial contamination and was enzymatically hydrolyzed using the commercial proteases Alcalase, Esperase and Flavourzyme. An ACE inhibitory peptide was isolated using membrane filtration, gel permeation chromatography, and normal phase and reverse phase high-performance liquid chromatography. The purified ACE inhibitory peptide was identified to be a tripeptide, His-Pro-Tyr (Lee and Song, 2003).

The physiological functions of enzymatic hydrolysates of collagen or keratin contained in livestock and fish waste have been studied and results demonstrated that the enzymatic hydrolysate of meat meal, a collagen waste, showed strong ACE inhibitory activity ($IC_{50} = 0.6–2.8$ mg/ml). In contrast, the enzymatic hydrolysate of a mixture of horn and hoof, a keratin waste, showed high antioxidative activity. Thus, collagen or keratin contained in livestock and fish waste may be convertible to useful products by enzymatic hydrolysis, thereby providing new physiologically functional food materials (Ohba *et al*., 2003).

Inhibitory activities against ACE of enzymatic hydrolysates of porcine skeletal muscle proteins have been investigated. Myosin B, myosin, actin, tropomyosin, troponin and water-soluble proteins extracted from pork loin were digested by proteases – including pepsin, alpha-chymotrypsin and trypsin. After digestion, hydrolysates produced from all proteins showed ACE inhibitory activities, and the peptic hydrolysate showed the strongest activity. In the case of myosin B, the molar concentration of peptic hydrolysate required to inhibit 50% of the activity increased gradually as digestion proceeded. The hydrolysates produced by sequential digestion with pepsin and cc-chymotrypsin, pepsin and trypsin, or pepsin and pancreatin showed weaker activities than those digested by pepsin alone, suggesting that ACE inhibitory peptides from peptic digestion might lose their active sequences after digestion by the second protease. However, the hydrolysates produced by sequential digestion showed stronger activities than those by chymotrypsin, trypsin or pancreatin alone. These results suggested that the hydrolysates of porcine meat were able to show ACE inhibitory activity, even if they were digested *in vivo*, and that pork might be a useful source of physiologically functional factors (Katayama *et al*., 2003).

Carnosine is a natural antioxidant present in skeletal muscle. A number of patents describe the potential use of carnosine as a therapeutic agent in applications such as: wound healing; treatment of hypertension and trauma; treatment of cataracts and stomach and duodenal ulcers; and also as a bactericidal, anti-inflammatory and immunomodulatory agent. A number of cosmetic applications for carnosine (e.g. with respect to skin aging) have also been described. Food applications for carnosine include use in food flavourings (beef/brothy) and as an additive to decrease deterioration in foods and discolouration rate of meat (reviewed by James *et al*., 1995).

Recent research has revealed that the degradation products of dietary sphingolipids are biologically active and have the capacity to inhibit the

development of colon cancer in mice. An investigation of the content of sphingomyelin and neutral glycosphingolipids in commonly consumed meat and fish products reported generally lower amounts of sphingolipids in fish meat than in red meat and poultry, with poultry being the richest source of this class of lipids. In fish, the sphingomyelin/neutral glycolipids ratio varied from 1 to 2.9, while in poultry the ratio varied between 5.2 and 19.2, and in red meat it varied from 1.6 to 8.3 (Hellgren, 2001). If there is future demand for these bioactive ingredients, meat/poultry processing may be a source of these compounds.

The polyamines putrescine, spermidine and spermine occur in the cells of living organisms where they fulfil an array of physiological roles, including a role in human cell growth and proliferation which has been of great interest in studies on tumour growth. However, polyamines could be useful for post-operation patients, during wound healing, and for growth and development of the neonate digestive system. Both endogenous and dietary polyamines participate in such processes. Data on polyamine contents in foods are limited in the literature. While putrescine content increases as a result of bacterial activity during inappropriate storage and processing of foods of animal origin, spermidine and spermine originate mainly from raw materials. Higher contents of spermidine, compared with spermine, are typical for foods of plant origin, while an opposite relation is characteristic of foods of animal origin. Legumes, cauliflower and broccoli are foods with high spermidine content; while meat and meat products are high in spermine (Kalac and Krausova, 2005).

Immediately after slaughter, spermine and spermidine have been detected in red and white meats. Spermine was the prevalent amine, ~70% of total, while low levels of histamine were also detected in chicken thighs. During storage at 4 °C, there was a decrease in spermine, spermidine levels remained constant, and putrescine, cadaverine, histamine and tyramine were formed. At 15 days, higher levels of amines were found in breast compared with thigh. An index based on the ratio of the polyamines spermidine and spermine was considered appropriate for the evaluation of chicken meat quality. Chicken-based meat products (mortadella, frankfurters, sausage, meatballs, hamburger and nuggets) were analyzed for bioactive amines. Nuggets were the only products with amine profiles similar to fresh chicken meat. There was a prevalence of spermidine over spermine for most of the products, suggesting the incorporation of significant amounts of vegetable protein in the formulations (Silva and Gloria, 2002).

Meat and meat products have effects on appetite and have shown highly satiating characteristics. If meat-based products could be designed to be have less energy (calorific) density, while remaining satiating and having sensory appeal, they could form the basis of functional foods to perhaps address the growing incidence of obesity (Fernandez-Gines *et al*., 2005).

During the last two decades, many food protein fragments have been demonstrated to elicit biological effects in various *in vitro* or *in vivo* test

systems. A considerable number of these bioactive peptides are opioid receptor ligands, which may be regarded as exogenous supplements to the endogenous opioidergic systems in humans. Most of these food-derived opioid receptor ligands are fragments of milk proteins; however, bovine serum albumin and haemoglobin, i.e. constituents of meat, have been demonstrated to contain fragments behaving like opioid receptor ligands. Practically all of these compounds display opioid agonist activity, but only very few of them behave like opioid antagonists. However, in terms of evidence-based dietary supplementation, more studies are needed to prove that oral administration of food protein-derived opioid receptor ligands or their precursors have any beneficial effects in humans in order to support a benefit for the consumer (Teschemacher, 2003).

Bioactive peptides, biogenic peptides, opioid peptides, immuno-stimulating peptides, mineral-soluble peptides, antihypertensive peptides and antimicrobial peptides can therefore originate from food materials and enzymatic hydrolysis of proteins. These peptides – which are produced in the enzymatic hydrolysate of treated food materials such as milk, animal and fish meat, and also from other products – have been recently reviewed (Yamamoto *et al*., 2003).

While meat ingredients have been added to a variety of foods, several functional (i.e. healthy) food ingredients have also been added to meat and meat products. For example, omega-3 fatty acids from fish oils, olive oil, soy proteins, antioxidants such as tea catechins, green tea extracts, phenolic compounds from rosemary, and dietary fibre (bran, oats, inulin) have all been added to meat products to enhance their nutritional profile (Fernandez-Gines *et al*., 2005). Furthermore, modifications to the feed an animal receives can modify the lipid, fatty acid and vitamin E content in meat (Aharoni *et al*., 2005).

13.5 Conclusions and future trends

The processing of red and white meat results in the generation of large volumes of waste material. This review has examined the various approaches that a modern meat processor should consider in 'managing' this waste material, including its minimization, responsible diposal and value-addition. All can play a part, either in isolation or in combination, in improving processing efficiency and effectiveness, addressing legislative requirements and enhancing the financial, social and environmental outcomes for a processor. In particular, this review has highlighted modern approaches to clean water and energy conservation, minimization of odours and high-BOD effluent streams (and their disposal), responsible management of high-risk waste materials and, perhaps of most importance, adding value to by- and co-products. It is this latter area that the authors believe could be the most lucrative for the processor, driven by the large and burgeoning

biotechnology and functional foods markets. In terms of trends, financial, legislative and community pressure will continue to be applied to meat processors for acceptable management of waste streams, and the industry must focus on solutions that provide 'triple bottom-line' (profit, people, planet) benefit for long-term sustainability.

13.6 Sources of further information and advice

Meat processing/meat waste
www.geosp.uq.edu.au/emc/CP/Res/Red_Meat/Meat_Manual.pdf
www.fpfaraday.com/Files/Waste+Report+Printed.pdf
www.defra.gov.uk/animalh/by-prods/FormerFoodstuffs/guidance_dispffs.pdf
www.dwaf.gov.za/Documents/Policies/WDD/AbattoirWasteHandling_Disposal.pdf

Government/regulatory bodies
Canadian Food Inspection Agency – http://www.inspection.gc.ca/
European Food Safety Authority – http://appl.efsa.eu.int/
Food Standards Agency (FSA) (UK) – http://www.foodstandards.gov.au/

- FSA BSE overview site – http://www.food.gov.uk/bse/what/about/report
- FSA BSE controls page – http://www.food.gov.uk/multimedia/pdfs/bse-leaflet.pdf

Food Standards Australia New Zealand – http://www.foodstandards.gov.au/
US Food and Drug Administration (USFDA) – http://vm.cfsan.fda.gov/

- USFDA – http://www.fda.gov/oc/opacom/hottopics/bse.html
- USDA BSE page – http://www.fsis.usda.gov/Fact_Sheets/Bovine_Spongiform_Encephalopathy_BSE/index.asp
- USDA–FSA site on BSE – http://www.fas.usda.gov/dlp/BSE/bse.html#BSE%20Information%20Sites

WHO Food Safety site – http://www.who.int/foodsafety/en/

- WHO BSE page – http://www.who.int/zoonoses/diseases/bse/en/

World Organization for Animal Health – http://www.oie.int/eng/en_index.htm

Legislation on BSE
EU legislation – http://www.who.int/zoonoses/diseases/bse/en/
European Commission – summary of TSE law – http://europa.eu.int/comm/food/food/biosafety/bse/roadmap_en.pdf

US Federal Meat Inspection Act – http://www.fsis.usda.gov/Regulations_&_Policies/FMIA/index.asp

Other organizations with information on BSE
Leatherhead Food International (UK) – http://www.foodsafetytoday.com/
National Renderers Association (US) – http://www.renderers.org
PrionData.org – http://www.priondata.org
The British Medical Journal's variant Creutzfeldt Jakob Disease collection – http://bmj.com/cgi/collection/mad_cow

13.7 References

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14

Waste management and co-product recovery in dairy processing

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14.1 Introduction

Dairy processing in major dairy producing countries has undergone rationalisation in recent years, with a trend towards larger yet fewer plants operated with less staff. Consequently, most dairy processing plants are quite large in Europe, the United States, Australia and New Zealand. Large processing plants have enabled the use of automated and more efficient equipment – including the use of specialised processes such as membrane filtration, ion exchange and modern drying processes, thus increasing the opportunity for the recovery of milk solids that were formerly discharged. In addition, sophisticated process control systems have allowed improved processing efficiencies and cost savings.

However, large-scale manufacturing increases the environmental burden on to smaller areas, loading the impact of liquid waste disposal, noise and gas emissions in the vicinity of the plant. Additionally, large-scale processing also increases the requirement for long-distance distribution, with centralised plants supplying large geographic areas, further adding to greenhouse gas emissions.

The dairy industry has focused much attention on the utilisation of sweet cheese whey over the years. However, several aspects of waste disposal continue to be troublesome in the dairy industry; including acid whey, nanofilter permeates, mother liquor from lactose crystallisation, clean-in-place waste and spent ion exchange regeneration brines. Although limited utilisation of many of these by-product streams is due to low returns and economies of scale, continued research and process optimisation is required

to overcome the remaining technological barriers for maximising the recovery of dairy by-product streams.

14.2 Worldwide dairy production trends

14.2.1 Milk production

Global milk production continues to increase at over 1% each year, reaching 514 MT in 2004 (see Table 14.1). Within these figures the trends differ between regions, as some of the dominant producers are losing market share to new players, with the most rapidly increasing production occurring in Asia (OECD 2004).

Europe (EU-25) is the dominant milk-producing area with 143 MT or 23% of total world milk supplies in 2004 (Table 14.1). In recent years milk production in the EU has been starting to lose world market share. This can be attributed to the Uruguay Round Agreement on Agriculture, which imposed quota restraints on milk production and limitations on subsidised exports.

The second major milk-producing nation is India with 88 MT of world milk supplies, of which 47 MT is buffalo milk. India is showing a steady growth in milk production up from 12.8% to 14% of the world milk supplies, overtaking the United States in 2000. India is self-sufficient in dairy products and is now starting to participate in world export markets (Cox & Youg Zhu 2003).

Milk production in the United States appears to have plateaued; reaching 77 MT in 2004, its world share has reduced from 12.8% to 12.3% in the 6 years to 2004. This reflects changes in production patterns in the rest of the world more than changes in the United States. The most significant change in recent years has been the rapid increase in milk production in China, which has tripled its milk production in the 6 years to 2004 to 19 MT. This increase can be attributed to changing diets of urban residents of China; associated with increasing income and awareness of the health benefits of milk, especially for the young and the elderly, and also to dairy product marketing (Fuller *et al.* 2005). China has introduced a national school milk programme and is expanding local production to meet demand.

There has also been a 50% increase in milk production in New Zealand, where much of the increased production has been sold as exports. New Zealand dairy production is based on grazing with very low production costs with about 97% of milk destined for the export market, principally as dried dairy products and cheese. Despite New Zealand's share of world milk output being less than 3%, its share of world trade is more than 30%. Similarly, Australia, with less than 2% of world milk production, supplies about 20% of global dairy trade (OECD 2004). Two of the least-populous nations, who produce less than 5% of the world supply, account for half of

Table 14.1 Worldwide milk production 1998–2004. Data from IDF-FIL (2004)

Country	Worldwide milk production ('000 tonnes)							Percentage of world production	
	1998	1999	2000	2001	2002	2003	2004*	1998	2004
European Union-25	144327	144434	143005	143685	143741	144217	142865	25.9	22.8
India	71300	74600	76490	79490	81855	84380	88000	12.8	14.1
United States	71373	73807	76004	75068	77139	77252	77220	12.8	12.3
Russia	33197	32274	31938	33000	33467	33300	33000	6.0	5.3
Brazil	21630	21700	22134	22580	22635	23000	23500	3.9	3.8
China	6621	7120	8420	10255	12998	17463	19000	1.2	3.0
New Zealand	10500	11900	12700	13300	13900	14450	14500	1.9	2.3
Ukraine	13738	13140	12658	13444	14142	13658	13200	2.5	2.1
Australia	10474	11172	10862	11609	10636	10404	10700	1.9	1.7
Turkey	8832	8800	8750	8490	8489	8500	8700	1.6	1.4
Argentina	9684	10329	9794	9475	8528	7975	8775	1.7	1.4
Japan	8572	8460	8497	8300	8385	8400	8400	1.5	1.3
Canada	8130	8289	7925	8148	8017	8013	8046	1.5	1.3
Belarus	5232	4762	4320	4300	4907	5000	5000	0.9	0.8
Romania	5160	5076	5002	5047	4450	4500	4500	0.9	0.7
Other countries	43568	42320	47320	45218	48379	48713	44319	7.8	7.1
World total	476862	483436	490611	495837	505746	512700	514000		

* 2004 is a forecast figure.

the global trade; thus indicating the importance of dairy products to their economies.

14.2.2 Dairy product production

Milk can be converted into a wide range of dairy products – including fresh products such as modified milk, yoghurt, desserts and custards, or longer-shelf-life products such as cheese or butter, long-life milk, milk powders and whey powders. Overall about one-third of world milk is consumed as fresh market milk; one-quarter is used in cheese making; one-fifth is processed into butter, milk powder and casein. The remainder is processed into soft or frozen products, condensed and evaporated milk, or other dairy products (Cox & Youg Zhu 2003).

Trends in production of cheese, butter, skim milk powder (SMP) and whole milk powder (WMP) have been reported by OECD FAO, with forecasts up to 2014 (see Fig. 14.1). These figures show that cheese production has been steadily increasing relative to other dairy products, up by 50% in the 20 years to 2004. Cheese production is forecast to continue to increase more than any other dairy product, reaching 21 MT by 2014 (OECD 2005a). Meanwhile, production of SMP and WMP has been stagnating. This reflects a trend in world dairy markets away from the supply-led bulk commodities (SMP, butter) to demand-driven value-added dairy products such as cheese (OECD 2004).

The dominant cheese-producing nations are Europe and the United States, accounting for 6 and 4 MT, respectively, of the 17 MT cheese produced (see Table 14.2). Increasing cheese production has been driven by changes in consumption. These changes in turn have been driven by per capita income growth, but also by globalisation of diets. There have been shifting trends towards fast and convenience foods, resulting in people consuming more cheese, for example in hamburgers and pizza. It is worth noting here that for every kilogram of cheese produced, 9 litres of waste whey is created.

14.2.3 Dairy processing overview

The dairy industry has long been directed towards maximising the recovery of complementary products from milk, wherein milk is separated and processed into a range of products that maximise the recovery of the various components of milk. At the most basic level milk can be separated to produce cream and skim milk. Cream can be converted into butter and buttermilk powder (BMP), while skim milk can be spray dried to SMP. Alternatively, milk can be processed into cheese with the whey converted into ricotta cheese and the remainder fractionated into whey protein, lactose and dairy salts. A typical mass balance for a range of products from milk is shown in Fig. 14.2.

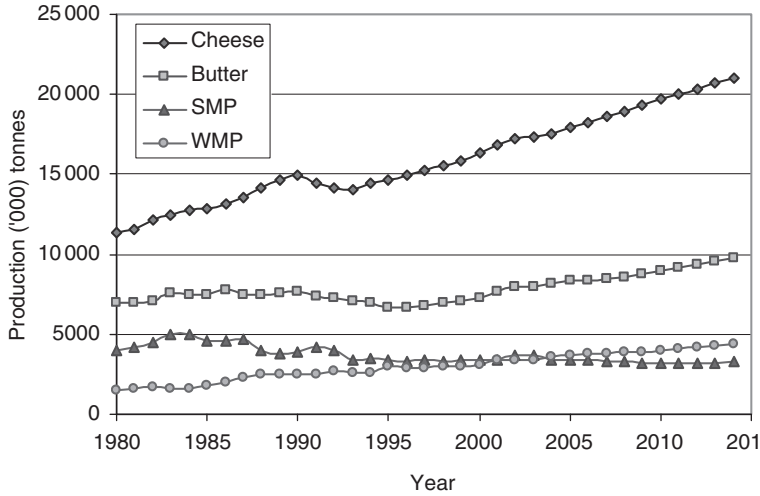

Fig. 14.1 Trends in dairy product production 1980–2014. Data from OECD (2005a, b).

Table 14.2 Worldwide cheese production 2000–2005. Data from USDA Foreign Agricultural Service (2005)

Country	Worldwide cheese production ('000 tonnes)					
	2000	2001	2002	2003	2004 (p)	2005 (f)
European Union-25	5861	5865	5993	6117	6292	6364
United States	3746	3747	3877	3900	4020	4150
Brazil	445	460	470	460	470	480
Egypt	380	395	410	450	455	457
Australia	373	374	413	368	391	400
Argentina	445	440	370	325	345	360
Russia	220	260	340	335	330	335
Canada	328	329	350	342	326	330
New Zealand	297	281	312	301	313	319
China*	194	202	210	217	225	233
Ukraine	67	105	129	169	200	200
Mexico	134	140	145	126	130	132
Japan	34	34	36	35	35	35
Korea	15	20	20	23	25	27
Other countries (c)	3832	4185	4189	4182	3941	4088
Total†	16371	16837	17264	17350	17498	17910

(p) Preliminary; (f) forecast; (c) calculated by difference.
* China data from Freeman (2005).
† World totals from FAO-GIEWS (1997–2005).

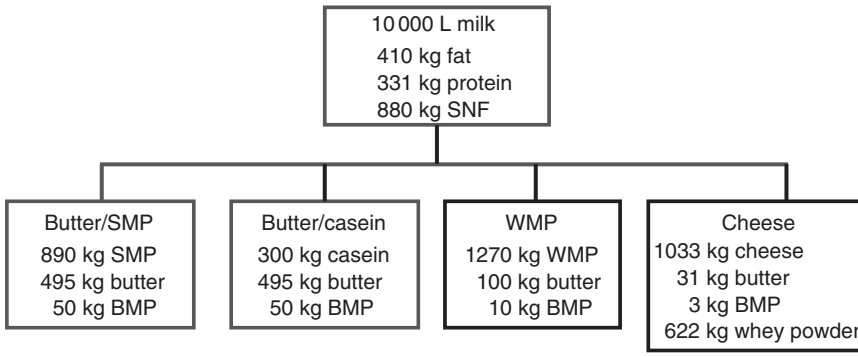

Fig. 14.2 Dairy product yield from 10 000 L of milk, 2003/2004. Source: Dairy Australia (2004).

Dairy manufacturing plants produce a range of dairy products depending on scale, location of plant and proximity to market. Dairy plants producing fresh milk and products with a short shelf-life, tend to be located on the fringe of urban centres close to consumer markets. In contrast, plants manufacturing items with longer shelf-life – such as butter, milk powders, cheese and whey powders – tend to be located in rural areas closer to the milk supply (UNEP 2000).

In recent years the dairy industry in most major dairy countries has undergone rationalisation, with a trend towards larger but fewer plants. Consequently, manufacturing plants located in Europe, the United States, New Zealand and Australia tend to be quite large and automated. Such technological developments have enabled more efficient processing, including the adoption of membrane processing enabling the recovery of dairy solids that would otherwise be lost. However, large plants can also lead to increased environmental loadings in areas in close proximity to the factory.

14.3 Current status of waste problems faced by the dairy industry

The impact of dairy processing on the environment has been summarised in the schematic in Fig. 14.3. This diagram shows the inputs and outputs for a typical dairy manufacturing plant producing market milk, butter, milk powder and cheese. Inputs include the raw milk, other ingredients, water, energy, detergents, refrigerants and packaging. Outputs include: dairy products; a range of dairy liquid effluents such as cleaning-in-place (CIP) cleaning waste, cheese whey and spills; air emissions such as combustion gases and milk powder dust; and solid wastes such as damaged stock or

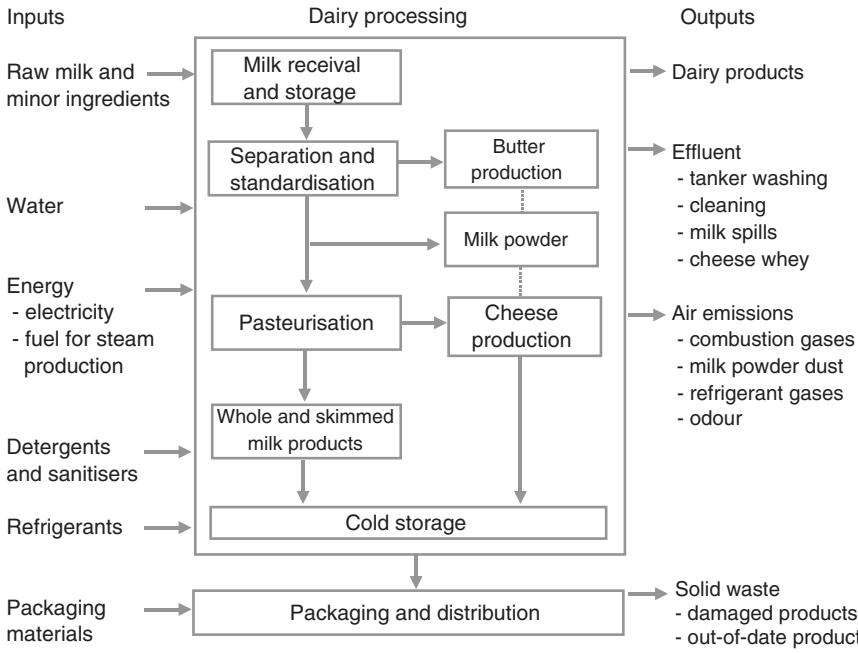

Fig. 14.3 Dairy processing impact on the environment – inputs and outputs. Source: UNEP (2000).

out-of-date product. Input–output comparisons form the basis of the life cycle assessment of dairy processing conducted by a number of researchers (Cederberg & Mattsson 2000; Berlin 2002; Hogaas-Eide 2002; Nicol 2004). These studies have identified the key issues that the dairy industry needs to address to ensure the environmental sustainability of the industry based on water and energy usage, and waste disposal.

14.3.1 Water

Water is an important input in dairy production, used for irrigation of pastures for milk production and for sanitation of milking and dairy processing facilities. Its availability often determines the viability of dairy processing in different geographic areas. The highest dairy production regions are often associated with high rainfall and/or low evaporation, such as New Zealand and Northern Europe. In future, changes in rainfall patterns and global warming may adversely affect the viability of dairying regions.

In dry climates such as Australia, water availability is a key constraint. Australian studies show that dairy farming has very high water consumption compared with other agricultural production, at 5902 GL/year or 39.5% of all irrigated water (National Land & Resources Audit, 2002); correlating to 1.3–50 kL/cow/year (Rogers and Alexander 2000). Practices such as

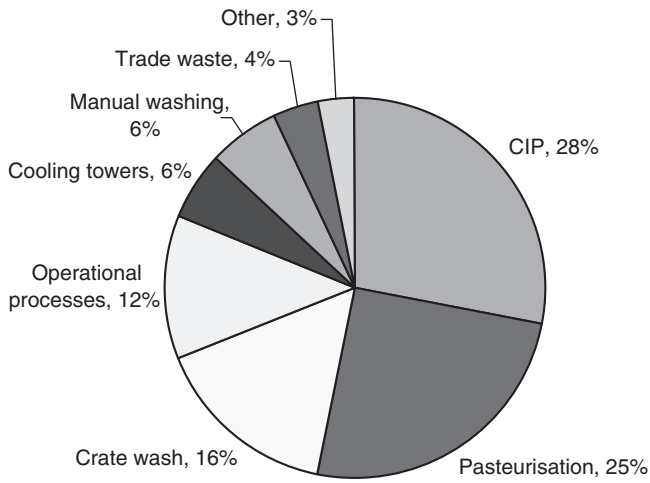

Fig. 14.4 Water use for market milk processing. Source: Prasad *et al.* (2004).

articulation of irrigation water in open channels and flood irrigation in high evaporation areas are largely responsible for these figures. More efficient irrigation practices are now encouraged.

Water usage in dairy processing is additional to farming requirements. Dairy factories use large quantities of water, mostly for cleaning and sanitation, and subsequently produce substantial volumes of liquid waste. Water usage is monitored by tracking the overall consumption of water compared with the raw milk intake. The water usage pattern for a typical market milk processor is shown in Fig. 14.4, and the water usage pattern of dairy plants surveyed by the Danish Environmental Protection Agency (EPA) is shown in Table 14.3. These figures show that the water usage varies considerably in different areas of the plant, e.g. the cheese room was reported to use between 0.06 and 20.89 L/kg product. Such variation highlights opportunities for improvements. In recent decades there have been considerable reductions in water usage, as higher charges for water and effluent disposal have now been imposed in some countries to reflect environmental costs. Recent data from Australia show average water consumption to be 1.44–1.64 L/L raw milk processed into market milk, cheese or powder products (see Table 14.4). Further discussion on the recovery, treatment and reuse of water is covered in Section 14.6.1.

The trend to reduce water consumption is a reflection of manufacturers realising the true cost of water. The components that make up the cost of water include: purchase price, treatment costs, heating, treatment of waste water, disposal of waste water, pumping costs, and maintenance and capital depreciation costs. An example of the estimated cost of water for dairy processing in rural regions of Australia is shown in Table 14.5.

Table 14.3 Areas of water consumption for a dairy processing plant, from a Danish EPA 1991 study. Source: UNEP (2000)

Area of use	Water consumption (L/kg product)	Percentage of total (%)
Locker room	0.01–1.45	2
Staff use	0.02–0.44	2
Boiler	0.03–0.78	2
Cold storage	0.03–0.78	2
Receipt area	0.11–0.92	3
Filling room	0.11–0.41	3
Crate washer	0.18–0.75	4
Cooling tower	0.20–1.80	5
Cleaning	0.32–1.76	8
Cheese room	0.06–20.89	13
Utilities	0.56–4.39	16
Incorporated into products	1.52–9.44	40
Total	2.21–9.44	100

Table 14.4 Water usage ratios for the production of different dairy products. Source: Prasad *et al.* (2004)

Product	Water consumption (L/L milk)		
	Minimum	Maximum	Average
White and flavoured milk	1.05	2.21	1.44
Cheese and whey products	0.64	2.90	1.64
Powdered products	0.07	2.70	1.52

Table 14.5 Costing for ambient and hot water for a dairy processor in Australia. Source: Prasad *et al.* (2004)

Cost component	Cost (AUS$/kL)
Water purchase	$0.54
Wastewater treatment	$0.75
Wastewater pumping	$0.05
Wastewater discharge (volume charge)	$1.09
Heating to 80 °C	$2.80
Cost of ambient water	$2.43
Cost of hot water (80 °C)	$5.23

14.3.2 Energy

Large dairy processing plants consume significant amounts of energy in the processing, packaging and transport of dairy products. Electricity is used for the operation of machinery, refrigeration, ventilation, lighting and the production of compressed air. As with water consumption, the use of energy for cooling and refrigeration is important for ensuring good keeping quality of dairy products. Storage temperatures are often specified by regulation. Thermal energy, in the form of steam, is used for heating and cleaning. As well as depleting fossil fuel resources, the consumption of energy causes air pollution and greenhouse gas emissions, which have been linked to global warming (UNEP 2000). Life cycle assessment, one of the tools within the ISO 14000 series, has enabled tracking of energy consumption throughout the life of a product by systematic analysis of all the inputs and outputs. Life cycle assessments of market milk in Sweden and Australia are compared in Table 14.6 using data from Nicol (2004) and Svenskmjolk (2004). The Swedish study shows that half the energy was used on the farm; this was due to fuel used by tractors, energy used in fertiliser production and electricity used for milk harvesting and cooling. Australia requires relatively less energy on the farm, as the dairy farming there is mainly pasture based. However, much more energy is required for transport and distribution in Australia, due to the greater distances between farm and processor, and the consumer. Table 14.6 also reviews the energy required for long-life milk, showing larger energy requirements for the more sophisticated processing, packaging and distribution. The shelf-stable nature of long-life milk enables the milk to be transported considerably greater distances (Nicol 2004).

Table 14.6 Life cycle energy consumption for market milk and long-life milk in Australia (Nicol 2004) and Sweden (Svenskmjolk 2004)

Activity	Australian market milk (GJ/tonne)	Swedish market milk (GJ/tonne)	Australian long-life milk (GJ/tonne)
Farm	1.79	2.99	1.77
Raw milk transport	0.51	0.10	0.50
Manufacturing	1.19	0.42	2.35
Packaging	1.19	1.51	2.55
Warehouse	0.17		0.54
Transport to market	0.26	0.24	2.47
Supermarket/consumer	1.19	0.73*	0.58
Consumer transport	1.79		1.94
Home refrigeration	0.43		0.50

* Swedish market and consumer energy consumption added together.

Energy consumption also depends on the type of product. Processes that involve the concentration and drying of milk, whey or buttermilk are very energy intensive. In comparison, the production of market milk involves only some heat treatment and packaging, and therefore requires considerably less energy, see Table 14.7.

Plants producing powdered dairy products employ a wide range of energy efficiencies, depending on the type of evaporation and drying processes used. Energy consumption depends on the number of evaporation effects (the number of evaporation units that are used in series) and the efficiency of the powder dryer (UNEP 2000). Energy consumption also depends on the age and scale of a plant as well as the level of automation. Table 14.8 shows examples of different evaporation and drying systems. Fuel consumption per unit of product decreases as the processing plants become larger and more energy efficient. However, the dairy industry needs to become more responsive by employing renewable sources of energy to ensure long-term sustainability of the industry.

14.3.3 Greenhouse gas emissions

The reduction of greenhouse gas emissions was one of the principal commitments made at the recent IDF World Dairy Summit (Begg 2005). Life cycle studies on greenhouse gas emissions for market milk in Sweden and Australia show that the bulk of greenhouse emissions occur at the farm, 87% in Sweden and 70% in Australia (Nicol 2004; Svenskmjolk 2004) (see Table 14.9). Farm emissions comprise methane from cows and nitrogen

Table 14.7 Energy consumption for various dairy products (GJ/tonne end product). Source: Joyce and Burgi (1993)

Product	Electricity consumption (GJ/tonne)	Fuel consumption (GJ/tonne)
Market milk	0.20	0.46
Cheese	0.76	4.34
Milk powder	1.43	20.60
Butter	0.71	3.53

Table 14.8 Energy consumption for different evaporation and drying systems (GJ/tonne product). Source: Joyce and Burgi (1993)

Type of evaporation and drying system	Energy consumption (GJ/tonne)
5-effect evap. and 2-stage drier	13–15
3-effect evap. and 1-stage drier	22–28
2-effect evap. and 1-stage drier	40–50

Table 14.9 Proportion of life cycle greenhouse gas emission for market milk produced in Australia (Nicol 2004) and Sweden (Svenskmjolk 2004)

Activity	Greenhouse gas emissions	
	Australia	Sweden
Farm	70%	87%
Raw milk transport	4%	1%
Manufacturing	5%	2%
Packaging	4%	4%
Transport to market	2%	2%
Market/consumer	15%	5%

oxide from effluent and fertiliser denitrification (Nicol 2004). Methane is formed when the cow ruminates and the feed is broken down in the first stomach and intestines. In one year, a cow produces 120–130 kg of methane through this natural process (Svenskmjolk 2004). The productivity of the cow has a direct impact on the relative contribution of greenhouse gas emissions to overall life cycle emissions (Svenskmjolk 2004). A high-yielding cow releases slightly more methane but, calculated per kilogramme of milk, the high-yield cow releases less greenhouse gases. Future reductions in greenhouse gases can be achieved by increasing cow productivity with improvements in nutrition, pastoral management and genetics.

Greenhouse gases are also released in other parts of the life cycle of milk. Transport is the next greatest contributor, primarily due to the use of the fossil fuels used to transport the raw milk to the factory then distribute it to market and the consumer, especially in Australia where greater distances are involved. Packaging also represents 4% of the greenhouse gases, with methane emissions from the disposal of milk packages representing approximately 30%, while 70% are emissions of carbon dioxide (Svenskmjolk 2004). The remaining greenhouse gas emissions arise from manufacturing processes, which are relatively low for market milk but increase considerably with energy-intensive processing such as evaporation and drying.

14.3.4 Disposal of waste

The dominant environmental problem caused by dairy processing is the discharge of large quantities of liquid effluent. Dairy processing produces waste from washing and cleaning steps, and from off-specification product. Examples of sources of waste from milk, cheese and dried powder processing are shown in Fig. 14.5.

The effluent loads from dairy processing depend on the type of product being produced, the scale of the operation and whether a plant uses batch

Milk processing
- Overfill
- Spillage
- Purge of product prior to CIP
- Retained product in poorly drained pipelines
- Heat deposited waste
- Product defects/returns
- Laboratory samples

Cheese processing
- Whey
- Cheese fines
- Separator de-sludges
- Cheese brine/salt whey
- Waste water from flushing pipelines
- Curd losses from curd transfer system

Milk powder
- Flushing at start-up and shut down (concentrates with extremely high biological oxygen demand (BOD))
- Deposit on heating surfaces (protein and $CaPO_4$)
- Exhaust loss of fines from driers

Fig. 14.5 Sources of wastage in dairy processing. Source: Hale *et al.* (2003).

Table 14.10 Summary of American dairy and milk processing plant effluent loadings. Based on DPPEA (1999)

Products	Waste water (ww) from processing	
	Range (ww kg/kg milk)	Average (ww kg/kg milk)
Milk	0.10–5.40	3.25
Cheese	1.63–5.70	3.14
Ice cream	0.80–5.60	2.80
Condensed milk	1.00–3.30	2.10
Butter		0.80
Powder	1.50–5.90	3.70
Cottage cheese	0.80–12.40	6.00

or continuous processes (see Table 14.10). Small batch processes require more frequent cleaning, with increased losses from product change-over, drainage and cleaning losses. Evaporation processes have the potential to create very high organic loads due to losses during start-up and shut down. Cheese whey, if not used as a by-product, is discharged along with other waste waters and can have a considerable impact on the organic load of the waste from the plant.

Dairy processing effluent generally exhibits the following properties: high organic load due to the presence of milk components; fluctuations in pH due to the presence of caustic and acidic cleaning agents and other chemicals; high levels of nitrogen and phosphorus and fluctuations in temperature (UNEP 2000). Dairy wastes include grease, sugars, nitrogen, phos-

Table 14.11 Composition of various dairy products. Source: Hale *et al.* (2003)

Product	Total solids (g/L)	Fat (g/L)	Protein (g/L)	Lactose (g/L)	Phosphorus (mg/L)	BOD_5 (mg/L)	COD (mg/L)
Whole milk	125	35	36	47	950	114000	183000
Skimmed milk	92	0.5	36	47	980	90000	147000
Buttermilk	87	5	35	40	900	61000	134000
Cream	379	315	28	33	672	400000	750000
Condensed milk	260	75	71	98	2050	271000	378000
Sweet whey	62	0.5	7.5	47	490	42000	65000
Acid whey	56	0.5	7.5	40	160	35000	60000
Casein whey permeate	48		0.7			26400	44000
Whey permeate	39		1.5			19800	33000
Skimmed milk powder	957	10	350	519	9950	700000	950000
Whey powder	950	12	123	732	6950	600000	929000

phorus, acidic and caustic cleaning chemicals; and they have high biological oxygen demand (BOD_5) and chemical oxygen demand (COD). The composition of the waste reflects the dairy product being processed and it is possible to identify the source of the waste problem by chemical analysis (see Table 14.11).

Organic loadings in dairy processing effluents vary from 180 to 23000 mg/L COD. Low values are associated with milk receipt and high values reflect the presence of whey from the production of cheese. Typical levels in the effluent stream from an ice cream and dairy products factory, as reported by Scott and Smith (1997), show loads in excess of 9500 mg/L COD. Milk loss to the effluent can amount to 0.5–2.5% of the incoming milk, but can be as high as 3–4% (UNEP 2000).

Treatment and disposal of dairy waste is often dependent on the location of the factory. Plants located near urban areas often discharge the effluent into municipal sewage treatment systems after primary treatment to remove fat and solids. For some municipalities, the effluent from local dairy processing plants can represent a significant load on sewage treatment plants. In extreme cases, the organic load of waste milk solids entering a sewage system may well exceed that of the township's domestic waste, overloading the system (UNEP 2000).

In rural areas, dairy processing effluent may be irrigated on to land. If not managed correctly, dissolved salts contained in the effluent can adversely affect soil structure and cause salinity. Contaminants in the effluent can also leach into underlying groundwater and affect its quality. In some locations, effluent may be discharged directly into water bodies via deep-ocean outfalls. Such dumping should meet the London Convention (1972), but dumping is hard to control and the effluent can have a negative impact on water quality due to the high levels of organic matter and resultant depletion of oxygen levels.

14.3.5 Legislation

Environmental legislation has become a strong driver for changing practices in dairy processing to achieve greater eco-efficiency (Honkasalo *et al.* 2004). New legislation is progressively being developed and implemented as the implications of environmental neglect become apparent and in response to public scrutiny about adverse environmental impacts of the dairy sector. Most countries around the world have introduced policies and regulations to control air and water emissions and waste disposal. Some useful links are given here:

- European Union – Waste Management Policies
  http://europa.eu.int/scadplus/leg/en/s15002.htm
- United States – Environmental Protection Agency
  http://www.epa.gov/epahome/rules.html
- Australia – Environmental Protection Agency
  http://www.environment.gov.au/
- China – Cleaner production in China
  http://www.chinacp.com/eng/cp_policy.html
- India – National Environment Policy 2004 (draft)
  http://envfor.nic.in/nep/nep.pdf

The policies cover a range of waste management priorities including: waste avoidance, waste reduction, waste reuse, waste recycling or reclamation, waste treatment and waste disposal. Regulatory standards, which are legally enforceable, provide a strong impetus for the industry to adopt waste avoidance practices to avoid fines and even jail sentences for serious offences.

The main driver for the emerging regulatory trends has been the European Union, in that sustainability serves as a core goal of all public policy (Commission of the European Communities 2001). The policies recognise the multifunctional role of agriculture in addressing non-trade concerns such as the environment and animal welfare (WTO 2000). The EU has also introduced regulations mandating recycling and disposal measures for the food industry that appear to be more stringent than internationally accepted levels. Environmental assessments are increasingly based on life cycle analysis.

In comparison, the United States has not advocated broad environmental goals in the regulation of food and agriculture (Osborne 2004). Individual states have their own laws reflecting varying community concerns; however studies have shown that states with more stringent environmental regulations tend to lose dairy manufacturing to those with less stringent policies (Isik 2004). Increased environmental responsibility is often a response to avoid litigation.

Harmonising regulations internationally would increase environmental performance. It has been shown that introducing waste minimisation practises is both environmentally responsible and results in micro-economic

reform; these practices are often highly profitable to firms, with short payback periods (Nguyen & Durham 2004). Multinational companies such as Nestlé advocate the harmonisation of environmental laws, regulations and standards in order to eliminate existing and future trade barriers (Nestlé 1999). Environmental policies, at the international level, are guided by the international environmental standard ISO 14001 and by the United Nations Environment Programme (UNEP) sponsored by OECD.

The international standard ISO 14001 was developed along the same lines as ISO 9000 but is concerned with environmental management systems (EMS). The aim is for companies to achieve sound environmental performance by addressing their attention to the potential environmental impact of their activities, products or services. The general purpose of this standard is to provide assistance to organisations implementing or improving an EMS. An EMS will provide order and consistency so that an organisation can address environmental concerns through the allocation of resources, assignment of responsibilities and ongoing evaluation of practices, procedures and processes.

The UNEP addresses the need for cleaner world production through the publishing of guides and information. UNEP has defined cleaner production as the continuous use of industrial processes and products: to increase efficiency; to prevent the pollution of air; water and land; to reduce waste at source; and to minimise risk to the human population (UNEP 2000).

14.4 Cleaner production in the dairy industry

Cleaner Production is a preventative approach to environmental management that encompasses eco-efficiency, waste minimisation and pollution prevention. Cleaner Production is a forward-looking, 'anticipate and prevent' philosophy. Cleaner Production does not deny growth; it merely insists that growth be ecologically sustainable (UNEP 2004). In this context, waste is considered a 'product' with negative economic value. Each action to reduce consumption of raw materials and energy, and reduce generation of waste, increases productivity and creates financial benefits. The definition of Cleaner Production adopted by UNEP (2004) is as follows:

> Cleaner Production is the continuous application of an integrated preventive environmental strategy to processes, product and services to increase overall efficiency, and reduce risks to humans and the environment. For production processes, Cleaner Production results from one or a combination of conserving raw materials, water and energy; eliminating toxic and dangerous raw materials and reducing the quantity and toxicity of all emissions and wastes at source during the production process.

The dairy industry is beginning to recognise the value of applying Cleaner Production strategies to improve the sustainability of operations

by reducing waste and implementing more efficient processes. UNEP (2000) released a major document providing guidelines for manufacturers wishing to apply Cleaner Production strategies. These guidelines categorised Cleaner Production techniques into the following areas: improved housekeeping, process optimisation, new process technology, and new product design.

14.4.1 Housekeeping

Improved housekeeping is based on improvements to work processes and proper maintenance, including preventing spills and wastage, and improved monitoring, training and inventory. Examples of improved housekeeping are:

- Collect first flush of product changeovers and where possible blend back into the next product or dispose of as animal feed; e.g. Dairy Farmers, Australia collects 500 L product/day during changeovers, this is used for blending, with payback of 1 month (Prasad *et al*. 2004).
- Elimination of rinsing between different flavoured batches of yoghurt, whereby the merge phase is drawn off and used as animal feed, resulted in reduced product loss by 50% and reduced CIP detergent and waste water by 20% (Huisingh and Baas 1991).
- Insulation of pipes and repair of steam leaks and steam traps; e.g. a 1 mm hole in a steam line at 700 kPa will lead to a loss of 3000 L fuel oil/year (Prasad *et al.* 2004).

14.4.2 Process optimisation

Process optimisation reduces resource consumption by optimising existing processes. Examples of improvements that are easily achievable in the dairy industry, as identified by Prasad *et al.* (2004), include:

- Optimising start-up/shut-down procedures and changeovers by fine tuning timers and accurately detecting product interfaces to reduce product waste; e.g. National Foods, Australia are now saving 60 000 L milk/year by such optimisation.
- Optimisation of production scheduling so that process capacity matches filling capacity.
- Minimise waste during separator desludging by optimising bowl opening frequency; e.g. Murray Goulburn, an Australian co-operative, are now saving AUS$40 000/year with payback of 1 year.

14.4.3 Process technology changes

Process technology changes involve the installation of new processes and technology to minimise waste and increase efficiency. Examples of installations of new technology include:

- Replacing batch pasteurisers with continuous process pasteurisers incorporating plate heat exchangers with less waste and counter current heat regeneration reducing energy consumption (UNEP 2000).
- Fully automated cleaning system in a milk processing plant to reduce water and energy usage; e.g. Bonlac Foods, Australia are achieving savings leading to payback of 5 months (Nguyen *et al.* 2003).
- Reliable instrumentation, such as turbidity meters to identify milk solids concentration, and a combination of temperature, pH and conductivity meters to monitor CIP frequency, effectiveness and chemical loss (Prasad *et al.* 2004).
- High efficiency boilers, with heat recovery equipment such as economisers to recover heat from the flue gases to preheat the boiler water. Fuel consumption can be reduced by 1% for each 4.5 °C reduction in flue temperature (Prasad *et al.* 2004).
- Mechanical vapour recompression (MVR) evaporators reduce energy consumption compared with thermal vapour recompression (TVR). For example, equipment capable of evaporating 15 000 kg/h skim milk requires 1610 kg/h steam for a 5-effect TVR, 1190 kg/h steam for a 7-effect TVR, while a 1-effect MVR/2-effect TVR requires only 375 kg/h steam plus 150 kW to drive the mechanical compression motor (Niro 2005).

14.4.4 New product design

New product design can result in benefits throughout the life cycle of the product, with reduced waste, reduced energy and water consumption and higher yields. The new process often requires new process equipment and marketing efforts to establish the new product in the market place successfully. An example is the ultrafiltration of milk for cheese manufacture which increases the recovery of whey proteins in the cheese and reduces whey volumes. Cheese produced from these processes is smoother and can have a more tangy flavour. The marketability of these new cheeses needs to be managed by introducing these changes to consumers as deliberate and desirable in a new product range (Jameson 1987).

Many of the examples of Cleaner Production discussed here demonstrated increased profitability with short payback periods. Continued implementation of Cleaner Production strategies in the dairy industry will create economic opportunities yet at the same time improve the long-term ecological sustainability of the industry.

14.5 Co-product recovery in dairy processing

14.5.1 Whey

The principal by-product from dairy processing is whey from cheese and casein production, with 9 kg whey produced from each 1 kg cheese.

Moreover, cheese production has been increasing and this increase is predicted to continue into the next decade, thus utilisation of whey will continue to be a pressing issue for dairy processors. The high BOD_5 of whey (40 000 mg/L) and of whey permeate (35 000 mg/L) presents a major pollution problem (Mawson 1994). The current estimates for transporting whey for disposal on to land or to animals are AUS$6000/ML (Zadow 2005), showing that costs are being borne by both the manufacturer and the environment when disposal is chosen as the method for dealing with whey. With increasing waste disposal penalties, alternative processes that add value to whey will become increasingly important.

Smaller factories often cannot justify the investment involved in manufacturing high-value, technologically challenging whey by-products. They seek cheaper alternatives with minimal processing or instead continue to spread whey on to fields and feed it to livestock. Very-large-scale cheese manufacturers produce enormous volumes of whey, therefore investment in large-scale process developments is more economically viable. Options for utilising whey from cheese and casein production include consideration of reuse within the factory with minimal processing or the development of new processes and co-products. Alternatively, joint ventures between several manufacturers create economies of scale enabling small–medium dairy processors to pursue more ambitious projects of co-product development.

Whey utilisation statistics

Early studies on dairy by-product utilisation show very low rates of whey utilisation. In 1968 an industrial waste profile of the United States dairy industry by the Federal Water Quality Administration found that the recovery of by-products from whey was only 53% compared with very high levels of by-product recovery for skim, buttermilk and cream (see Table 14.12). Overall this situation has shown some improvement with worldwide utilisation figures now estimated at 60% (Zadow 2005). Whey utilisation across the world is variable: North America, Oceania and Europe report high levels of utilisation, while South America reports lower levels of utilisation (see Tables 14.13 and 14.14).

Table 14.12 By-products from dairy wastes in the United States in 1968. Source: Gillies (1974)

Dairy by-product	Percent utilised	Conclusion
Skim from butter manufacturing	91	Efficient utilisation
Buttermilk from butter manufacturing	91	Efficient
Cream from cheese manufacturing	99	Efficient
Whey from cheese manufacturing	53	Improvement needed

Table 14.13 World annual whey balance and utilisation in 2001. Source: Zadow (2005)

Region	Total whey		Industrially utilised		WP and lactose		Demineralised whey		WPC	
	(%)	(MT)	(%)*	(MT)	(%)†	(MT)	(%)†	(MT)	(%)†	(MT)
USA	24.8	36	80	28.8	50	14.4	10	2.9	40	11.5
EU	41.5	60	60	36	60	21.6	10	3.6	30	10.8
Canada	2.8	4	80	3.2	50	1.6	10	0.3	40	1.3
Argentina/Brazil	6.2	9	40	3.6	70	2.5	5	0.2	25	0.9
Australia/New Zealand	5.9	8.5	90	7.7	40	3.1	10	0.8	50	3.8
Rest of world	19.0	27.5	25	6.9	75	5.2	5	0.3	20	1.4
Total	100	145	59	86.1	56	48.3	9	8.1	34	29.7

* Percentage of total whey volume.
† Percentage of whey utilised.

Table 14.14 Utilisation of whey and permeate in Australia in the period 1991–2004. Source: Zadow (1992, 2000, 2005)

Dairy by-product	Year		
	1991	1999	2004
Whey production (ML)	1361	3022	3778
Whey utilisation (%)	50.4	62.2	87.8
Permeate production (ML)	118	746	1510
Permeate utilisation (%)	83.1	62.3	78.1

NB Whey utilisation does not include whey used as animal feed, or spread over land as fertiliser.

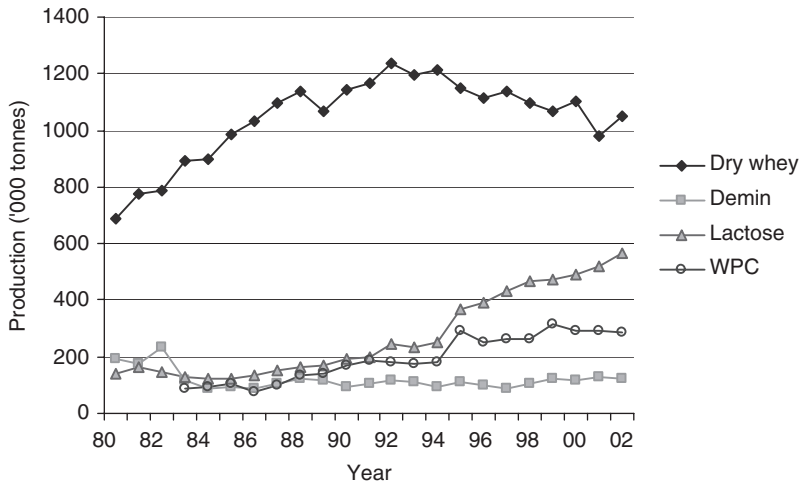

Fig. 14.6 Production of dried whole whey and by-products, including demineralised whey (Demin), whey protein concentrate (WPC) and lactose between 1980 and 2002. Source: Zadow (2005).

Increasing whey utilisation reflects the growing awareness of the economic opportunities for high-value by-products from whey. Rather than producing only non-hygroscopic whey powder, larger amounts of whey are fractionated to produce whey protein concentrate and whey protein isolates (see Fig. 14.6). As whey protein production increases, the permeate fraction containing the bulk of the whey solids (lactose and minerals) is increasingly underutilised; some is used for milk standardisation and lactose manufacture but much is still being lost in the system, risking environmental problems.

Whey composition

Whey contains 95% of the original water, most of the lactose, 20% of the milk protein and traces of fat. The remaining milk solids, about 50%, are

Table 14.15 Composition of sweet and acid whey, and ultrafiltration (UF) permeate. Source: Durham (2000)

Composition	Sweet whey cheddar	Acid whey		UF permeate cheddar
		HCl	Lactic	
Solids (%)	6.6	5.1	6.0	5.5
pH	6.1	4.7	4.0	6.1
Lactose (%)	4.8	3.7	3.9	4.7
Protein (%)	0.9	0.73	0.72	0.01
Ash (%)	0.59	0.60	0.72	0.53
Lactic acid (%)	0.13	0	0.60	0
Fat (%)	0.06	0.05	0.003	0
Calcium (ppm)	430	1200	1140	375
Phosphorus (ppm)	440	680	900	275
Potassium (ppm)	1460	1200	1530	1450
Sodium (ppm)	430	270	400	430
Chloride ppm	970	2600	910	940

incorporated into cheese. The composition of whey depends upon the type of cheese produced. Factors such as the season, location, and type and health of dairy cattle also affect whey composition. There are two main types of whey: sweet whey and acid whey. Sweet whey (pH>5.6) is produced from the manufacture of rennet cheese such as cheddar or mozzarella. Acid whey (pH<5.1) is produced by lactic acid fermentation to produce fresh cheese such as cottage or cream cheese or by hydrochloric acid casein production. Acid whey contains higher levels of calcium phosphate compared with sweet whey, as shown in Table 14.15.

The mineral composition of sweet and acid whey is determined by the method of curd formation, either acid precipitation or rennet coagulation. Acid whey is formed as the pH is lowered and the colloidal calcium phosphate is solubilised, the casein micelle structure of milk is disrupted and the casein proteins aggregate releasing calcium phosphate into the whey (Brown 1988). Sweet whey is produced when rennin cleaves κ-casein on the surface of the casein micelle, destabilising the casein complex, releasing casein-macropeptide into the whey, the remaining hydrophobic α, β paracasein flocculates together in the presence of calcium to form the cheese curd.

14.5.2 Utilisation of whole whey

Whey is highly perishable and needs to be collected hygienically and utilised soon after manufacture. Whey is first clarified to remove cheese solids and pasteurised to stop starter culture activity. If these steps are taken, the whey can be used as food in a wide range of products, with the dairy manufacturer's choice determined by scale, availability of equipment and markets for the products. Most of the costs associated with processing whey arise

from evaporation and drying due to the low solids levels in fresh whey. Liquid applications are less capital intensive but require rapid, flexible production capabilities able to deal with the variable quantities of whey available throughout the day in the cheese factory. Options for processing whey have been summarised in Figure 14.7.

Whey drinks

Whey drinks are an attractive option for small-scale cheese makers, utilising equipment generally available in most dairies. Early reports of whey drinks describe the formulation of fresh pasteurized fruit flavoured whey drinks and lactic-fermented whey drinks (Jelen *et al*. 1987; Schwab 1995). However, these have not been taken up commercially on a significant scale. A widely accepted whey drink in Switzerland, Rivella, is marketed as a de-proteinated carbonated drink, but is not identified as a whey drink (Sciancalepore *et al*. 1992; Johnson *et al*. 1996). Opportunities continue to exist to market the nutritional advantages of whey in a refreshing drink. Anji and Durham (2005) report development of a nutritious, flavoursome, pineapple and mango whey drink based on ultrafiltered, fresh sweet cheese whey. The drink was given a 90 °C/4 s heat treatment for a refrigerated extended shelf-life (ESL).

Cheese whey can also be incorporated into many of the fresh products currently produced in the dairy factory. Studies by Nguyen *et al.* (1997) demonstrated that small dairy manufacturers can employ membrane concentration to recover cottage cheese whey for use in ice cream or yoghurt, with a payback period of less than 10 months.

Whey in dairy product standardisation

An increasingly significant application of milk and whey permeate is milk protein standardisation (Jelen & Michel 1999; Nissen 1999). The standardisation of the protein content of milk was approved by the Codex Alimentarius Commission in July 1999 (Heggum 1999). Milk protein standardisation studies by Rattray and Jelen (1996) showed that skim milk permeate and sweet whey permeate increased the freezing point of milk due to the lower lactose levels, while acid casein whey permeate had the opposite effect. It was suggested that using combinations of permeates or addition of lactose may help to achieve the desired solids levels. It was also noted that permeates from lactic acid fermentations were unsuitable due to the presence of starter culture metabolites, which affected the flavour and storage stability of the milk.

Whey fermentation

There are a multitude of whey fermentation by-products that utilise the nutrient-rich liquid whey (see Table 14.16). The attributes required for commercialisation are exacting. Commercially successful whey fermentations include processes for the production of cheese starter cultures,

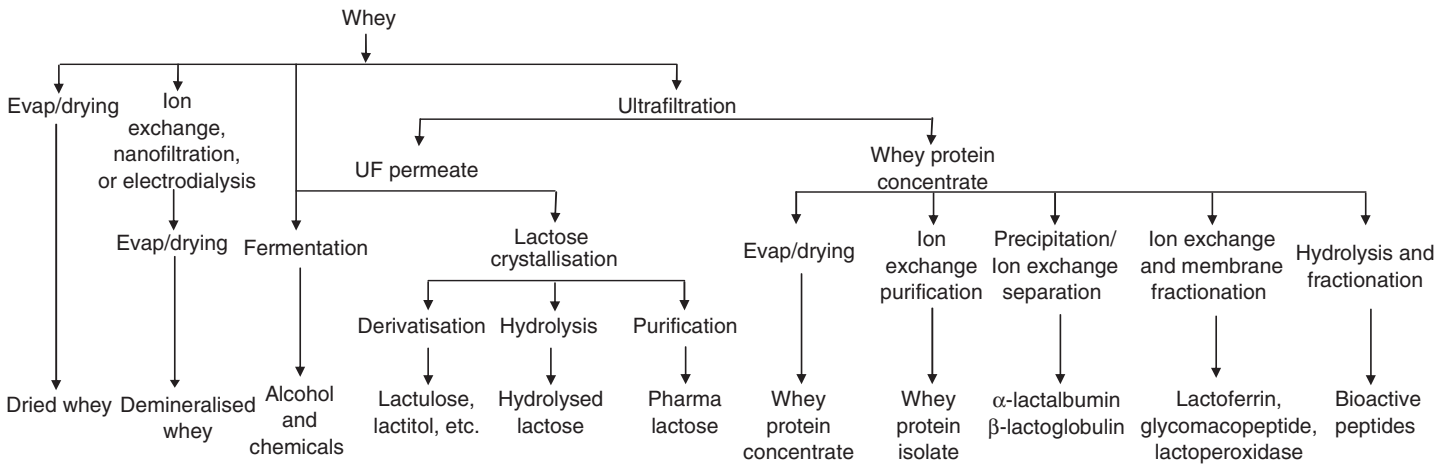

Fig. 14.7 Process alternatives for creating by-products from whey.

Table 14.16 Fermentation by-products from whey produced from a range of bacterial and yeast fermentations

Product	Organism	Reference
Ethanol	Saccharomyces *cerevisiae* (immobilised β-galacto-sidase), Kluveromyces *fragilis,* Candida *pseudotropicalis*	Lewandowska *et al.* 2002
	Recombined Saccharomyces *cerevisiae* (genes from *K. marxianus*)	Domingues *et al*. 2001
Butanol/acetone	Clostridium *acetobutylicum*	Haddadin *et al*. 1997
Acetate	Lactococcus *lactis*/Clostridium *formicoaceticum*	Huang & Yang 1998
	Lactobacillus *helveticus*/ Gluconobacter *oxydans*	Poget *et al.* 1994
Galactitol	Aspergillus *niger*/ Mycobacterium *smegmatis*	Muniruzzaman *et al*. 1994
Glycerol	Kluveromyces *marxianus*	Rapin *et al*. 1994
Lactic acid	Lactobacillus *salivarius*	Vasala *et al*. 2005
	Lactobacillus *lactis*	Liu *et al*. 2004
	Lactobacillus *casei*	Buyukkileci & Harsa 2004
	Lactobacillus *helveticus*	Fitzpatrick & O'Keeffe 2001
	Lactobacillus *delbrueckii*	Gassem & Abu-Tarboush 2000
Citric acid	Aspergillus *niger*	El-Samragy *et al.* 1996
Vinegar	Kluveromyces *fragilis*/ Acetobacter *pasteurianus*	Parrondo *et al.* 2003
Propionic and acetic acid	Lactobacillus *helveticus*/ Propionibacterium *acidipropionici*	Haddadin *et al.* 1996
	Propionibacterium *jensenii*	Tuckett *et al.* 1996
	Propionibacterium *acidipropionici*	Sorlini & Daffonchio 1995
Gluconic acid	Aspergillus *niger*	Mukhopadhyay *et al.* 2005
Succinic acid	Anaerobiospirillum *succiniproducins*	Lee *et al*. 2000
Diacetyl	Lactococcus *lactis* subsp. *lactis diacetylactis*	Gutierrez *et al.* 1997
Nisin	Lactococcus *lactis*	Hickmann & Monte Alegre 2001
Single cell protein	Kluveromyces *fragilis*	Ghaly *et al.* 2005
	Schizosaccharomyces *pombe* (mutant strain)	Abou-Zeid & Zaied 1999
Exopolysaccharide	Streptococcus *thermophilus*	Vaningelgem *et al*. 2004
	Streptococcus *thermophilus*	Zisu & Shah 2003
	Lactobacillus *delbrueckii* subsp. *bulgaricas*	Briczinski & Roberts 2002
	Pseudomonas *elodea*	Dlamini & Peiris 1996

Table 14.16 cont'd

Product	Organism	Reference
Lactan	Rahnella *aquatilis*	Pintado *et al*. 1999
Xanthan gum	Xanthomonas *campestris*	Nitschke *et al.* 2001
Bacterial slime	Lactococcus *lactis*	Christiansen *et al*. 2001
Biosurfactant	Pseudomonas *aeruginosa*	Dubey & Juwarkar 2004
Lipids	Apiotrichum *curatum,* Cryptococcus *albidus,* Lipomyces *starkeyi*	Akhtar *et al*. 1998
β-Galactosidase	Kluveromyces *marxianus*	Santiago *et al*. 2004
Lysozyme	Kluveromyces *lactis* (recomb. human lysozyme)	Maullu *et al*. 1999
Proteinases	Serratia *marcesens*	Romero *et al.* 1997
Transglutaminase	*Streptoverticillium mobaraense*	Zhu *et al.* 1995
Ribonucleotides	Kluveromyces *fragilis*	Belem & Lee 2002
Riboflavin	Ashbya *gossypii* Torulopsis *candida*	Erturk *et al.* 1998 Buzzini & Rossi 1997
Carotenoids	Rhodotorula *rubra*/ Kluveromyces *lactis*	Frengova *et al*. 2004

enzymes, nisin, ethanol and nucleotides. Most of these products can be sold for a premium.

Many of the organisms selected to ferment whey are able to utilise lactose as their primary energy source. However, to increase the range of fermentation by-products, some micro-organisms have been grown with immobilised β-galactosidase, or co-fermented with another organism able to utilise lactose, e.g. lactose fermenting *Lactobacillus helveticus* are grown with *Gluconobacter oxydans* to produce acetate. In recent years, micro-organisms have been manipulated so they are able to ferment lactose. For example recombinant DNA techniques can be used to convert *Saccharomyces cerevisiae* using genes from *Kluveromyces marxianus* to enable them to utilise lactose. Alternatively some organisms have been challenged to form mutations, so creating mutants able to utilise lactose, e.g. *Schizosaccharomyces pombe*.

Fermentation also creates waste by-products, such as spent fermentation liquor or stillage from alcohol production. These require further treatment to recover biomass for use as stock feed, or wastewater treatment prior to disposal over paddocks as fertiliser.

Whey powder

Evaporation and spray drying are the generally accepted methods for the disposal of large quantities of whey and a whey drying plant is often an integral part of a cheese factory (Caric 1994). Whey powder is used in a wide range of foods, mostly as a lower cost skim milk replacer, and in animal food. The properties of the whey powder are mainly governed by

the amount of lactose converted to α-monohydrate crystals before drying, creating a non-hygroscopic free-flowing product. If the lactose in the liquid whey is not crystallised the resulting high levels of amorphous lactose in the dried whey will lead to caking and poor storage stability (Listiohadi *et al.* 2005a, 2005b).

Whey is evaporated to 50–60% total solids, then pre-crystallised by cooling with mixing for 15–24 h to create a large number of crystal nuclei. This ensures non-hygroscopic α-lactose monohydrate is the dominant crystal form. The slurry is spray dried and then finished off in a fluidised bed drier to slowly equilibrate the moisture and maximise the content of α-lactose monohydrate.

New technology, known as the Paradry process, has been developed to improve the drying of hygroscopic liquids. This process has been designed for evaporation of liquids with high total solids contents, high viscosity or tendency to fouling. It is continuous, has low operating costs and produces a non-caking powder (Anon. 2003). The Paradry process commences with concentration to higher solids (75% for whey powder) using the Paraflash evaporator. The concentrate is continuously crystallised with mixing to obtain 90% crystallisation, forming a dough or granulated paste which is rapidly dried in the spin-flash drier (Anderson 2001).

14.5.3 Fractionation of whey

Whey fractionation imposes greater processing demands, but results in a wide range of products with unique functionalities and high value in the market place. Fractionated whey products include: whey protein concentrate; whey protein isolate; α and β fractions; bioactive proteins such as immunoglobulins, lactoferrin, lactoperoxidase and glycomacropeptide (GMP); and hydrolysed bioactive peptides. By-products from the ultrafiltration permeate include edible-grade lactose, pharmaceutical-grade lactose, hydrolysed lactose, lactose derivatives and milk salts. Applications of whey by-products are summarised in Table 14.17.

14.5.4 Demineralisation of whey

Whey is supersaturated with calcium phosphate and contains high levels of potassium and sodium. Calcium phosphate precipitation can cause problems such as fouling of the membrane or evaporator. High levels of minerals can inhibit lactose crystallisation which can adversely affect non-hygroscopic whey production and reduce yields and purity for lactose manufacture. The presence of high mineral levels limits the use of whey powder in infant formula and adversely affects the flavour and range of applications for whey products. A range of demineralisation techniques have been developed including precipitation, ion exchange, electrodialysis and nanofiltration. Some techniques preferentially remove divalents (e.g. calcium

Table 14.17 Properties and uses of whey and permeate co-products. Based on Durham *et al.* (1997b)

Product	Properties	Usage
Whey powder	Low-cost milk solids	Skim milk replacer
Demineralised whey powder	High-quality protein	Infant formula
Whey protein concentrate (35–85% protein)	High-quality protein, gelation, adhesion, emulsification, foaming	Infant formula, sports diets, non-fat milk replacement, processed meats, desserts
Whey protein isolate (90%+)	High-quality protein	Infant formula, sports diets
α-Lactalbumin (α-fraction)	High-quality protein	Infant formula
β-Lactoglobulin (β-fraction)	Gelling solubility and nutrition	Restructured meat or fish Sports and dietetic beverages
	Aeration	Meringues, desserts
Lactoferrin	Antibacterial	Infant formula
Lactoperoxidase	Anticaries	Toothpaste
Glycomacropeptide	Bifidobacteria, enhanced immunity	Infant formula
Immunoglobulins	Anticancer	Cancer prevention, treatment
	Enhanced immunity	Diets for AIDS patients
Peptides (hydrolysates)	Bioactive	Convalescent diets, athletes
Edible lactose	Carrier, filler	Colour, flavour carrier
	Free-flow agent	Instantised powdered foods
	Maillard browning, crumb texture	Bakery, coffee whitener
	Protein stabiliser	Milk standardisation
Pharmaceutical lactose	Bulking agent, binder, flavour	Tabletting excipient
Lactulose	Bifidobacteria enhancement	Infant formula
	Laxative	Laxatives
	O_2 uptake, NH_3 reduction	Diet for athletes
Lactitol	Bifidobacteria enhancement	Infant formula
	Non-caloric sweetener	Chewing gum
Lactobionic acid	Bifidobacteria enhancement	Infant formula, baby foods
Oligosaccharides	Bifidobacteria enhancement	Infant formula, baby foods
Hydrolysed lactose	Sweetener	Yoghurt, ice cream
Milk salts	Calcium, potassium	Diet supplement
	Flavour	Table salt substitute, health drinks

phosphate) or monovalents. These processes can be combined to remove a greater proportion of minerals.

Precipitation/whey pretreatments

Calcium and phosphate are present in milk, whey and permeate at concentrations where precipitation is inevitable (Schmidt & Both 1987). Calcium phosphate precipitation is enhanced by raised pH, heat (Brule *et al.* 1978) and concentration. This leads to evaporators fouling, shortened process runs and additional cleaning losses. Removal of protein further destabilises calcium phosphate, causing more fouling to occur during the evaporation of ultrafiltration permeate (Schmidt & Both 1987). Calcium phosphate precipitation is also responsible for fouling of ultrafiltration membranes (Ramachandra Rao *et al.* 1994).

Calcium phosphate precipitation is not rapid and involves a number of stages. Initially the solution becomes opalescent with the formation of hydrated amorphous calcium phosphate. This is followed by one of the precursor phases – dicalcium phosphate dihydrate (DCPD), dicalcium phosphate (DCP), tricalcium phosphate (TCP) or octacalcium phosphate (OCP) – the structure and composition depending on the pH, temperature and concentration. Then finally recrystallisation to hydroxyapatite (HAP) occurs (Feenstra & de Bruyn 1979).

The fact that this process is slow and continues through stages is central to the problem of calcium phosphate removal. Rapid removal of calcium and phosphate prior to heat and concentration is the preferred option, but this is not always possible when the whey protein needs to be protected. Researchers have described heat treatments and pH adjustments (Hayes *et al.* 1974; Hickey *et al.* 1980; Hobman 1984), and additives such as calcium (Hayes 1982; Karleskind *et al.* 1995), phosphate (Hiddink *et al.* 1981) and ethylene diamine tetraacetic acid (EDTA) (Ramachandra Rao *et al.* 1994) to force the equilibrium one way or the other.

Many dairy manufacturers are now recovering calcium phosphate from whey to produce dairy calcium supplements, marketed to combat osteoporosis. The precipitated calcium phosphate can be recovered using centrifugal separators, followed by washing, drying and milling.

Nanofiltration

Nanofiltration is a membrane process used to separate small molecules such as mineral ions and water salts but retain larger molecules (including lactose and proteins), coupling demineralisation with concentration. By allowing relatively free passage of monovalent ions, nanofiltration membranes are able to reduce the build-up of the osmotic pressure gradient. High fluxes are possible at lower pressures, with energy and equipment costs lower than those for reverse osmosis (Hoppe & Higgins 1992).

Nanofiltration using 300 Da membranes preferentially removes up to 65% of the monovalent ions (Na^+, K^+, Cl^-), with further reductions achieved

by diafiltration. Removal of monovalents improves lactose crystallisation from whey permeate. Guu and Zall (1992) reported that lactose recovery was improved by 8–10% after nanofiltration of permeate or sweet whey.

Nanofiltration is also advantageous for the production of demineralised whey powder for infant formula, as calcium phosphate is retained, yet monovalents are removed. The limited removal of divalent ions is due to their hydrated size, with up to 94% calcium and magnesium retained (van der Horst *et al.* 1996).

The nanofiltration permeate contains about 0.3–0.5% solids including potassium, sodium, non-protein nitrogen (NPN) and lactose. The permeate can be cleaned by reverse osmosis to produce clean water with the remaining solution being a 'dairy salt' concentrate. This monovalent salt mixture could be a useful by-product as a natural low-sodium table salt substitute or it could be used in sports and health beverages. This salt has also been reported to be recovered and used to regenerate ion exchange resins, as described in the following section (Durham *et al.* 2004).

Ion exchange demineralisation

Ion exchange has been used for the industrial demineralisation of whey and permeate since the 1970s. Ion exchange is capable of removing up to 95% of the minerals that are present in whey, with the demineralised whey mostly used in infant formula. Ion exchange can be conducted on either mixed-bed or sequential cation and anion resin-filled columns. The process involves passing the clarified whey or ultrafiltration permeate through a cation exchange resin whereby the cations in whey – such as sodium, potassium and calcium – are adsorbed on to the resin, displacing the hydrogen counter-ions on the cation resin. Subsequently, the decationised whey is passed through an anion exchange resin which absorbs the anions – such as chloride, sulphate and phosphate – displacing the hydroxyl counter-ions on the anion resin (Houldsworth 1980). When all of the fixed ionic sites on the resins are saturated with whey cations and anions, the whey is rinsed from the column and the resin is regenerated with strong acid and alkali solutions to remove the adsorbed ions and replace them with H^+ and OH^- (Jonsson & Arph 1987).

There are many difficulties with the ion exchange process, such as short running times between regeneration, high consumption of regenerant chemicals and associated waste problems, high water requirements to remove excess regenerant, losses of whey protein due to irreversible adsorption and loss of protein functionality due to pH fluctuations during processing (Jonsson & Arph 1987).

Ion exchange resins can also be used just to decalcify whey and permeates, by employing cationic resins in the sodium or potassium form; thereafter the decalcified whey or permeate is nanofiltered to remove the excess sodium and potassium. The ion exchange resin can be regenerated using the concentrated permeate from the nanofilter, thereby recycling the

salt from within the process, and avoiding the cost and pollution associated with purchasing salt to regenerate the resin (Durham *et al.* 2004; Groupe Novasep 2005).

Decalcification improves downstream processing, eliminating problems associated with calcium phosphate precipitation fouling evaporators and membranes. Decalcified whey can be further deionised by ion exchange with reduced regeneration costs; decalcified whey permeate can be further treated by ion exclusion chromatography to produce purified lactose.

Electrodialysis

Electrodialysis is an electrically driven membrane demineralisation process and has been employed in the dairy industry since the early 1970s. Electrodialysis is used to partially demineralise whey, mostly for infant formula. Monovalents are preferentially removed, enhancing the Ca : Na ratio in electrodialysed whey and minimising chloride levels, thus providing a nutritional advantage for use in infant formula.

Anion and cation membranes (ion exchange resins cast into thin films) are stacked alternatively with plastic spacers, with an anode or cathode at each end. When a direct current voltage is applied, the anions move towards the anode and the cations move towards the cathode. The membranes form alternating compartments of ion diluting and ion concentrating channels. The whey is pumped through the ion diluting channels, while the whey ions collect in the ion concentrating channels and are removed (Batchelder 1987). At the mineral levels normally found in whey, electrodialysis is more suited to partial (50%) demineralisation as higher levels of demineralisation require a disproportionate increase in recirculating time and electrical power, with membrane fouling also a problem at high levels of demineralisation.

14.5.5 Whey protein fractionation

Whey protein concentrate

Following its introduction in the early 1970s, ultrafiltration of whey has grown to become commonplace throughout the dairy industry today. Ultrafiltration membranes with a molecular weight cut-off in the range of 10 000–30 000 Da are used to concentrate the whey proteins, while the lactose and minerals readily pass through the membrane into the ultrafiltration permeate. The ultrafilter concentrates the whey protein concentrate (WPC) to 15–20% total solids. The spray-dried WPC contains approximately 34% protein, dry weight, the remainder consists of lactose and minerals. WPC 34 has a similar gross composition to skim milk powder and can be used in foods as a skim milk replacement.

Diafiltration can be employed to remove lactose and minerals, purifying the whey protein up to 90% protein dry weight. In this process, water is

added to the whey retentate during ultrafiltration to wash out lactose and minerals. There are a range of WPCs on the market, with enhanced protein levels such as WPC 50, WPC 75 and WPC 90.

WPCs possess a range of functional properties: gelation, water binding, foaming and emulsification. The 'quality' or functionality of the protein concentrate depends on the processing history of the whey, including the time/temperature history, pH, fat content, pump shear experienced and the whey pretreatments.

Whey contains many high-value biologically active proteins and peptides, with health and nutritional benefits that are attractive to an increasingly sophisticated market. In response, dairy processors continue to develop technologies to recover these fractions, with separations based on size, density, charge or hydrophobicity of the different components (see Table 14.18). Techniques include salting out, selective precipitation, heating at low pH, affinity chromatography, anion exchange chromatography, cation exchange chromatography using conventional resins or ion exchange membranes, size exclusion chromatography, hydrophobic chromatography, or combinations of enzymatic hydrolysis and membrane filtration. Different techniques are often combined to develop elaborate processes of sequential fractionation and purification resulting in an array of high-value by-products.

Whey protein isolate

Whey protein isolates (WPI) containing over 90% protein dry weight, are prepared using ion exchange. Whey protein purification by ion exchange adsorption utilises the amphoteric nature of whey proteins. Whey proteins have a net positive or negative charge depending on the pH of the medium. The isoelectric point of whey proteins is less than pH 5.5 and sweet whey is pH 6.1. Whey proteins therefore have a net negative charge at the pH of

Table 14.18 Types of proteins found in whey. Source: Marshall (1982)

Whey protein	Concentration (g/L)	Proportion of protein (%)	Isoelectric point (pH)	Molecular weight ($Da \times 10^3$)
β-Lactoglobulin	3.0	50	5.3–5.5	18.3
α-Lactalbumin	0.7	12	4.2–4.5	14
Proteose peptones	1.4	23	–	4.1–40.8
Bovine serum albumins	0.3	5	5.1	69
Immunoglobulins	0.6	10	5.5–8.3	15–1000
Lactoferrin	0.05	0.8	9.0	81–84
Lactoperoxidase	0.03	0.5	9.6	89
Glycomacropeptide*			4.2	7

* Data from Michel *et al.* (2002).

sweet whey and can adsorb on to the positively charged anion exchange resins.

The ion exchange process is carried out in a stirred tank, a batch column or on a continuous separator such as a simulated moving bed or using annular chromatography. Typically the whey is eluted through an anion exchange resin to adsorb the whey protein. The lactose and minerals are rinsed from the resin, then the protein is desorbed with acid or brine solutions. Variations on this process may employ cation exchange resins with whey adjusted to less than pH 4.5 so the proteins carry a positive charge, the purified whey proteins can then be eluted from the resin by raising the pH above 5.5. For example, a high-flow process described by Doultani *et al.* (2004) uses Sepharose big beads to adsorb the whey proteins, which are eluted with a single buffer for WPI, or with combinations of buffers to separate α-lactalbumin, lactoperoxidase and lactoferrin fractions.

There are some difficulties associated with ion exchange protein separation. Large volumes of dilute rinse and regenerant solutions are required, particularly for batch separations. The protein fraction needs to be ultrafiltered to separate the protein from the regenerant. The highly concentrated brine, acid or alkali used for more efficient protein desorption can affect protein functionality and solubility. Contaminants in whey can also foul the resin, necessitating regular cleaning cycles (Morr 1989).

Separation of α-lactalbumin and β-lactoglobulin

α-Lactalbumin is the major protein in breast milk and has a large market in the manufacture of infant formula. The β-lactoglobulin fraction is a highly functional protein that is soluble in acidic beverages, suitable for egg-white replacement in meringues and for heat-set gels in manufactured meat products.

There are many methods developed for the separation of the α-/β-fractions of whey protein with a few commercially practicable, such as that developed by Pearce (1995). This process involves heating a membrane-concentrated whey to ≥55 °C at pH 4–4.5 resulting in reversible aggregation of the α-lactalbumin, which is then separated by centrifugation. The solubilised α-fraction can then be purified by microfiltration to remove phospholipids, while the supernatant is recovered as β-fraction. Another method for the separation of α-/β-fractions is based on precipitation using acidified sodium citrate to precipitate α-lactalbumin; this is separated, re-solubilised with calcium chloride and then dialysed to give a 70% pure α-lactalbumin fraction; the liquid β-lactoglobulin is dialysed to give a 90% pure β-fraction (Alomirah & Alli 2004). There are many more processes described in the literature, for example: α-/β-fraction separation based on size exclusion chromatography (Garcia Rojas *et al.* 2004); two-stage ultrafiltration with 30 000 and 100 000 Da membranes in series (Beelin & Zydeny 2004); continuous annular chromatography (Giovannini & Freitag 2002); colloidal gas aphrons from cationic detergent (i.e. surfactant stabilised microbubbles)

(Fuda *et al.* 2005); ceramic hydroxyapatite chromatography and size exclusion chromatography to further purify and recover lactoferrin and immunoglobulins (Schlatterer *et al.* 2004).

Bioactive proteins

Whey contains a range of bioactive proteins. Lactoperoxidase is a prominent enzyme in whey that has a natural antimicrobial action. It is used to catalyse the inactivation of micro-organisms, yet is harmless to mammalian cells (Kussendrager & Hooijdonk 2000). Lactoferrin is an iron-binding protein with health benefits related to intestinal health, anti-inflammatory and anti-tumour activity (Naidu 2005). GMP is a κ-casein-derived protein naturally free of phenylalanine. It has physiological functions such as promotion of bifidobacteria, and immunomodulatory and bacterial toxin-binding effects (Thoma & Kulozik 2004).

Ion exchange chromatography is normally employed for the separation of lactoferrin and lactoperoxidase from whey. These proteins have isoelectric points above pH 9 and so are positively charged at the pH of whey and can be separated on cation exchange resins, then purified using ultrafiltration (Heeboll-Neilsen *et al.* 2004).

Novel ion exchange techniques are also being developed to recover lactoferrin and lactoperoxidase. Noh *et al.* (2005) describe the use of reverse micelles formed by cationic detergent to separate lactoferrin, based on solubilization behaviours of the proteins manipulated by pH, salt and surfactant concentrations. Fuda *et al.* (2004) describe the use of colloidal gas aphrons from anionic detergent, i.e. surfactant-stabilised microbubbles that act as ion exchangers for separation of lactoferrin and lactoperoxidase from whey.

Membrane adsorbers are also suited for separation of large volumes containing small amounts of the desired compound – such as whey growth factors, lactoferrin and lactoperoxidase (Regester *et al.* 1996). Ion exchange microporous membranes bind the desired component, yet allow passage of the remainder of the whey through the membrane, the desired component is then eluted from the membrane using dilute brine, acid or alkali and then concentrated by ultrafiltration (Splitt *et al.* 1996).

Uniform transmembrane pressure (UTP) filtration is opening up scope for more control over membrane separations for whey protein fractionation. In the past, concentration polarisation at the membrane surface limited selectivity of the membrane. In this mode the permeate is recycled to maintain a uniform pressure differential across the membrane (Kulozik & Kersten 2004). So far the application has been limited to microfiltration separation of native caseins, but there is scope for fractionation of many of the proteins in whey.

GMP is normally separated using ion exchange chromatography, whereby GMP carries a negative charge in whey at acidic pH, thus the GMP flows through the ion exchanger while the other positively charged proteins

bind to it. The GMP is then recovered from this final liquid (Tek *et al.* 2005). An alternative method, which reduces the volume of ion exchange resin and regeneration brine required, involves separating the GMP by ultrafiltration with a 100 000 Da membrane, then recovering the GMP by anion exchange chromatography (Xu *et al.* 2000).

Whey protein hydrolysates

Milk and whey proteins have been found to have different physiological functions due to the numerous bioactive peptides that are encrypted within the intact protein. Whey peptides contain 3–20 amino acid residues with the sequence of amino acids defining the function. Whey peptides have been found to exhibit various properties such as antioxidative, antihypertensive, antimicrobial, cytomodulatory, immunoregulatory, opioid, angiotensin-converting enzyme inhibition and mineral carrying capacity (Korhonan & Pihlanto 2003).

Bioactive peptides are commonly produced by enzymic hydrolysis of the whole protein, batch or continuously with immobilised enzymic membrane reactors. Hydrolysis is coupled with various separation procedures depending on the target peptide. Stepwise ultrafiltration (with diafiltration) can be used to fractionate the peptides based on size, while ion exchange/size exclusion chromatography is used for separations based on size and charge (Korhonan & Pihlanto 2003). New techniques have been reported by Groleau *et al.* (2004) who describe peptide fractionation by electro-nanofiltration which involves inserting a cathode into the permeate compartment of nanofiltration to increase separation of neutral and basic peptides.

14.5.6 Lactose from whey

Lactose manufacture

Lactose is recovered from whey permeate as edible-grade lactose in most dairy manufacturing countries. Edible-grade lactose is used in infant formula, chocolate and confectionery, baked goods and as a flavour/colour carrier (Rajah & Blenford 1988). Pharmaceutical-grade lactose is further refined and converted into a range of products: milled lactose crystals, spray-dried lactose, anhydrous β-lactose and micronised lactose. It is mainly used in pharmaceuticals as a tableting excipient, but also as the high-price, dry-powder inhaler. Pharmaceutical lactose and edible lactose are also used as a raw material for lactose derivatives such as lactulose, lactobionic acid, lactitol, galacto-oligosaccharides, or hydrolysed to liquid lactose syrup.

The process of lactose manufacture typically involves concentration of the ultrafiltration permeate to 60% solids in a multiple effect evaporator. The concentrate is transferred to large crystalliser vats and slowly cooled over 20 h. The crystallised lactose is then separated, creating a mother liquor containing 20–30% of the lactose plus 90% of the ash. The lactose

crystals are washed in a refiner washer with water in a ratio of 1:1 (water: lactose), then dried, milled and bagged.

Pharmaceutical-grade lactose undergoes further purification. The washed edible-grade lactose crystals are redissolved to 60% solids, fining agents are added, then filtered, recrystallised, separated and washed again (Pritzwald-Stegmann 1986). The process is less economically attractive than edible-grade lactose due to the large-scale investment required for the double crystallisation. It is also less environmentally attractive due to the larger waste streams from the double crystallisation.

The efficiency of the lactose crystallisation processes has been improved. Most dairy manufacturers use nanofiltration to reduce mineral impurities, as advocated by Guu and Zall (1992). Whey pretreatments to remove calcium phosphate as 'dairy calcium', which is sold as a dietary supplement, also reduce fouling of the evaporator (Hobman 1984). However, there is much more that needs to be done. The disposal of mother liquor is a major problem, it has limited use as stockfeed as it is perishable and the animals need to live close to the factory. Animal feeding also needs infrastructure, transport and feed lots. Mother liquor can be used as fertiliser by metering into irrigation, however it has a very high BOD_5, and has been found to kill fish if leakage occurs directly into streams. The best way to deal with mother liquor is to limit its production.

Chromatographic separation of lactose

Chromatographic separation of mother liquor has been proposed to reduce the problem of mother liquor disposal. Patents by Harju and Heikkilae (1989) describe a process that increases lactose yield from mother liquor, similar to the chromatographic separation of beet sugar molasses applied commercially. Further developments of this technology by researchers at the University of Western Sydney (UWS)/Food Science Australia (FSA) and licensed by Applexion (Groupe Novasep 2005) have extended the use of chromatography to recover lactose from whey permeate prior to crystallisation, thus avoiding production of mother liquor in the first place.

The ion exclusion lactose (IEL) process recovers pure lactose by ion chromatography, yielding pure lactose and a range of by-products, including calcium and soluble mineral fractions and a surplus of potable water. It is based on a four-step process wherein the whey permeate is first decalcified by ion exchange; secondly it is concentrated by nanofiltration and thirdly separated into purified lactose and mineral fractions by chromatography. The fourth step is the recovery of the monovalent ions from the nanofilter for regeneration of the ion exchange column; thus avoiding the cost of buying salt to operate the ion exchange, and reducing disposal of salty waste (Durham *et al.* 1997a). The process removes the problem of the highly pollutive mother liquor associated with traditional lactose processes and increases efficiency of downstream processing of lactose creating new

high-value lactose products. The liquid lactose fraction can be directly used in infant formula without the need to crystallise and redissolve, or can be used for lactose derivatives. Alternatively the purified lactose can be crystallised into pharmaceutical-grade lactose crystals (<0.01% ash) without mother liquor waste. Due to the purity of the liquor, the crystallisation can be controlled to create lactose designed to meet customer specifications for particle size and shape (Durham *et al.* 2004).

Derivatives of lactose

Hydrolysed lactose

Lactose can be enzymically hydrolysed into glucose and galactose with β-galactosidase, resulting in a syrup that is more easily digested by those who are lactose intolerant. Hydrolysed lactose is sweeter and more soluble than lactose and can be used for sweetening syrups in ice creams, yoghurts and drinks without lactose crystallisation problems (Hourigan 1984). Galactose isolated from hydrolysed lactose is also finding a market as an endurance sports drink additive (G-Push) (Alexander 2002). However, cost and difficulty of storage limit the production and use of hydrolysed lactose syrup.

Galacto-oligosaccharides

Oligosaccharides are carbohydrates of three to ten linked monomer sugars most commonly produced by enzymic transglycosylation reactions of lactose (Playne & Crittenden 1996). Oligosaccharides pass through the colon undegraded, where they encourage the growth of bifidobacteria in the intestine. Oligosaccharides also contribute to the development of the immune system, in human milk they have a protective effect against viral and bacterial infections, stimulate the immune response and enhance the bioavailability of minerals (Geisser *et al.* 2005). Oligosaccharides are water soluble and mildly sweet, they have a high viscosity and can contribute to the mouth-feel of the product. They can be used as a humectant, to control Maillard browning or inhibit starch retrogradation (Crittenden & Playne 1996).

Lactulose

Lactulose is produced by the alkaline isomerisation of lactose. It stimulates the growth of *Lactobacillus bifidus* in the large intestine, which has the same functions as oligosaccharides, i.e. lowering the pH of the colon and repressing the growth of pathogenic bacteria (Visser *et al.* 1988). Lactulose is widely used in hospitals for chronic constipation (Alexander 2002). Methods developed for the production of crystalline forms of lactulose have improved the range of applications of lactulose (Carobbi & Innocenti 1991; Dendene *et al.* 1995). New developments in lactulose production include the use of electro-membrane isomerisation followed by demineralisation by electodialysis to produce high yields of lactulose from lactose (Evdokimov & Alieva 2004).

Lactitol
Lactitol is produced from the catalytic hydrogenation of lactose to produce the sugar alcohol (Visser *et al.* 1988). Lactitol is used as a low-calorie sweetener and also acts as dietary fibre, competing against sorbitol and maltitol. Lactitol is less cariogenic than sucrose. It is not absorbed through the small intestine, so it does not raise blood glucose levels and is thus suitable for diabetics. It is fermented in the large intestine by the natural microflora to give 50% of the energy value of lactose (Booy 1987).

Lactobionic acid
Lactobionic acid is produced from the chemical oxidation of lactose or enzymically by lactose dehydrogenase (Rand & Hourigan 1975). Lactobionic acid is a complexing agent for metal ions (Visser *et al.* 1988). It is used as an organ preservative and more recently has been used as a skin cream additive (Alexander 2002).

14.6 Improving end-waste management in dairy processing

14.6.1 Water

Water is a limited resource, yet dairy processing characteristically requires very large quantities of fresh water. Water is used primarily for cleaning process equipment and work areas to maintain hygiene standards. The dominant environmental problem caused by dairy processing is the discharge of large quantities of liquid effluent. For plants located near urban areas, effluent is often discharged to municipal sewage treatment systems. In extreme cases, the organic load of waste milk solids entering a sewage system may well exceed that of the township's domestic waste, overloading the system. In rural areas, dairy processing effluent may also be irrigated on to land. If not managed correctly, dissolved salts contained in the effluent can adversely affect soil structure and cause salinity. Contaminants in the effluent can also leach into underlying groundwater and affect its quality (UNEP 2000).

Dairy processing effluents generally exhibit the following properties:

- high organic load due to the presence of milk components;
- fluctuations in pH due to the presence of caustic and acidic cleaning agents, plus other chemicals;
- high levels of nitrogen and phosphorus;
- fluctuations in temperature.

Meanwhile, large volumes of water are available for reuse within the typical dairy factory. Milk contains 87% water, whey contains 94% water, yet when converted into dried products the bulk of this water is lost in the waste as condensate, steam or permeate. Dairy processing also generates large

quantities of waste water. At start-up, during interruptions and rinsing, the waste is diluted milk, whey or cream without chemicals and could potentially be recovered. The development of processes for the recovery and reuse of water from dairy processing, requires consideration of the following: assessment and maintenance of quality of the recycled water; reuse options for recycled water within the dairy plant; treatment options for recycling water such as membrane filtration, ion exchange, etc.

Water quality

Water recovered from condensate, steam or permeate often contains low levels of organics from the medium being concentrated. The type and amount is dependent on the process equipment, the product and the degree of concentration achieved. Monitoring quality parameters such as: pH, conductivity, solids, BOD, total plate count and coliforms, is necessary for safety and quality, with the degree of testing determined by the intended reuse of the water. Water containing even small amounts of organic matter poses a biological hazard if stored without treatment for any length of time. Recycled-water storage tanks need to be monitored with regular flushing and cleaning cycles.

Guidelines for the hygienic reuse of process water have been published by the Codex Alimentarius Commission (2001) and by the New Zealand Food Safety Authority (NZFSA, 2003) (see Fig. 14.8). These guidelines can be used to set the framework of water reuse within the factory. Matching water quality requirements with the type of water available requires analysis of the critical control points and an evaluation of the potential contamination of the food products. For example, water used to wash floors does not need to be treated to the same level as water used to wash equipment that is in contact with the food. The water reuse plan should be integrated with existing Hazard Analysis and Critical Control Points (HACCP) programmes, with programmes for monitoring and control of recycled water (Kirby 2004).

Pursuant to the publication of the water reuse guidelines, the Codex Committee for Food Hygiene decided during the 36th session in 2004 to discontinue the consideration of the draft guidelines for the time being, with the understanding that this decision would be reviewed at a later time (Codex Alimentarius Commission 2004). As yet, the Codex committee have not yet revisited the water reuse guidelines.

Water reuse options

In the dairy factory there are two main scenarios for water reuse, either reused water will come into contact with food (raw, intermediate or final product) or it will not come into contact with food (Kirby 2004). The intended use of the water will therefore determine the degree of hazard and type of water treatment required.

The CODEX guidelines for the hygienic reuse of processing water in plants specify the following:

- Reuse water shall be safe for its intended use and shall not jeopardise the safety of the product through the introduction of chemical, microbiological or physical contaminants in amounts that represent a health risk to the consumer.
- Reuse water should not adversely affect the quality (flavour, colour, texture) of the product.
- Reuse water intended for incorporation into a food product shall at least meet the microbiological and, as deemed necessary, chemical specification for potable water. In certain cases, physical specifications may be appropriate.
- Reuse water shall be subjected to ongoing monitoring and testing to ensure its safety and quality. The frequency of monitoring and testing are dictated by the source of the water or its prior condition and the intended reuse of the water; more critical applications normally require greater levels of reconditioning than less critical uses.
- The water treatment system(s) chosen should be such that it will provide the level of reconditioning appropriate for the intended water reuse;
- Proper maintenance of water reconditioning systems is critical;
- Treatment of water must be undertaken with knowledge of the types of contaminants the water may have acquired from its previous use; and
- Container cooling water should be sanitised (e.g. chlorine) because there is always the possibility that leakage could contaminate the product.

Fig. 14.8 Proposed draft guidelines for the hygienic reuse of processing water in plants. Based on: Codex Alimentarius Commission (2001).

Food-contact applications include: cleaning/rinsing of equipment; diafiltration water for ultrafiltration or nanofiltration processes; ion exchange resin rinsing or elution; decanter washing of lactose crystals or calcium phosphate precipitates; and dissolution of ingredients such as lactose for secondary crystallisation. Non-food-contact applications include: counter current cooling processes; boiler water and steam generation; floor washing and general sanitation; and fire fighting. It should be noted that these applications may lead to incidental contact with food.

Treatment of recycled water

The choice of treatment is determined by the intended reuse of the recycled water and the quality of the available water. It is possible to treat water to such an extent that it reaches potable quality. Wastewater treatments can be classified as primary (physical), secondary (biological) or tertiary (chemical). Waste water that has undergone all three levels of treatment and tested to meet the standards is classified as potable (Palumbo *et al.* 1997).

Early treatment options for water reuse include the addition of disinfectants to stop bacteria and mould growth such as silver ions, chlorine and chlorine compounds, or mixtures of peracetic acid and H_2O_2. Such chemical addition may have enabled water to be used for cleaning, but not for food

contact. Other treatment options include carbon filtration to remove organic contaminants and ion exchange to remove minerals, particularly for boiler feed water (IDF 1988).

Improvements in membrane materials, cost and process efficiency have enhanced the options for water reuse. Gungerich (1996) examined the use of ceramic ultrafiltration to clean up evaporator condensate from milk concentration. The ultrafilter removed all the micro-organisms and residual protein material thereby improving downstream processing by reverse osmosis. Without pretreatment, the reverse osmosis rapidly lost flux and needed cleaning after only 15 h, with ultrafiltration pretreatment the reverse osmosis maintained flux at 50 L/m^2/h and could be run over 24 h. Additionally, the ceramic membrane can be backflushed and sanitised with chlorine or steam without damaging the membrane. Recent studies by Vourch *et al.* (2005) examined the treatment of dairy rinse waste water and found that single-stage reverse osmosis or two-stage nanofiltration/reverse osmosis was sufficient for water reuse as heating, cooling, cleaning or boiler feed water. Higher quality water, which complied with the drinking water limit, was produced using a two-stage reverse osmosis/reverse osmosis process.

The final water quality relies on continued integrity of the membrane during repeated cycles of processing and cleaning. Membrane failure can occur, either as a slow leak or as sudden failure. Water quality monitoring is integral to the membrane process to assure water quality and safety, and identify leaks as they occur. It is recommended that the recycled water be pasteurised prior to reuse, to reduce microbial contamination and delay microbial growth during storage. Finally storage time of water before use should be minimised (e.g. less than 1 day) at cool to ambient temperatures to minimise microbial growth. The storage tank should also be flushed with a regular cleaning programme to prevent the build up of contamination over time.

14.6.2 CIP cleaning solutions

Dairy equipment is regularly cleaned using clean-in-place (CIP) operations. CIP is a system of cleaning and sanitising based on circulating chemicals and water without taking the equipment apart (IDF 1979). The first step in the cleaning cycle is a water rinse, followed by a caustic wash to remove most of the organic deposit. After a short water rinse, an acidic wash is circulated to remove the mineral soils, followed by a sanitising step (Henck 1995). In the dairy industry, CIP operations make a substantial contribution to water consumption and are responsible for 50–95% of overall waste stream volume and high pH (9–11) (Gesan-Guiziou *et al.* 2002). According to the work of Hogaas-Eide (2002), cleaning processes are also major contributors to the total eutrophication potential from dairy processing at 80%; while the corresponding contribution to energy use is approximately 30%.

CIP systems rely on sensors to monitor the cycle and the adequacy of cleaning; therefore it is possible to divert the spent cleaning fluid for recycling. To prolong the life of detergent, it is common practice to eliminate the soil particles by sedimentation, centrifugation or by membrane technology (Schindler 1993). In recent years membrane filtration processes such as microfiltration, ultrafiltration and nanofiltration have been applied to CIP solution regeneration, resulting in significant removal of both suspended solids and soluble solids (Dresch *et al.* 2001).

A number of studies (Dresch *et al.* 2001; Merin *et al.* 2001; Gesan-Guiziou *et al.* 2002; Rasanen *et al.* 2002) have examined the use of nanofiltration to recycle spent caustic solutions, whereby milk solids are removed by nanofiltration and the cleaned caustic collected in the nanofiltration permeate for reuse. Studies by Lundekvam and Flaten (1997) show that membrane recycling of cleaning solutions leads to energy savings of 16%, water savings of 10% and also detergent savings (alkaline 32% and acidic 26%). Life cycle assessment of CIP by Hogaas-Eide *et al.* (2003) also confirms that membrane filtration of cleaning solutions reduces detergent use, energy use and emissions.

Studies on the operation of nanofiltration CIP recycling have been directed towards optimising the choice of membrane, volume concentration ratio and whether to operate the plant in batch-fed or continuous mode. Rasanen *et al.* (2002) report that nanofiltration using Desal-5 DL membranes reduced the COD content of caustic washing solution by 80% with a 16–21 volume reduction; the cleaned caustic nanofiltration permeate was then concentrated by reverse osmosis from 0.2% to 0.5–0.7% NaOH prior to reuse.

Recycled caustic solutions have also been found to have better cleaning efficiency compared with newly prepared NaOH solutions (Merin *et al.* 2002). Microfiltration and nanofiltration permeates of recycled cleaning solutions tested on ultrafiltration membranes fouled with whey proteins, were found to have lower surface tension due to residual milk components hydrolysed by the caustic solution. Consequently the recycled cleaning solutions had a higher wetting capacity and cleaned more efficiently.

However, variable payback periods for the installation of nanofiltration CIP recycling equipment have been reported. Dresch *et al.* (2001) estimated that savings from reduced caustic usage, reduced energy for heating the total volume of the CIP and reduced HNO_3 for neutralising waste water, had a payback of 14 years. Koch International who market the AlkaSave® Recovery System estimate payback at 1.5 years (but assume much higher disposal rates for CIP solutions for single-use CIP than Dresch *et al.* (2001)). Meanwhile Henck (1995) estimated a 7.7 year payback, but also with assumptions of high disposal rates for single-use CIP solutions.

14.6.3 Spent ion exchange brines

Ion exchange is widely used in the dairy industry for protein fractionation, a demineralisation or decalcification of whey and permeates. Ion exchange is also used for softening boiler feed water and water treatment. However the effectiveness of ion exchange relies upon having an adequate supply of brine to regenerate the resin and maintain ion exchange capacity at a functional level. The amount of regenerant chemicals required and the disposal of spent high-salt regenerant imposes significant environmental costs. To counter these problems, methods to minimise the use of regeneration chemicals or recycling strategies need to be implemented.

Alternative ion exchange systems

Several alternative ion exchange systems have been proposed with reduced regeneration requirements; two examples based on the use of weak anion or cation resins include the Svenska Mejeriernas Riksforening (SMR) process and the Sirotherm® (ICI Aust. Ltd) process.

The SMR process employs a weak anion resin in the bicarbonate form. The whey anions are first exchanged for HCO_3^- counter-ions, then the whey enters a weak cation exchange column in the ammonium form, where the cations are exchanged for NH_4^+ counter-ions. The demineralisation efficiency of this process is about 90% (Jonsson & Olsson 1981). After the resins have been saturated with whey salts, the resins are rinsed and regenerated with ammonium bicarbonate. The NH_4HCO_3 remaining in the whey after the ion exchange is a thermolytic salt, and decomposes to NH_3, CO_2 and H_2O when heated. Thus it is possible to recover the NH_3 and CO_2 stripped off from whey for recycling into regenerant (Jonsson & Olsson 1981). Another advantage is the reduced pH fluctuations of the whey during ion exchange, maintaining the pH range of 6.5–8.2. However, after every three to four cycles the cation resin requires a strong HCl treatment to regenerate the resin due to the partial retention of Ca^{2+} and Mg^{2+} on the cation resin after NH_4^+ regeneration.

The Sirotherm process was developed by Weiss *et al.* (1966) employing thermally regenerable ion exchange resins that are regenerated with hot water instead of acids and bases, thus reducing operating costs and effluent pollutants (Parrish *et al.* 1979). The Sirotherm process uses weak basic and weak acidic ion exchange resins for the adsorption of salts from an aqueous solution (Weiss *et al.* 1966). In the process described by Parrish *et al.* (1979) whey permeate is pretreated with Duolite S-761 to remove all residual proteins and riboflavin which may cause irreversible fouling of the Sirotherm resin. Next the permeate is passed through Sirotherm TR-10 which removes 76% Ca^{2+} and 90% Mg^{2+} ions and an equivalent amount of anions. The remainder of the Ca^{2+} and Mg^{2+} is removed with Duolite C-20. Further treatment with Sirotherm TR-20 removed 99.5% of the Na^+ and K^+. Lactose

was crystallised from the deionised solution and the yield increased from 43% (untreated) to 56% (deionised).

Recycling spent ion exchange brine

A number of recycling strategies to recover spent ion exchange regeneration brine have been developed by researchers. These employ various combinations of electrodialysis and nanofiltration to recover spent brine from either anion or cation exchangers.

Byszewski *et al.* (1995) proposed a method whereby spent anion exchange regenerant is sent to a three-compartment electrodialytic water splitter, having at least one bipolar ion exchange membrane, to produce an electrodialytically depleted regenerant solution and an amount of acid and base that is about equal to the amount required to regenerate the anion exchange column.

Noel (1994) proposes a three-step method for the demineralisation of whey by a strong monovalent cation exchange resin before passing through electrodialysis. The removal of divalents improves the operation of the electrodialyser. They recover the brine from the electrodialysis step and use this to regenerate the ion exchange resin.

Rocha San Miguel Bento (1995) propose a process for the recovery of spent anion exchange regeneration brine by nanofiltration; whereby nanofiltration is used to concentrate the colourants from sugar decolourisation into the retentate while the permeate can be used as recycled anion exchange regeneration brine. Further examples on recycling of spent anion exchange brines by nanofiltration have also been reported by Wadley *et al.* (1995) and Cartier *et al.* (1997).

The recovery of spent anion exchange brine using nanofiltration fits naturally with the functionality of polymeric nanofiltration membranes, which carry a negative surface charge at neutral pH, wherein salt rejection characteristics are governed by anion repulsion (Baticle 1997). Patents exploiting the negative charge of nanofiltration membranes for the recovery of spent anion exchange brines rely on the ability of nanofiltration membranes to reject large multivalent anions (e.g. colourants) while the monovalent chloride ions pass through into the permeate for use as recycled anion regeneration brine.

Polymeric nanofiltration membranes are amphoteric with ionisable carboxyl and amine functional groups on the membrane surface with an isoelectric point in the range of pH 3–6 (Hagmeyer & Gimbel 1999). Recent research has been directed towards utilising the positive surface charge of nanofiltration membranes at acidic pH to maximise rejection of multivalent cations (Durham *et al.* 2003; Teixeira *et al.* 2005). Studies have shown that nanofiltration rejection characteristics can be manipulated with changes in pH, anion composition and brine concentration by diafiltration to maximise divalent cation retention and maximise recovery of monovalent brine for regeneration of ion exchange resin.

14.7 Future trends

Milk production continues to increase, steadily in developed countries (Europe, the United States, Canada, New Zealand and Australia) but with the highest rate of increase in developing countries (China and India). As standards of living increase, dairy products are becoming a staple in diets for people across the world. Dairy product consumers are also changing the way that they are eating dairy foods, shifting towards modified milks, yoghurts and especially cheese; this trend is predicted to increase into the foreseeable future, resulting in increasing quantities of whey which needs to be utilised or sent to waste. Dairy manufacturers are also required to respond to increasing legislative and environmental pressures (waste disposal, greenhouse gas emissions, fossil fuel and water availability), yet remain profitable in a very competitive market.

In response to these pressures, dairy farms are amalgamating to create larger farming units; smaller factories are closing, with the remaining large dairy factories aiming at increasing economies of scale. This has both positive and negative effects on the environmental impact of dairy processing; large-scale production concentrates the impact of waste and emissions on to a small area, yet also allows greater efficiencies and capabilities for implementing processes for recycling and producing co-products.

One future option to increase co-product recovery is to create networks of manufacturers to collect together their resources, increasing economies of scale for the utilisation of waste by-products (Durham *et al.* 1998). For example, a centralised whey processing facility pooling whey from several cheese manufacturers is more able to implement new technologies for processing and marketing a range of whey co-products. High-value whey products aimed at the nutraceutical and functional food markets (e.g. whey protein isolates, bioactive peptides, whey protein hydrolysates, pharmaceutical α- and β-lactose, galacto-oligosaccharides and dairy minerals), increase the profitability of such ventures. Lower cost co-products utilising the bulk of the whey, and employing cleaner production practices, would ensure the continued environmental viability of the industry. Examples of these co-products include: liquid permeates used in dairy product standardisation; hydrolysed lactose syrups used in soft drinks and whey drinks; and demineralised and whole whey powders used in infant formula, ice cream and baked foods.

14.8 Sources of further information and advice

International
IDF-FIL International Dairy Federation – http://www.fil-idf.org
International Dairy Foods Association – http://www.idfa.org/
United Nations Environment Programme – http://www.unep.org/
Food and Agriculture Organisation (FAO) – http://www.fao.org

FAO – Dairy Links –
http://www.fao.org/livestock/agap/lps/dairy/URL/Url.htm
International Organisation for Standardisation – http://www.iso.org/

European Union
EuroMilk – European Dairy Association/European Whey Products Association – http://www.euromilk.org/
European Union – Waste Management Policies –
http://europa.eu.int/scadplus/leg/en/s15002.htm
Leatherhead Food Research Association – http://www.lfra.co.uk
Dairy UK – http://www.dairyuk.org/
Danish Dairy Board – http://www.mejeri.dk
Swedish Dairy Association – http://www.svenskmjolk.se/
National Dairy Products Research Centre, Cork – http://www.teagasc.ie

North America
American Dairy Products Institute – http://www.adpi.org/
American Dairy Science Association – http://www.adsa.org/
US Environmental Protection Agency –
http://www.epa.gov/epahome/rules.html
Dairy Management Incorporated – http://www.dairyinfo.com/
Institute of Food Technologists – Dairy –
http://www.ift.org/divisions/dairy/dairyrcenters.html
Canadian Dairy Information Centre –
http://www.dairyinfo.gc.ca/cdicmain.htm

Oceania
Dairy Australia – http://www.dairyaustralia.com.au
Dairy Process Engineering Centre – http://www.dpec.com.au
Australia Environmental Protection Agency –
http://www.environment.gov.au/

Asia
Cleaner Production in China –
http://www.chinacp.com/eng/cp_policy.html
India – National Environment Policy 2004 –
http://envfor.nic.in/nep/nep.pdf
Dairy industry in India –
http://www.indiaagronet.com/indiaagronet/DAIRY/Dairy.htm

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15

Waste management and co-product recovery in fish processing

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15.1 Introduction

Fish processors face significant problems when disposing of waste materials. For much of the last century, the waste from fish processing was transported to rendering plants where it was converted into meal, or sold to mink farms or pet-food manufacturers. The rendering plants have since been recognized as significant polluters and most are now closed; there are few mink farms remaining and pet-food manufacturers take very little waste from fish processors. Disposal of fish waste at sea or in landfills is both expensive and highly regulated, as is pumping ground waste into coastal waters. Fish processors must therefore find new ways to address the problem of waste disposal.

There are good economic reasons to consider co-product recovery. With fewer fish being caught, the pressure to maximize profit by using every part of each fish is intense. While not every species or type of fish can be used in its entirety, a large number can. This chapter outlines the current status of seafood waste management and co-product development.

15.2 Co-product recovery and development

The spectrum of potential co-products (or by-products) is enormous, ranging from the very high end (fish oil capsules) to the very low (compost), and encompassing mince, gelatin, roe and other specialty organs, leather, liquid fertilizer, flavorants, and fish meal. Shellfish wastes lend themselves to some of the same products – namely flavorants, liquid fertilizer, meal, and compost – while shrimp, lobster, and crab shells contain chitin, which

has unique benefits. The simplest and most functional way to think about what is currently waste is to separate the options into three categories: those that eliminate all or part of the waste, those that use all of the waste, and those that use a portion of the waste.

15.2.1 Eliminating waste

The most straightforward and economical way to deal with waste would appear to be elimination. For example, any producers who can sell their product whole will not have a waste problem and, in some cases, can achieve the highest price for the product. Restaurants and markets in Asia often sell seafood live. But while this is relatively straightforward for crab and lobster, it becomes more difficult for suppliers of live fish. Fishing boats will need holding tanks, the fishermen must be taught how to handle the catch without harming it, and facilities and transport on land will need to be altered to accommodate live catch. Those who can enter this market most easily are aquaculturists who raise relatively small fish such as tilapia or flounder, since they start out with the facilities to maintain the fish alive, have collecting systems that cause minimum trauma to the animals, and offer species that are recognized and valued by Asian markets.

Despite the obvious benefits of selling products alive and whole, shellfish dealers are finding that they can maximize profits by further processing, even products such as lobsters, which have traditionally been sold alive and whole (particularly in the United States). However, further processing almost always generates waste, and that waste must be dealt with.

Some waste can be eliminated by changing the form of the product or by developing a market for a new product. Korean markets are interested in whole flatfish containing roe, so the roe does not become a waste product. The head and tail are cut off but the roe is left in the body, a cut known as 'karimi'. Another example, popular in some countries, is roe-on scallops. Unlike the flatfish example, which only involves finding a market and minimal re-training of the workforce, marketing roe-on scallops is more difficult due to regulatory issues, particularly concerning red tide toxins. Scallops are more usually sold as pure muscle tissue without the roe. Muscle tissue does not take up toxins, but roe does, so producers of roe-on scallops must comply with testing and certification.

There are large Asian populations, with their own markets and restaurants, in many cities around the world. Different populations buy different products. Fish processors who wish to reduce waste by developing new products or modifying old products should explore the different markets and see if they can supply the demands of these potential new customers, as well as those they have served traditionally. For example, Asian consumers buy fish heads, whereas most Western consumers prefer headless product. However, while the price for fish heads in Asian markets may

seem very attractive, once it is recognized that a significant amount of neck meat must be left on the head, thus reducing the weight of steak or fillet, the profit will shrink. Thus, producers must make careful economic decisions when trying to reduce waste by creating products for more than one market.

15.2.2 Utilizing all the waste

There are three main categories of co-products that utilize all, or almost all, seafood processing wastes. These are compost, meals, and hydrolysates or digests. These products are often produced by independent entrepreneurs, who have the time and interest to learn the technology and to develop markets that, for the most part, have nothing to do with seafood. We will define each technology, explain how to get started on a small scale, and discuss the pros and cons of each.

Composting

Composting is the controlled, microorganism-mediated breakdown of organic materials containing both carbon and nitrogen, to produce a humus-like material that can be used as a fertilizer and soil amendment. Composting can be aerobic (requiring oxygen) or anaerobic (without oxygen). Aerobic composting reduces odors and improves nitrogen retention, compared with the anaerobic process.

The biggest advantage of composting is that it is the only process that accommodates all organic waste generated by fish processing: spilled or excess breading, crustacean shells, rotten material, smoked scraps, etc. Hard shells, such as those of clams and mussels, are not broken down by composting but they are cleaned by it and can be screened out later, and may have value as driveway fill. Composting will sometimes even break down toxic contaminants, but this must be tested for each individual contaminant. Where contaminants are not broken down, they will at least be diluted. It should be noted, however, that there is a real danger that the breakdown products of known contaminants will be unknown or lesser known contaminants, which may be difficult to test for. The disadvantage of composting for our purposes is that seafood waste cannot be composted by itself. Seafood is an excellent source of nitrogen but to compost it, a source of carbon must be added. This means that composters negotiate for and handle very large quantities of autumn leaves, sawdust, grain hulls, peat, shredded paper – whatever carbon source is available. To prevent odor, the carbon source must be available as soon as the seafood waste comes in to be composted, and so it usually needs to be stored onsite. Thus composting seafood wastes requires a lot of space, along with logistics and material handling expertise. There are in-vessel systems that greatly reduce the space requirements, but they are much more expensive and often require greater skills to operate.

Composting is a slow process, and may take anywhere from a few weeks to several months, after which the compost must 'cure' before it can be sold. Curing generally means that it must be stored, sometimes for as long as a year. The economic and space consequences of this are evident.

Although home composting is simple, running a commercial composting operation is not. Professional composters make about half of their income from selling compost and the other half from fees paid by businesses disposing of compostable waste. Composting might be a good solution for seafood processors who generate a relatively small amount of waste, and who have access to carbon sources and land. It is worth noting that crustacean shells may make the finished compost particularly valuable since they contain chitin, which provides slow-release nitrogen.

Another method for those generating a relatively small amount of waste is vermicomposting, or worm composting. Although called composting, this process actually comprises feeding worms with the waste and harvesting their excrement (professionally known as 'castings'). Worm castings are a particularly valuable fertilizer (due mostly to the microorganisms they contain rather than the amount of nitrogen and other nutrients), and worm composting has some real advantages over the more conventional process. First, it is much quicker. Second, unlike real compost, which has to cure prior to sale, worm castings have a limited shelf-life so the sooner they are sold, the better. Third, the worms are housed indoors, they do not take up huge amounts of room and multi-tiered housing can be constructed cheaply. Fourth, there is no odor and therefore the issues of land, permits, and litigation are minimized. Finally, there are no corrosive chemicals or high temperatures and pressures to deal with, no dangerous (and expensive) equipment, and the process is safe for humans. Unfortunately, the latter is not true for standard composting, where employees must wear appropriate protective breathing equipment to prevent the inhalation of mold and bacterial spores.

Unlike meal manufacture or hydrolysis, there is an enormous body of literature on composting, and a surprisingly large sub-section of this is on seafood composting. However, like all seafood by-product ventures, a fair fraction of seafood composting operations start out looking profitable but run into problems. Successful ventures in the literature should be researched, not least to see if they are still operating, and, since composting is rarely done in a proprietary way, operators are usually willing to share their problems and expertise. There is also a lot of information on vermicomposting, much of which is online. A number of references are included at the end of the chapter, but it should be noted that while the publications for beginners may have titles that sound more cute than serious – *Worms Eat My Garbage* (Appelhof, 2001) and *Compost This Book* (Christopher and Asher, 1994) – they tend to be the best places to start, since they explain everything a small-scale startup needs to know, use non-technical language, and are inexpensive.

Meal manufacture

Fish meal (or, for that matter, shrimp or crab meal) is basically the result of cooking, dehydrating, partially de-oiling, and grinding the raw material. Since fish is typically 65–80% water (depending upon its oil content), this process provides a huge reduction in bulk and renders the material shelf stable, so that it can be bagged, stored, and shipped at ambient temperatures. It also supplies feed mills (by far the largest users of seafood meals) with fish in the dry granular form they prefer. This section focuses on the production of fish meal because it far outweighs the production of any other type of seafood meal.

Modern fish meal manufacturing usually consists of several processes: coarse grinding, cooking, pressing, drying, stabilizing with antioxidant, and fine milling. In addition, there is often a short curing period which allows the meal to cool down before it is packed into containers for shipment. The liquid that is pressed out of the cooked fish is separated into oil and water streams. The oil becomes a product and, as we will see, the treatment of the water stream (which contains significant amounts of protein) varies, although it is generally evaporated down to a thick paste.

In meal production, the fish is cooked for two reasons: to sterilize it and stop any decay processes, and to denature the protein and free-up bound water. Fish decays rapidly at even slightly elevated temperatures, so how the fish scraps are stored prior to cooking is as important as the cooking process.

One of the main reasons why the old rendering plants were such bad polluters was due to raw material handling and storage. These plants collected scraps, including poultry and meat as well as fish, from multiple sources, many of which did not bother to refrigerate what they saw as waste. Containers might be left out in the sun, and might be held overnight or even over a weekend. Upon arrival at the plant, they were rarely refrigerated and were often left outside. Raw material delivery was not timed to plant operation, nor was raw material quantity limited to the plant's capacity. Poultry and meat wastes come from animals whose body temperature is close to 100 °F/38 °C. If cooled to around 45 °F/7 °C, the process of decay slows dramatically. But fish body temperature is more likely to be around 45 °F/7 °C, and so storage at that temperature or a higher one will allow enzymes and bacteria to break down tissue rapidly and hasten decay. In fact, as discussed later, warming fish tissues causes them to liquefy, digested by endogenous enzymes.

For a rendering plant, improper (i.e. warm or extended) storage causes two problems. First, as enzymatic autolysis causes more liquefaction, the yield of solid meal is reduced. Second, advanced bacterial decomposition causes the formation of highly odiforous compounds, such as cadaverine and putrescine. Fish also contain significant quantities of an odorless compound called trimethylamine oxide (TMAO) which, upon death and decay, is broken down to trimethylamine (TMA). It is the strong and

very unpleasant odor of TMA that is diagnostic of spoiled fish. Hence the old style of rendering plant caused serious odor-pollution in neighboring areas.

Modern meal plants rarely take in scraps from more than a few processors because it is too difficult to maintain quality control. Most meal plants no longer take in scraps at all; they work with dedicated fisheries of what are called 'industrial' fish, usually oily fish such as menhaden in the US Gulf, sand eel in the North Sea, capelin off Iceland, or anchovy off Peru. These are typically small fish, which are not in great demand as human food, and are available in large quantities. The mealplants that take them have enclosed refrigeration facilities to hold fish that have been delivered but cannot be processed immediately. It should be noted that industrial fish are major components of their ecosystem's food chains and whether problems within those ecosystems are due to the removal of large quantities of such fish is currently hotly debated. These concerns, plus increasing regulations banning other forms of disposal and demanding total utilization of the catch, all add to the push for adapting meal production to processing waste.

Meal plants that do use processing scraps have found ways to keep those scraps in good condition. In areas like Alaska, where single corporations run enormous plants, there are several good-sized fish meal plants operating on the waste from a single processing operation. This arrangement makes sizing the meal plant to the primary operation straightforward. Processors in other areas have formed cooperatives to operate meal plants as a group. Since the cooperative benefits from the meal plant, it is in each member's interest to keep their contribution fresh. Unlike the small boats that catch the industrial fish, fish plants usually have refrigerated holding facilities where they can store waste that cannot be utilized immediately.

A different set of operating conditions exists on factory trawlers, which often run on-board meal plants. These on-board plants operate differently from the land-based ones, and will be discussed later.

Modern meal plants are usually totally enclosed, so that odors cannot escape. Some plants go to the extreme of having negative air pressure inside the plant, so that when the doors are opened, air can enter but not escape. However, with fresh raw material and enclosed processing machinery, this is of secondary importance. The most likely stage for the escape of odors is from the process itself, and plants today have built-in mechanisms for eliminating those odors, usually via condensation of the vapor followed by incineration at very high temperatures, which oxidizes the volatile odor compounds to their odorless products.

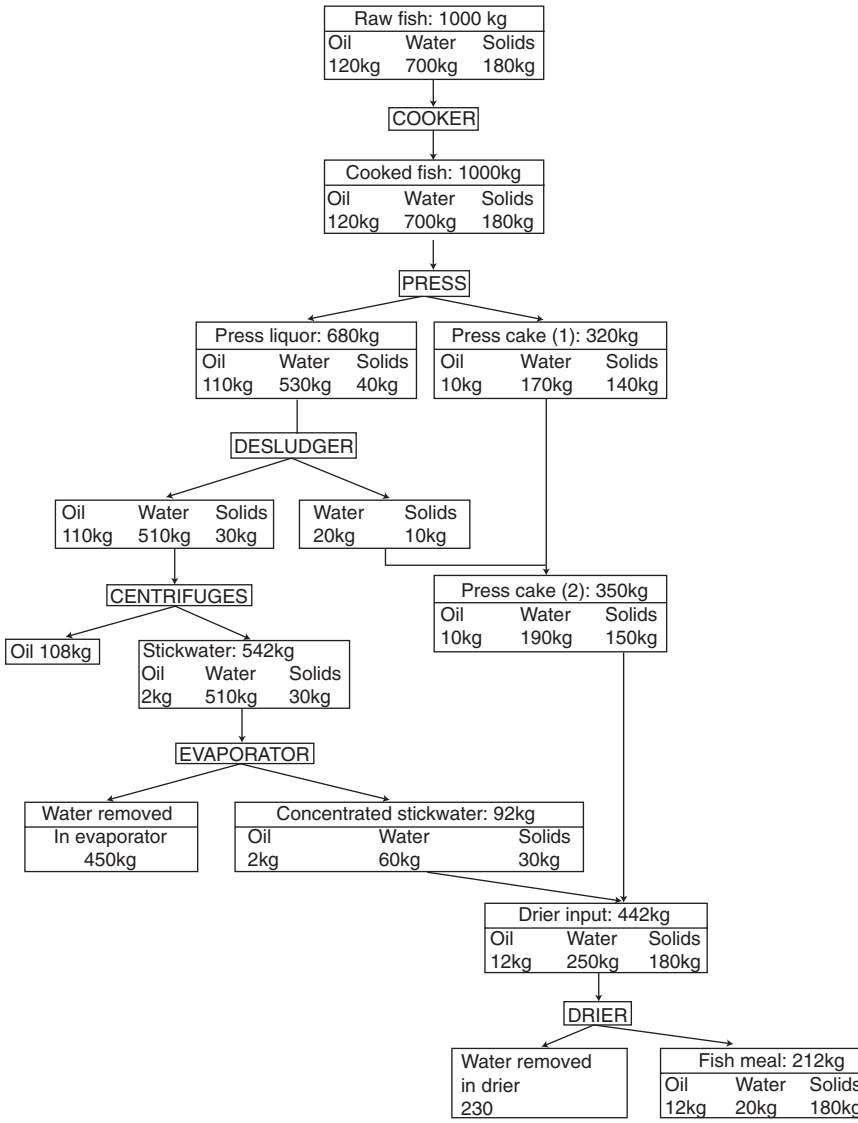
As with all food plants, good housekeeping is the key to both good products and good relations with neighbors. Even a small amount of fish left to rot can cause unpleasant odors. Unlike most food plants, industrial fish meal plants are designed to operate continuously for the entire season.

The processing machinery is not easily accessible for cleaning after each shift, and each stage of the process is continuous with the preceding and subsequent stages. While some parts, such as the holding tanks, can be cleaned quickly and easily, other parts, such as the dryer, must be dismantled before they can be cleaned. This is a painstaking and time-consuming job. Because the dryer works continuously, it is often constructed in such a way that when it is turned off, wet fish is left in the first section. Since it is assumed that the dryer will only be turned off at the end of a processing season, when all the machinery is taken apart for cleaning, this is not as unreasonable as it first appears. But it does mean that if processing stops for a week or two, because of storms or work stoppages, the operators will often decide to keep the burner running to dry what is left in the machine while the plant is down, rather than pull everything apart for cleaning or deal with odor problems when the plant is re-started and the rotten material in the dryer heats up again.

In addition to fish meal, this type of processing plant produces fish oil and a product called fish solubles, or concentrated stickwater. After the coarsely ground fish has been cooked, it is pressed to remove as much oil and water as possible. This liquid is decanted to separate the aqueous and oily fractions. In some cases, a triple decanter is used, which separates the aqueous stream into high and low solids fractions. The resulting oil will be discussed below. The pressed meal goes into a dryer, while the aqueous stream, known as stickwater or presswater, is taken to an evaporator, where its water content is reduced from over 90% to about 50%. The evaporator removes water far more efficiently than the dryer, particularly modern multi-stage evaporators (Fig. 15.1).

On-board meal plants do not have evaporators. Their press stream goes through a double decanter, the presswater is pumped overboard, and the oil is added to the ship's fuel after polishing to remove any last bits of water and solids. At least 20% of the diesel fuel can be replaced by fish oil without any problems or modifications. By modifying the burner, fish oil can replace most or all of the diesel (see below). Unlike factory trawlers, land-based plants cannot dump their presswater unless they have the appropriate discharge permits to release it into local waters, although some plants have hired vessels to carry it out to sea and discharge it.

When the fish is pressed, the soluble proteins come out in the presswater. Prior to evaporation, the protein content of the presswater is about 6–7%, a figure that can increase greatly as the raw material ages. When the concentrated solubles emerge from the evaporator, they are thick and gluey, with the consistency of tomato paste. Traditionally, these were added to the dry meal, which was then re-circulated to the dryer. Such a product was called a 'full meal'. More recently, the prices for full meals have dropped and producers have found other markets for the concentrated solubles, usually the fertilizer market, where fish solubles compete with hydrolysates.



Composition of fish material during the process

Material	Water (%)	Solids (%)	Fat (%)
Raw fish	70	18	12
Press cake	53	44	3
Press liquor	78	6	16
Dilute stickwater	95	5	<1
Concentrated stickwater	65	33	2
Fish meal	9	85	6

Fig. 15.1 Composition of fish material during the process (from Windsor and Barlow, 1981).

The old markets for fish meal were as ingredients in pig and chicken feeds. The current markets for fish meal are in aquaculture feeds, where they have high value, especially for carnivorous fish. This has resulted in fish meal prices fluctuating independently of soybean meal prices for the first time in fish meal's long history. However, the use of fish meal in fish farms has placed new demands on meal manufacturers.

Fish farms have big problems with water pollution, and so require fish meal that contains very few soluble proteins and has a low bone content. Bone contains significant amounts of phosphorus, much of which is indigestible. It passes through the fish and ends up in the water, where it acts as a fertilizer and can cause massive algal blooms. When these algae die, their decay uses up the oxygen in the water (the potential for this is called 'biological oxygen demand' or BOD), and causes local anoxia which can kill fish (and all other animals). In meal made from fish caught solely for that purpose, bone content is less of an issue since the meat to bone ratio of these small fish is relatively high. However, in meal plants using processing scraps, the bone level can be very high, as heads and frames may constitute much of the input. With aquafeed markets paying a premium for low ash (i.e. low bone) meals, many plants today screen or air-classify their meal after drying, to separate out the larger pieces of bone. This creates an additional product, fish bone meal, which can be used in fertilizers or feeds. Fish bone meal is currently fairly low in value, but the economics as a whole are affected by the fact that certain aquafeed markets see white fish meals (i.e. those made from scraps from food fish as opposed to the 'brown' meals made from industrial fish) as of special value. These markets also place a high value on freshness of raw material and digestibility of the finished product, both of which are routinely tested by buyers.

Historically, many meal plants were built to produce fish oil, with meal as a low-value by-product. Today, meal is the primary product and oil the by-product, despite the market for high-priced fish oil capsules and omega-3 fatty acids promoted as dietary supplements. High-priced consumer products containing fish oils are usually made in specialized refineries and not in meal plants, so meal plants, especially those in isolated areas like Alaska – where fuel prices are high and most coastal communities are off-road – burn the oil to run their boilers, rather than shipping it to a buyer. The oil is usually mixed 50:50 with diesel, but it can replace diesel completely, provided all solids are taken out and the boiler retro-fitted. Burning fish oil produces only 80% of the British thermal units (BTUs) from an equivalent amount of diesel, and requires a different burner, but currently the economics of burning fish oil are quite favorable. In addition, as the price of fuel rises, they will become more favorable and the use of fish oil for fuel ('biodiesel') may spread (Steigers, 2003). Markets and the prices they command fluctuate constantly, and if the value of fish oil, either as a human nutraceutical or as an aquafeed ingredient, goes up and the price of fuel comes down, the oil will go back on the market.

One big change in meal production over the last 50 years is the use of antioxidants to prevent oxidation. The long-chain polyunsaturated fatty acids that confer so many benefits on fish oil are extremely prone to oxidation when exposed to heat and oxygen. This causes oxidative rancidity, which makes the meal (or the oil) less appetizing and even harmful in feed. Extreme oxidation may actually cause meal to spontaneously combust. This has happened often enough to mean that high oil fish meal is still considered a hazardous cargo, and the manufacturer may have to prove to shippers that sufficient antioxidants have been added. Antioxidants are added to the meal as it emerges from the dryer and before milling and bagging. The oil may be protected with antioxidants as well, although most buyers prefer flushing with nitrogen or argon followed by air-tight packaging.

Both fish processing plants and meal plants will need to adapt to increase the utilization of processing wastes in meal. Few fish plants are large enough to justify their own meal manufacturing facility. This means that new meal plants will only be built in locations where there are sufficient fish processors within a workable radius to justify their construction. The fish processors will have to cooperate with the meal plant by keeping fish scraps refrigerated and delivering them to the meal plant as rapidly as possible. This is particularly important where viscera form a significant fraction of the raw material. The ideal situation is where all processors are sufficiently close to the plant that material can be pumped from one building to another. If the fish scraps have to be transported, the issues of noise, odor from the trucks, and leakage onto the roads must be resolved. In most communities, building permits will not be issued until the meal plant has guaranteed very high levels of hygiene and cleanliness.

A new meal plant must also assure itself that there is sufficient raw material available to make a profit. The profit may be less critical if the plant is run by a group of processors whose major concern is complying with waste disposal regulations, but independent owners will not operate at a loss. Meal plants are bulk businesses and need to take advantage of economies of scale, whereas fish processing can be profitable at almost any scale. Equipment in a large fish processing plant tends to be multiples of the same equipment items that a small plant would purchase singly. Small family businesses avoid the labor problems of large operations, and can make money by sticking to high-value species, providing customers with special services, and developing value-added products. This is not true of meal.

Meal plants sell into commodity markets, via brokers, and the most profitable plants are not necessarily those that produce the best product, but those that have a large capacity and operate up to that capacity as many days out of the year as possible. Since most fisheries have seasons, plants operating on waste are most efficient when the season for one fishery immediately succeeds another, so that processing is more or less continuous throughout the year. The most difficult situations in which to operate a profitable (or at least break-even) meal plant are those that occur, for instance, in wild

salmon fishing, processing and canning communities, where the total season may be 4 months, but where peak production lasts for only about 6 weeks. It is extremely difficult to establish a profitable plant under these circumstances, although there are possibilities. One option is to aim for a more moderate level of production, and dispose of excess fish waste during the peak season externally, for instance via ocean- or land-dumping, paying a composter's tipping fees, or some other solution, depending on the individual situation. Another possibility is to source reasonably priced equipment that can handle peak loads, but which can also operate easily with smaller loads. It might be worth looking at rendering equipment, which can be both smaller in scale and cheaper than modern meal plant equipment. However, it is important to determine if the raw material to be processed matches the requirements of the machinery; for example, some rendering presses will not work unless the raw material contains 20% bones.

Bones can be a problem for fish meal producers. When whole fish are turned into meal, the ratio of meat to bones is high, and these small fish, targeted by the fishing fleets that service meal plants, have small light bones. However, scraps from processing plants, which may contain a high proportion of heads and frames, often contain large bones and have a high bone to flesh ratio. The larger the proportion of bones in the raw material, the higher the ash content of the finished meal will be. There are ways to reduce ash content in a finished meal, such as air-classification (similar to winnowing chaff from grain) and screening, which could allow entry into certain markets, that set limits on ash content (such as those for fish feed). Cat food manufacturers often specify low ash because high ash can cause urinary tract problems in neutered male cats and, although acidification of the feed can solve this problem, this has not been sufficiently marketed to cat owners.

Preferences for specialized meals for very specific markets bring up an important point. It was mentioned above that fish meal is a commodity, handled through commodity brokers. In general, the smaller meal manufacturer will not sell directly to the user but, rather, to a broker who is often also a blender. The broker will blend high ash meal with lower ash meals to achieve a saleable mix. While some customers will demand an unmixed meal that meets their specifications, most bulk customers will buy blended meals. The premium price for low ash white fish meal may not justify the extra costs of the machinery required to lower ash content. In addition, although removing ash may increase the price per ton of the meal, the volume produced and sold will fall. When all factors are taken into consideration, manufacturing a superior high-quality meal may not compensate for the added costs of production.

Hydrolysates

Hydrolysates are sometimes called 'digests', and they are also often referred to as fish protein hydrolysates (FPH), or as 'liquid fish', as in 'liquid fish

fertilizer'. Unlike fish meal, which refers to a reasonably consistent process and product, hydrolysates are extremely variable – both in their processing and in the form, function and pricing of the final product. (Fish silages, a sub-group of hydrolysates, will be discussed later in this section.)

What unifies all of these products is that they use protein-digesting enzymes to liquefy the fish flesh, after which bones and other indigestibles can be screened out. The two main sources of variability are the enzymes and conditions under which digestion takes place, and whether the resulting liquefied fish is left as a liquid, concentrated to a paste, or dried. Unlike meal, which is almost always sold as a bulk commodity, hydrolysates offer the possibility of small-scale production, value-added processing, and individual marketing.

Hydrolysates left as liquids are acidified to prevent spoiling. The raw material to produce liquid fish hydrolysates is digested rapidly under optimal conditions, pasteurized to kill any microorganisms present and to inactivate the enzymes and stop the digestion, and then acidified. The acid(s) used are chosen according to cost, functionality, and flavor. For example, liquid fish fertilizer is invariably stabilized with phosphoric acid, which is cheap and adds phosphorus to a product where phosphorus content is a plus. However, phosphoric acid, like other inorganic acids, is ineffective as a mold retardant, so a small amount (e.g. 0.25%) of propionic acid or a propionate salt is added as well. Hydrolysates for the cat food market (where they are sprayed on to dry feed to enhance palatability) are also acidified with phosphoric acid because cats like this acid. Phosphoric acid is also a good acidifier for concentrated fish hydrolysates destined for the salmonid feed market, because salmon as well as some other species tested seem to prefer the taste of phosphoric or formic acid to other acids. Phosphoric acid is not only far cheaper than formic acid, it is less volatile and thus easier to work with – not just in the hydrolysing facility, but also in the feed plant, where heat is usually applied to form pellets. However, phosphoric acid should be limited or not used at all where water pollution with excess phosphorus could cause problems. The producer must balance the requirements for the market, the target organism, and product stability.

Determining the correct amount of acid needed to stabilize the product usually takes a few trials. This can be particularly tricky in products containing bits of bone, which dissolve over time and neutralize inorganic acids. Those just beginning production of liquid fish products, as well as most small-scale operators, typically produce fish fertilizer. This is always put into plastic containers because, if stabilization was incomplete, there is a possibility of gas build-up and consequent rupturing of the container. In extreme cases, the container might explode and a glass container would be much more dangerous than a plastic one.

Concentrated fish solubles, made as a by-product of fish meal, are invariably cheaper than purpose-made fertilizer, and offer the additional advantages of cheaper shipping and storage since they have a much lower water

content. They are not the same as hydrolysates, since solubles are made of the soluble proteins expressed after cooking, while hydrolysates include all the proteins solubilized by digestion. However, there have been no studies to demonstrate any differences in quality between these two products, despite various claims, such as those from small producers of liquid fish fertilizer who suggest that the processing method makes their products superior. It is difficult to make objective judgments about different products because most studies are carried out using fertilizers of unknown composition, as producers tend to treat their manufacturing process as a trade secret. Scientific research in this field could be of benefit, but despite this lack of quantitative comparisons, the liquid fish fertilizer market remains buoyant.

There are two things that make fertilizer production attractive to beginners. One is that the product can be sold wet, thus greatly reducing capital costs. The other is that the choice of enzymes is a lot simpler than it is for products where flavor is the key point. The types of enzymes used to digest fish wastes are protein-digesting enzymes, or proteases. The simplest and cheapest way to digest seafood is to include the viscera, grind and mix, and then raise the temperature to around 140 °F/60 °C. This is the temperature at which the endogenous enzymes of even cold-water fish work fastest. The enzymes do not remain active at this temperature for very long, but long enough to liquefy the raw materials. This method will not work if the fish has been frozen or cooked, as both these processes denature the proteins. Using such an endogenous digestion gives specific flavors, which are useful in certain products such as fish feed ingredients. However, the use of viscera is not approved for human food use (nor would the flavors be appropriate) and it is too coarse a method for producing fertilizers, because fertilizers must not clog the nozzles of large-scale delivery systems.

Proteases are of two general types, endopeptidases and exopeptidases. Endopeptidases chop up proteins in the middle, while exopeptidases snip off single amino acids from the ends. Random endopeptidases cut proteins or peptides (pieces of proteins) in random places; others separate the protein at particular amino acids. When a large number of free amino acids are desirable, a combination of endo- and exopeptidase is used because the endopeptidase creates many more peptides for the exopeptidase to act on. At the outset, it is important to find out if these two enzymes can be used in combination or if they will digest and thus inactivate each other. If the latter, then the endopeptidase should be used first, and de-activated before the exopeptidase is introduced.

Unlike the endogenous enzymes, which are of course free, other enzymes used to produce hydrolysates must be purchased. Exopeptidases tend to be particularly expensive. The enzyme of choice for fertilizer production is the cheapest, fastest-acting endopeptidase on the market. Papain is used when the only requirement is for a smooth, fine hydrolysate that will pass through a fertilizer delivery system, but the price of papain fluctuates according to

the papaya crop. Where flavor is important – whether for fish, pets, or humans – the choice of enzymes is critical and a long period of research and development may be required to find the optimum mix.

Flavor – and the specific flavors that tell us whether we are eating fish or broccoli – is largely due to the presence of free amino acids in food. Digestion may increase the amount of free amino acids, thus intensifying flavor, but the flavor produced may be an inappropriate or unpleasant one. Exposing certain amino acids, particularly hydrophobic amino acids, by cutting the protein or the peptide at hydrophobic linkage sites, creates bitter flavors. While fish, as a substrate for hydrolysis, tends to generate less bitterness than, for example, soybeans or casein, this may still be an issue. For products such as fertilizers, bitterness is irrelevant. For the production of high-value palatability enhancers, bitterness in fish hydrolysates may be reduced by removing the gall bladder from the starting material (Dauksas *et al.*, 2004). Hydrolysis, sometimes in combination with fermentations, has been widely used to produce flavors for pet foods (note the ubiquity of 'digests' in pet food ingredient lists), although these have commonly used poultry or beef parts as starting materials; hydrolysis has also been used to produce high-value flavorants for human foods (In, 1990).

Peptones or microbial growth media are another high-value class of products produced by enzymatic hydrolysis (Aspmo, 2005) As industrial fermentations increase in number and variety, the demand for growth media increases as well, and fish has been a satisfactory feedstock for peptone production. Additionally, fish-derived peptones may be uniquely suited to support the growth of marine organisms. Indeed, there are no limits to the creation of novel products from fish waste via controlled hydrolyses (Gildberg, 2004).

A typical protocol for small-scale liquid fish fertilizer production is shown in Fig. 15.2. The wastes are ground, typically to a quarter-inch or hamburger consistency, because a very fine grind may make it difficult to screen out the resulting small bone fragments which can clog nozzles. If the raw material has a high oil content, an antioxidant such as ethoxyquin may be added. The raw materials are then transferred to a jacketed kettle or other tank where they can be heated and continuously mixed. The temperature is based on the optimal operating temperature for the protease used, generally in the range of 140–160 °F/60–71 °C. The pH may have to be adjusted for some enzymes, but most of the random endopeptidases work well in the neutral pH range of most fish. The viscous mass will turn to soup surprisingly quickly. The operator decides when to stop the digestion, and does so by raising the temperature to inactivate the enzyme molecules and to destroy any microorganisms (200 °F/94 °C for 10 min is typical). The digest is then screened, stabilized with acid(s), and cooled.

Fish silage is a very different product, in that acidification is the first step, rather than the last. In ensiling, the fish is ground, mixed with acid, stored,

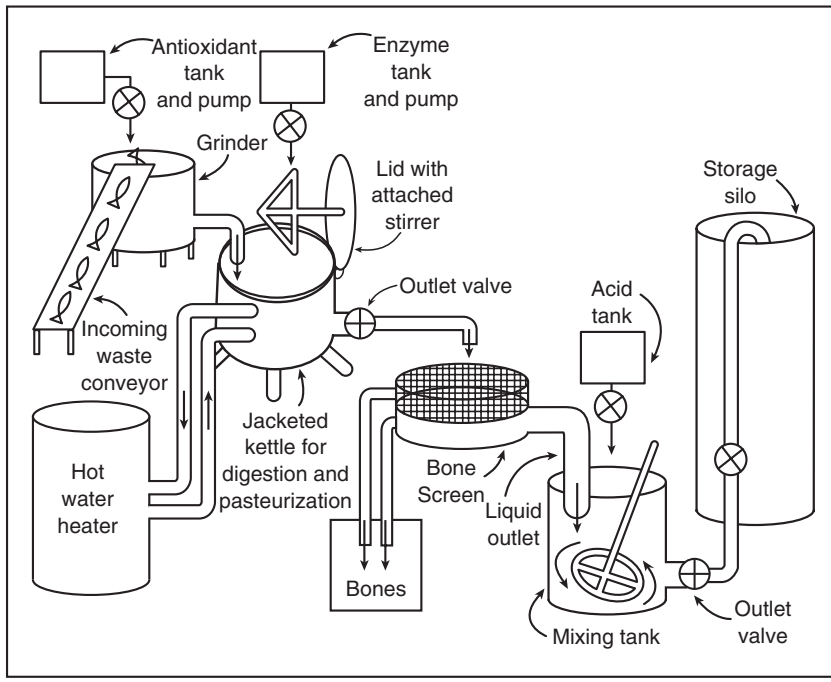


Fig. 15.2 A process for simple fish protein hydrolysis (Goldhor, 1992).

and occasionally stirred, usually at ambient temperatures. Over time and after self-digesting, it separates into a watery top layer consisting of soluble proteins and peptides, and a bottom layer of sludge. This is a very old method and there are a number of variations.

Ensiling fish is a primitive process, and the products of ensiling are usually primitive as well. Because the process is poorly controlled, digestion may proceed too far, leading to the breakdown of protein into ammonia and thus loss of feed value (Hardy *et al.*, 1983; Stone & Hardy, 1986; Stone *et al.*, 1989). Because the mix needs to be stored (and stirred at least occasionally) for weeks, it requires space. It is a useful method for less developed areas where the quantities of fish waste to be ensiled are not too large, and where livestock must be fed; it should be withheld from pigs and chickens prior to slaughter to avoid the meat tasting of fish. However, ensiling (or, more accurately, acidification of minced fish) is also sometimes used as a short-term method of preserving the material, prior to enzymatic hydrolysis. In situations where waste is to be collected from many generators for hydrolysis, grinding the fish and mixing it with acid is a reasonable way of ensuring that it will be in good condition when it arrives at the plant. This method is also used in Norway to preserve material on board vessels prior to delivering it to a land-based plant (Jangaard, 1987). Any operator

planning to follow this method should consider the choice and amount of acid very carefully. It may be handled by unskilled workers, it will probably have to be at least partially neutralized prior to mixing the silage into a finished feed, and – if it is volatile – heating during hydrolysis may become a health issue in the plant or in the neighborhood.

When dairy farmers talk of silage, they are generally referring to that produced by the anaerobic fermentation of corn with endogenous or added sugars, which generates acid. The raw mix may be inoculated with cultures of specific microorganisms to ensure that the acid generated is the one that the farmers require. In making fish silage, acid is generally added directly. However, it is possible to carry out fermentations where at least some of the acid is generated by the fish materials, by adding a source of sugar and the appropriate microbial cultures and storing the mixture anaerobically, usually for about 48 h. The acid produced is generally lactic, but sometimes propionic (van Wyk & Heydenrych, 1985; Hassan & Heath, 1986; Giurca & Levin, 1992; Cao *et al.*, 1997). It should be noted that in order to ensure that the acid penetrates the mass of fish and that no spoilage occurs inside the fish chunks in this process, it is usually necessary to carry out an enzymatic hydrolysis prior to fermentation. This thus becomes a fairly expensive process and one that is quite different from acid silage, which is cheap and well suited to primitive conditions; ensiling fish using this method is therefore only carried out under particular circumstances, such as when liquid acids are not available or are expensive to transport, or where lactic acid fermentation adds significant palatability. An early weaned pig feed might be an example of the latter. In fact, fish ensiling is as capable of being upgraded and used to generate sophisticated products as is enzymatic hydrolysis, of which it is a variant (Gildberg, 2004).

The earliest fish digest that we know of is the ancient Roman condiment called ‘garum’. Worcestershire sauce contains a direct descendent of garum, and what is arguably the most popular fish digest in the world today – Asian fish sauce – has similarities. Known as ‘nam pla’ in Thailand, ‘nuoc nam’ in Vietnam, ‘patis’ in the Philippines, and ‘tuk trey’ in Cambodia, fish sauce provides a major portion of the protein and vitamin B, as well as flavor, in diets that consist largely of white rice. It also provides a big dose of salt. One major way in which fish sauce differs from silage is in using salt rather than acid as the preservative. It is made by combining whole fish and/or shellfish with at least 20% of their weight in salt, and then storing the mixture underground or in sealed containers for months while it ferments and digests. The fish sauce sold is the clear (usually amber colored) supernatant from this process, and the sediment is either discarded or re-used to make a lower grade of sauce. The strong flavors and aromas of fish sauce are due to the long fermentation, and the process may be compared with that of wine-making (Lopetcharat *et al.*, 2001).

The market for fish sauce is enormous. Many of the traditional areas for its manufacture have experienced a dramatic decline in fisheries, while the

United States and Europe have seen big increases in populations from countries where fish sauce is popular. Surprisingly, manufacture of fish sauce has not moved easily to other countries, partly because of the need to suit traditional palates, and partly because of the need for a warm climate where the long storage and fermentation can occur. Western attitudes and regulations covering fish processing may also play a part.

The many ways in which digested and/or fermented fish products are manufactured are reflected in how many markets there are for such products. These markets include aquaculture feeds, livestock feeds, pet foods, human foods, fertilizers and foliar sprays, and microbial growth media. In the first four categories, it should be noted that digests are used largely as flavorants, attractants, and palatability enhancers, i.e. as minor ingredients playing major roles. Because of the immense plasticity of hydrolysis as a process, other specialty products are expected to emerge.

15.2.3 Utilizing part of the waste

There are three reasons to utilize some portion of the waste generated in fish processing. First, the removal of one specific type of waste may significantly reduce the total bulk that must be disposed of. This can reduce tipping fees or can bring the processor below a regulatory cap for dumping at sea. Second, processing one potentially valuable organ may increase revenue. Finally, pulling particular organs out of the mass of waste may increase the value of the remainder. All of these cases will be considered below, on an organ by organ basis.

Skins

Skins are particularly interesting since they may fit all three of the reasons cited above. Skin often forms a significant fraction of the total waste, and the current trend for manufacturing value-added products at the point of origin means more skinless fillets are being produced, and so more skins are being added to the waste streams of plants, whereas previously the skins were shipped off as part of the product. Additionally, because skins have a high ash content, their inclusion can reduce the price of fish meal, although it increases the volume produced. Skins can actually be profitable, if conditions are right.

There are two major products for which fish skins are the raw material: gelatin and leather. Gelatin production is suited to very large volume processing plants and leather to plants with relatively small production. In both of these cases, the processing of the skins is likely to be by separate entities, due to the heavy capital investment in equipment and the expertise required for each of these ventures.

Fish gelatin has potentially large markets in the United States, because it offers food manufacturers the possibility of kosher and halal labeling. While the percentage of the population actually keeping kosher is small, a

surprisingly large percentage of consumers prefer to purchase food products labeled as kosher in the belief that such products are superior. For this reason, food manufacturers try hard to achieve kosher certification. Not all fish are kosher; only those with removable scales. This eliminates sharks and other elasmobranchs, eels, swordfish, and catfish, to name the most common. Additionally, fish certified as kosher must be processed in a plant where there can be no admixture with non-kosher species. The non-kosher fish species can be used to make halal gelatins for the Muslim market, which comprises 1.3 billion people worldwide.

Fish gelatin is currently used mostly in refrigerated or frozen products. The major reason for this is that the melting point of many fish gelatins is lower than that of beef or pork gelatin, and therefore they tend to liquefy at room temperature. While this is undesirable in a product that should hold its shape on the table, it is highly desirable for a product designed to melt in the mouth. However, fish gelatins are surprisingly variable, with different species having different melting points as well as different gel strengths, often called 'bloom strength' (Choi & Regenstein, 2000; Gudmundsson, 2002; Gómez-Guillén *et al*., 2003). Fish gelatins therefore offer not only a range of gel strengths or firmness, but also the possibility of blending those from different species to achieve very specific melting points. This does however mean that some species' skins are unlikely to have commercial value; for example, salmon skins have so far yielded relatively small amounts of very low bloom strength gelatin.

In the past, industrially produced fish gelatin was mostly used for non-food purposes, such as photoresists in printing. However, food and pharmaceuticals now represent the larger market for fish gelatin, and researchers are working to increase both yield and bloom strength. Gelatin is essentially denatured collagen, so fish skins are also a source of collagen – a product used in cosmetics, nutraceuticals, and pharmaceuticals (Johns & Courts, 1977; Nagai & Suzuki, 2000).

In addition to being a source of collagen and gelatin, fish skins make surprisingly strong and beautiful leathers. Salmon skins were traditionally used by northern natives to make boots, and many fish skins take dyes well and can be produced in a range of brilliant colors. The technology for tanning fish skins is essentially the same as that for tanning hides, although smaller equipment is needed.

Shark and ray skins are famous for their toughness and textures, and are occasionally used in expensive boots and accessories. Shagreen, a sharkskin product used to cover expensive decorative products in the nineteenth century, recently had a small revival. One unique property of sharkskin leathers is their unidirectional denticles, which make them valuable where a non-slip grip is required. The classic use for this has been sword handles. It has also been used for 'pickpocket-proof wallets', but the problem here is that the owner will have as much difficulty removing them from his pocket as the thief. When the denticles of certain sharks are polished, the

skins can be used to make an extremely expensive and rare leather known as 'boroso' (Kreuzer & Ahmed, 1978). Barramundi skin from Australia, which has a unique texture, has been used in a variety of products ranging from barstool upholstery to bikinis. Eel skins (actually those of hagfish) are used in the manufacture of wallets, belts, etc., despite their narrow size. Although the scales must be removed from fish skins destined to make leather, the patterns that they leave are similar to scale patterns in endangered and costly snake and lizard skins, which they might be able to replace.

If fish skin leather is so beautiful and useful, why is it not more popular? This is a hard question to answer. A number of ventures have been started to tan fish skins and to turn them into products, and many of these have failed. The tanning itself is not the critical element, but the small size of fish skins, especially when compared with hides, is often cited as a problem. Another issue is the difficulty of getting the processors to provide the skins in the desired form, which may necessitate re-training the workers or buying new and expensive skinning equipment. Based on internet searches, however, fish skin leathers are surprisingly popular, although often in small niche markets.

Scales

Johnson & Associates (2002) state that:

> Pearl essence are crystals produced from fish scales through a process that removes, collects and purifies the crystals for use in paint pigments, cosmetics and a host of other products where unique luster is important. Soft, cloudy lotions and shampoos often contain pearl essence. Pearl essence pigments are also found in some high-end automotive paints.
>
> It is difficult to quantify the market for fish scales. The leading US buyer of fish scales, Mearl Corporation, is now a part of Engelhard Corporation, a Fortune 500 company that develops, manufactures, and markets technology-based performance products and engineered materials for a wide spectrum of industrial customers. Mearl purchases fish scales from the North Atlantic herring fishery and produces pearl essence for paint pigmentation and consumer products. While the markets for these products are huge (the pearlescent pigment market for automobiles is growing at a rate of 12% per year), it is difficult to pin down the value of the raw material, the fish scales.
>
> New entrants may find it difficult to break into the fish scale market. The only major company known to purchase fish scales in the eastern United States is closely tied to the sardine industry in Maine. It is likely (although not confirmed) that this business is viable only when it is a part of a high-volume secondary processing operation (in this case the production of sardines). In effect the fish scales are produced as a by-product that is sold to a plant that converts the scales into a marketable material (pearl essence). The barriers to entry in this business would seem insurmountable for new players.

Fish scales are a good organic fertilizer, providing slow-release nitrogen. They must be dried to render them stable and acceptable to the marketplace. Although grinding is recommended, this may not be necessary. They would be an appropriate addition to fish bone meal fertilizer and would allow the processor to get rid of two by-products at once.

Heads

Almost all fish processing removes heads. Heads or portions of heads may be used in a variety of ways, depending on the species in question. In the case of cod, there are ethnic markets (largely in the Atlantic provinces of Canada) for tongues and cheeks. Heads are often sold as bait, both for sport and pot fishing. Salmon heads are an excellent source of oil, currently used in human food supplements. The heads of many species are also saleable as food in some areas. Fish heads as food would appear to be the most attractive option for most processors, but it is important to note, as mentioned earlier, that the heads for this market must be cut further back than is normal for headed and gutted or filleted fish, to include sufficient meat. The price obtained for the head must therefore compensate for the loss of high-value fillet meat, and may change the shape of the fillet in ways that are not acceptable to primary customers.

Livers

A number of fish with lean flesh use their livers as oil storage organs. This includes cod, haddock, pollock, ling, halibut, and sharks, among others. The livers of these species can be composed of 50% or more oil, and this oil can be easily rendered out. The authors have demonstrated (Regenstein *et al.*, 2003) that the liver oil of Alaskan pollock can be separated out at room temperature if the livers are allowed to age, under refrigeration, for 24 h after death, at which point autolysis is occurring. Since livers used for oil storage tend to be quite large, they can be segregated during processing and handled separately.

Fish-liver oils contain fat-soluble vitamins as well as valuable omega-3 fatty acids. The first human health market for fish oils was due to their content of vitamin D and its ability to prevent rickets. A century or more ago, cod fishermen gutted the fish prior to salting, but saved the liver, which was thrown into a 'gurry butt' (see Rudyard Kipling's *Captains Courageous* for a surprisingly accurate description) and left to rot for the months-long voyage. Those old enough to remember early cod-liver oils will therefore recognize that the nauseating flavor was due to oxidation and impurities as a result of the poor handling. The discovery of effective and rapid methods of oil release and the use of antioxidants has completely changed fish oils, which today are mild-tasting and inoffensive (Stansby, 1990). For those wishing to access the lucrative human supplement market, it is important to recognize that before being enclosed in a capsule, the oil is refined,

cleaned of pollutants such as polychlorinated biphenyls (PCBs) or heavy metals, and the omega-3s often concentrated.

Certain fish livers, particularly those of sharks, are so rich in vitamin A that sharks were hunted during World War II specifically for their vitamin A content and its supposed role in improving night vision in fighter pilots. Luckily, the development of synthetic vitamins put an end to this before the shark populations were decimated. It should be noted that fish-liver oil is so rich in the fat-soluble vitamins that acute fish liver intoxication has been described in the medical literature and is assumed to be caused by hypervitaminosis A.

Where production is sufficient, liver oil can be burned to replace some or all diesel fuel, as discussed in the section on fish meal above.

Roes and milts

The value of fish roe (eggs) is best exemplified by that of sturgeon, whose caviar is so highly valued that this ancient group of fishes is close to extinction. Luckily, sturgeon or black caviar is not equally highly valued in all cultures. Salmon or red caviar is very popular in Japan and is considered superior to black caviar by many Russians and Eastern Europeans. Middle Easterners process and eat 'butarga', the smoked or salted and dried eggs of grey mullet or cod. Alaskan processors ship whole unprocessed egg sacks and milts (sperm sacs) of cod and pollock to Asian markets, although far fewer milts are sold than roes. American processors throw flatfish roes and milts away as waste, but Korean buyers will pay higher prices for flatfish cut so that these organs remain inside (karimi, as mentioned in Section 15.2.1).

Gonads are the only part of the sea urchin that is eaten, and European customers pay top prices for scallops with the brilliant red roe sacs attached. Herring roes are shipped to Japan from North Pacific spawning grounds, with the highest prices often paid for herring roe on kelp, where the fish have spawned and glued their eggs to the kelp. To produce this latter product, either pre-spawning herring are captured alive and penned in enclosures containing harvested kelp, or harvested kelp fronds are hung from floating rafts in known spawning areas. Although this product is little known in Europe, the average North American harvest of roe on kelp is about 500 metric tons (http://www.caviarguide.com/fishroe/herring-on-kelp.htm). Herring roe on kelp is not a typical by-product, since there is no primary product – the herring swim away. On the other hand, the roe stripping of herring, where everything but the roe became waste, created such a disposal problem and public outcry that it has been outlawed in most of the United States. Those who want to take the roe must find a use for the rest of the herring, even if that use is only fish meal.

Mince

Most fish processing operations discard a significant amount of edible flesh. This may be in the form of pieces removed from fillets, to meet size or

shape standards, or as cheeks, or as the meat left on the frame when the fish are turned into boneless fillets. The meat industry and, more recently, poultry processors, have increased yield and profitability by producing minced products from what is left after the high-value parts have been removed. Fish processing has been slower to take up this practice, perhaps because of the rapidity with which minced fish loses quality.

The most commonly used deboning machines, such as Baader and Bibun, work by pressing the fish parts, which are placed on a rubber belt, against a revolving perforated metal drum. The soft parts go through the holes, while skin, bones, eyeballs, etc. do not. The quality of the mince can be varied by altering the size of the perforations (generally between 1 and 10 mm, with 3–5 mm being most commonly used for fish) and by changing the belt tension.

Frozen fish mince is an accepted international commodity, sold in 35 lb (16 kg) blocks. It can be used to manufacture low-quality fish sticks or fish fingers or, at a set percentage, to fill spaces in frozen fillet blocks that are destined to be used in higher quality fish sticks/fingers or portions. There are both generic and specific issues that must be addressed by those wishing to produce mince. Generically, there is the balance between yield and quality. For example, trim mince is a higher quality product than frame mince, because frame mince may contain blood and other pigmented and strongly flavored materials. By increasing belt tension, the yield – especially of frame mince – will increase but the quality will decrease. More specifically, most mince is produced from the gadoid species, such as cod, haddock, Pollock, and hake. When gadoid flesh is frozen at temperatures common in the US seafood industry (e.g. above –22 °F/–30 °C), shelf-life is poor and the flesh tends to become rubbery and tough, due to an enzyme that is still active below freezing. Regenstein (personal observation, 1982) has shown that this gadoid reaction can be eliminated by initial freezing at a very cold temperature (–40 °F or –40°C). This appears to kill the enzyme and the mince can be made available to the market, and its higher freezer temperatures.

Worms (known as cod worms or seal worms) tend to accumulate in the flesh of gadoid fish. These must be removed from fillets individually, typically using light tables to make the worms visible and tweezers to pull them out, but this process would be uneconomical for mince. However, worms can pass intact through the mincer, and can be seen in the resulting mince. Although the worms represent harmless protein, they may not be tolerated by the end user. Reppond and Babbitt (1991) showed that worms could be eliminated by grinding, followed by passage through a Brown Finisher, a machine generally used in surimi production.

As with hamburger mince, fish mince has a surface to volume ratio approaching infinity, and this maximizes problems of contamination, oxidation, and spoilage. Frame mince – which contains blood, pigment, and mixed tissue types – is particularly challenging. Regenstein and Regenstein (1986) have suggested that one way to avoid some of these problems is to

cook the mince, using such natural antioxidants as rosemary extract, prior to freezing. The saleability of the frozen cooked mince depends upon forming a collaboration with an end user, such as a company preparing institutional foods. Baker and Regenstein's work has shown that white fish mince can replace beef in many popular foods, such as spaghetti, chili, tacos, etc., and confers both economic and nutritional advantages (Regenstein, 1980). Surprisingly, consumers do not recognize the fish in such preparations, as the strong colors and flavors of the accompanying ingredients act as masking agents and the minced fish offers a meat-like mouthfeel. Frame mince, with its stronger flavors, is more difficult to mask, but offers the health advantages of more omega-3 oils and bioavailable iron, which is in short supply in white fish meat.

Mincing is rarely practical for smaller operators and rarely economical for fillets, which are minced only as a first step towards surimi manufacture. Mincing can make sense for larger processors looking for a way to utilize some of the flesh cut off the fillets during trimming, or left on the frame after filleting. On the other hand, smaller operators often make higher profits by selling small chunks of trim as 'chowder fish', or by developing one or a few specialty food items (such as pates, mousses, or spreads) that can be sold locally. The latter are especially attractive secondary products for smokeries.

15.3 Shellfish

Shellfish are disparate members of four different phyla: Arthropoda (which includes crustaceans such as crabs, shrimp, and lobster); Mollusca (which includes clams, oysters, whelks, etc. as well as the squids and octopods); and – as we enter more Asian markets – Echinodermata (which includes sea urchins and sea cucumbers); and Coelenterata (which includes jellyfish). What this means in practice is that their biochemistry, and thus the potential and treatment of their by-products, is incredibly varied.

Coelenterates and echinoderms are rarely processed in the United States or Western Europe. Processing sea urchins produces large quantities of waste (and waste water) of low value. The shell of the urchin is mostly calcium carbonate. Urchin waste, if not too salty from the inclusion of sea water, can be used as a fertilizer; particularly for crops requiring calcium. The problem is in transporting large quantities of wet, rapidly degrading material. It can be a reasonable addition to compost and so can the waste water. Again, salt content may be a barrier, but this is ameliorated by diluting the waste with large amounts of other materials.

Molluscan shells also consist of calcium minerals and can present a disposal problem where clams, oysters, or mussels are processed. Where permitted, dumping at sea may be the best alternative, and may provide good cultch (a hard surface for larval settlement) for the next generation

of molluscs, especially where they are farmed. Cultch is sufficiently important that some oyster farmers who sell their catch in the shell have found it worthwhile to import shells from a sea clam processor and to dump those (Wellfleet Shellfish Department, 2005). Shellfish processors faced with huge piles of shells often hope that these will provide a useful and perhaps lucrative source of calcium for laying hens, because hens are given oyster shells as a calcium source; however, oyster shells for hens are mined from ancient, fossilized beds where the material is soft, abundant, unpolluted, and cheap.

A more interesting shellfish by-product could be made from some of the waste waters, particularly those with the highest organic load, which otherwise often create disposal problems. A good example of this is clam post-grind wash water. When large clams are processed, the pieces of meat are chopped and then washed. Because the chopping breaks so many cells, a large amount of protein comes out in the wash water. While this particular stream makes only a minor contribution to the plant's total waste water, it contributes a large proportion of the total BOD, which may cause problems in a wastewater treatment plant. However, the pollutant in this case is protein, and is actually a useful clam flavor. If post-grind wash water were used to pack the clam meats (rather than the plain water that is generally used), the waste stream would be cleaner, and the product would be superior. Note that this use of wash water is most appropriate in a product such as chopped clams which will be sterilized by cooking (S. Goldhor, personal observation, 1995–6).

While using selected process water streams as packing material is a viable option for the primary producer, such streams are generally too dilute for the flavor market and would require concentration by evaporation, co-drying with a carrier such as dextrose, or (more experimentally) through ultrafiltration. Flavor markets actually prefer dry powders which are shelf-stable and easy to handle. However, chefs and small industry users will often accept a frozen, concentrated flavor slush (rather like frozen orange juice concentrate), which can be used a spoonful at a time.

Flavors can be produced out of other wastes as well – some of the more intense and interesting flavors are produced from shellfish body parts; e.g. clam viscera (particularly from clams that are dug out of mud in deeper waters and are unaffected by red tides), brown crab meat, lobster bodies, etc. As more value-added shellfish is produced (with meat to be sold canned or frozen) and fewer are sold live and whole, more of these sorts of raw materials become available.

It should be noted that although some flavors are produced through a sort of tea-bag process, by simply leaching the smaller molecules out into a water soak, some flavors are intensified by enzymatic digestion of the materials. In fact, flavor production – whether for human or pet food use – is one of the most sophisticated sets of by-product processes, and may use digestions and/or fermentations. Thus, although a small processor could

simply boil shells and body parts to make stocks and soup bases for sale to local chefs, large-scale industrial sales require greater capital expenditure and sophistication. Again, it is important to emphasize that viscera are not only rich in flavor, they are also rich in industrial pollutants such as PCBs and dioxins (deep-sea clams seem to be an exception), and in seasonal pollutants such as red tide toxins. A careful testing program should be part of any startup operation working with viscera.

Crustacean waste waters, such as shrimp peeling or cook waters, may contain not only flavors but also astaxanthin, the pigment that gives salmon its color. If this can be captured (usually in oil), it may be surprisingly valuable as a salmon feed additive (Meyers *et al*., 1990).

The shells of crustaceans (e.g. shrimp, crab, and lobster) are partly composed of calcium carbonate. They also contain significant quantities of protein and chitin. Roughly speaking, each of these components comprises a third of the shell composition. Chitin is a polysaccharide (a long-chain carbohydrate composed of many linked sugars) that is very similar to cellulose, except that chitin contains nitrogen. Cellulose is the most abundant organic molecule in the world; chitin is the second most abundant. Chitin is an extraordinary molecule with many different properties. It appears to be able to stop bleeding from deep wounds even when only applied to the surface of the skin, and the US Army and Navy have supported two separate chitin business ventures, both of which are now producing hemostatic pads. While the Navy's pads are made of material produced by biotechnology, the Army's are produced from shellfish waste.

Chitosan, a soluble form of chitin produced by deacetylation, can clean up waste water. Chitosan captures polluting biological molecules, such as fat and protein, but can also clean up PCBs and heavy metals. Chitosan pills are available as a food supplement to bind fat in the intestine as an aid to dieting. Chitin can also be converted into polyglucosamine, which is widely taken to halt arthritic degeneration.

Unlike proteins and many other carbohydrates, chitin does not arouse any immune responses in humans. Since it can be spun, woven, felted, etc., it has been made into items such as intraocular lenses, biodegradable sutures, and second skins for burn victims. For these types of uses, it is essential that all the protein, even that most tightly bound, is removed from the chitin because protein does cause an immune response.

Despite the promise of chitin and the large amounts of shellfish waste, few chitin-/chitosan-based businesses have succeeded in North America. They have, however, succeeded in Japan, where chitin is well known, highly regarded, and – perhaps most importantly – legally mandated by the Government for certain tasks, such as cleaning up food-plant waste waters. Such businesses have also succeeded in China, where labor is cheap and environmental monitoring minimal. In the United States there have been two barriers to success. One is the cost of the labor and chemicals required to turn shells into chitin, a process requiring treatment with both acid and

alkali. The second is the natural variability of chitin itself, which has changing proportions of chitin and chitosan, and which differs depending upon the species and the developmental stage of the animals. Batch to batch variability makes it extremely difficult to sell products into markets that are used to uniform products adhering to strict standards (Muzzarelli & Pariser, 1978).

Crustacean shells are excellent raw materials for compost. Chitin provides not only slow-release nitrogen but is believed to act as a nematocide, and to provide some level of organic pest control – presumably by inducing chitinases in plants, which act against both insect and fungal pests. At least one Canadian company composting shrimp shells with peat was able to position its product as a very high end soil amendment with great success.

15.4 Future trends

The quantity, quality, diversity, and profitability of fishery by-products are likely to increase. The factors driving this are many. First, fish and shellfish offer unique flavors and nutrients that are in increasing demand, while wild stocks decrease or – at best – remain stable. As the catches diminish, the need to use all of the catch will become more evident. Second, regulatory pressure will encourage greater utilization. Most of these regulations will be environmental, for instance, banning excessive amounts of fish wastes being dumped at sea, pumped into inlets and harbors, or disposed of in landfills. Some regulations will be based on moral considerations, such as Alaska's waste laws which prevent roe stripping and trophy hunting. Third, the markets for healthy foods and nutraceuticals are booming. Fish body parts that are now seen as waste are rich sources of omega-3 fatty acids and of flesh which, while not in the shape of steaks or fillets, can still provide food for those who cannot afford or do not like the fillets but will gladly eat dishes made with minced fish that does not taste 'fishy'.

Fish by-products have become commercially viable over the last few decades, and some fishery by-products businesses have grown more steadily and more profitably than the primary fish processing businesses from which they sprung. By-products businesses are less dependent upon local stocks and the exigencies of local fishing. They can offer products for human consumption that are sometimes unique or at least, less generic, allowing some entrepreneurs to access venture capital that would have been unavailable to a primary processor.

Even those by-products that are generic commodities, such as fish meal or feed-quality oil, are becoming increasingly specialized, with higher demand and greater profitability. This is due to a combination of factors, of which the enormous global growth of the farming of carnivorous fish is the greatest. However, the loss of market share of competing products such as meat and bone meal, due to fears of bovine spongiform encephalopathy (BSE), has also played a role.

In short, the future for fishery by-products is positive, and it is difficult to see limits to product development and pricing.

15.5 Sources of further information and advice

General

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Fish meal

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16

Recovery and reuse of trimmings and pulps from fruit and vegetable processing

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16.1 Introduction

Fruit and vegetable processing (preservation or production of juice, wine, sugar, starch, oil, etc.) generates huge amounts of wastes; in the European Community (EC) approximately 150 million tonnes are produced annually (Awarenet, 2004). Solid waste represents around 30% of the processed vegetables and can reach up to 85% for sugar production from sugar beet. Until recently in Europe, the principal end-use of these wastes was in the animal-feed sector. However, the rules that regulate the world agricultural exchanges jeopardised this market as imported competitive fodder cereals became cheaper and therefore more attractive for cattle breeders. Vegetable wastes are also often spread on land or composted. These wastes have however a high water content and are consequently perishable and microbiologically instable. This can result in environmental issues such as noxious odours or microbiological hazards. In addition, legislation is strongly encouraging industry to find new end-uses for these by-products. Upgrading these wastes is also of great interest because of its economic profitability, since residues may be available in large quantities at a relatively low cost.

Wastes arising from fruit and vegetable processing industries are rich in cell wall materials. Such plant-derived wastes are generally heterogeneous as they may contain entire vegetable, leaves, stems, roots, tubers, seeds, stones, etc., which makes their upgrading rather difficult. Many plant by-products are still commonly and traditionally used as animal feeds or fertilisers. Some can however provide exploitable products or supplements for the food and other industries (chemicals, cosmetics, pharmaceutics).

Traditional uses include exploitation as sources of hydrocolloids, dietary fibre and ethanol. Nevertheless, for economic as well as environmental reasons, there is a continuous pressure to exploit such residues and to identify products with attractive properties and with potential markets (Laufenberg *et al.*, 2003).

This chapter will consider the upgrading of plant-derived food-processing by-products (mainly vegetable trimmings and residues from extraction). After an inventory of the main origins of the by-products, the upgrading of these plant wastes as a whole, of the constituting polymers, and of the monomeric components, will be addressed.

16.2 Origin and general characterisation of the by-products

16.2.1 Fruit and vegetable juices

Juice production concerns a large variety of fruits and vegetables and generates annually around 5.5 million tonnes of solid waste and between 0.5 and 6 m^3 of wastewater per tonne of raw material. The amount of solid waste depends strongly on the vegetable or fruit (from 5% for tomato to 75% for passion fruit) (Schieber *et al.*, 2001) but represents an average of 30–50% of the raw material. Using enzymatic liquefaction can reduce the quantity of waste; however the degradation of cell wall material by enzymes decreases the added value of the by-product, by reducing the fibre content of the pulp.

The major solid waste is obtained by pressing of fruits or vegetables and solid–liquid separation. This 'pomace' can contain pulp, peels, seeds and stones. The further utilisation of pomace depends on the fruit or vegetable type. Pomace is generally used for extraction of valuable compounds and for production of foodstuffs. For instance, pectin can be found to a greater or lesser extent in most fruits and is, after extraction and purification (see Section 16.4.1), added as gelling agent in numerous food products (including jams, fillings, sweets, etc.). Pomace can also provide other food additives – such as dietary fibres (see Section 16.3.1), lactic acid, pigments, vinegar, natural sweeteners and cellulose (see Section 16.4.2) (Nawirska and Kwasniewska, 2005). Some tropical fruits contain protein-degrading enzymes (papain in papaya, or bromelain in pineapple) which are used as meat tenderisers or washing powders or used in beer brewing. Pharmaceuticals and phytochemicals are also produced, depending on the nature of the fruit or vegetable: essential oils and antioxidants (see Section 16.5.2) like flavanones are extracted from orange or citrus peels (Tomas-Barberan *et al.*, 2004); the antioxidant lycopene, a bioactive terpenoid pigment, is extracted from tomato pomace (Al-Wandawi *et al.*, 1985; Tomas-Barberan *et al.*, 2004); polyphenols are extracted from apple pomace (Schieber *et al.*, 2001). Some studies now deal with the combined recovery of dietary fibres and antioxidant compounds, to obtain antioxidant dietary fibres (Saura-Calixto,

1998; Schieber *et al.*, 2003). Oil contained in the stones of some fruits (mango, apricot, peach) have culinary or cosmetics applications. The final solid waste is frequently used as animal feed, for composting or landfilling.

16.2.2 Grape and wine

With 60 million tonnes per year, grape is the first fruit production in the world. Most of the grape is used for wine production. Some 60% of the world's wine is produced in the EC, especially in France, Italy and Spain. Wine production generates a large amount of solid waste (around 20–30% of the processed material; estimated at between 5 and 9 million tonnes annually worldwide and 4.5 million tonnes in the EC) (Awarenet, 2004; Schieber *et al.*, 2001) and wastewater (1 litre for 1 litre of wine produced).

The majority of the solid wastes are produced during the first steps of the processing of the grapes. Grapes are harvested and crushed, resulting in mash and stems (2–8% of processed material). The mash is then pressed and separated into juice and pomace (10–20%). Stems and pomace are the principal by-products of wine production and are the source of valuable components (ethanol (see Section 16.5.4), tartrate, citric acid (see Section 16.5.5), hydrocolloids (see Section 16.4), dietary fibres (see Section 16.3.1)) (Schieber *et al.*, 2001). High-value antioxidants can be extracted from stems – such as resveratrol used in nutraceuticals, cosmetics or biopharmaceuticals. Pomace also contains other antioxidants (procyanidins, polyphenol extracts) and pigments (anthocyanin) (Tomas-Barberan *et al.*, 2004) and can be used for production of grape seed oil.

In the main wine-producing countries, solid wastes have to be treated in distilleries, which recover spirits by distillation and extraction of grape seed oil and incinerate the final waste. In other countries solid wastes can be used as a fertiliser.

16.2.3 Fruit and vegetable processing or preservation

In order to preserve them and increase their shelf life, fruits and vegetables are usually canned, frozen or dried. These processes generate around 6 million tonnes of solid waste every year and large quantities of wastewater (3.5–8.5 m^3 per tonne of raw material) are also necessary because of specific hygiene and legal constraints. The proportion of waste varies depending on the fruit or vegetable, ranging from 1% of raw material for cranberries to 20–30% for broccoli or carrot. Waste consists essentially of stems, leaves and stalks, which are more often spread on land, composted or used as animal feed. Some vegetable peels have been shown to contain valuable phenolics and bioactive components (Rodriguez *et al.*, 2004; Suutarinen *et al.*, 2004). Fruit stones and kernels are used for natural oils in the food industry, cosmetics or pharmaceuticals. Some leafy by-products (from

cauliflower, artichoke, lettuce, chicory, celery, etc.) are rich in dietary fibre (Femenia *et al.*, 1998), carbohydrates (Rupérez and Toledano, 2003, 2004), antioxidants or prebiotics (Llorach *et al.*, 2003, 2004), but their utilisation is still limited (Larrosa *et al.*, 2002).

16.2.4 Sugar production

Sugar is mainly produced from beet (in Europe) or cane (in warmer climates). In the EC, sugar beet production generates more than 100 million tonnes per year of solid waste and around 0.5 m^3 of wastewater per tonne of raw material; these wastes are generally spread on land. For every tonne of sugar beet, 140 kg of sugar is extracted, resulting in 86% waste. Beets are first flumed by water and separated from leaves, weeds, beet tails and soil. This represents around 100 kg of waste for a tonne of sugar beet and is used for animal feed or composting. Beets are then sliced and pressed, leading to sugar beet pulp (50 kg). This pulp can be dried and used as animal feed or as a raw material (rich in cellulose) for paper production (see Section 16.4.2). Pulp contains and can provide foodstuffs such as protein, prebiotic, cellulose, pectin or hemicellulose. Ferulic acid can also be extracted from pulp and bioconverted to vanillin (Thibault *et al.*, 1998) (see Section 16.5.3). The juice obtained by pressing is purified by adding milk of lime and carbon dioxide. This process step generates 60 kg of carbonation lime that can be used as a soil improver or as absorbent material. Finally the crystallisation step separates sugar from molasses (38 kg). Molasses are used for animal feed, pharmaceuticals extraction (betaine, vitamins, etc.) or as a source of carbon in fermentation processes (e.g. production of ethanol and citric acid). The rest of the waste is the water initially contained in the beets. In the case of sugar cane, processing produces large quantities of bagasse, commonly used as fuel or as a source of arabinoxylans (Schieber *et al.*, 2001).

16.2.5 Starch production

Starch is extracted from corn (60%), wheat (20%) and potato (20%). Starch production generates around 8 million tonnes of waste every year in the EC. This section will focus on potato starch extraction as this process produces the majority of the waste (5.5 million tonnes per year). Some 200 kg of starch is extracted from 1 tonne of potato: wastes represent 80% of the raw material. They are essentially produced during rasping of the potatoes and extraction of starch, resulting in a mixture of pulp (2%), potato juice (76%) rich in sugar and protein, and starch (20%). Proteins can be separated from potato juice by coagulation and used for animal feed. Potato juice is then concentrated and used as a fertiliser. Pulp is used for animal feed, but also for ethanol production. Production of dietary fibre has also been proposed.

16.2.6 Vegetable oil production

Vegetable oil production generates 15 million tonnes of waste every year. This process will be treated in Chapter 20.

16.3 Use of the whole by-products

16.3.1 Dietary fibre

The concept of dietary fibre is well established (even though the definition of dietary fibre may still be the subject of debate) and the nutritional benefits (protection against certain types of cancer, regulation of food transit through the digestive system, blood cholesterol lowering) of dietary fibre intake are generally accepted (Lee and Prosky, 1995). Whatever the definition, plant cell walls are the main sources of dietary fibre as they are composed of polysaccharides (mainly cellulose, hemicellulose and pectins) that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Our dietary fibre intake comes primarily from cereal products, fruits and vegetables that are consumed in their traditional presentation. However, foods can also be supplemented with dietary fibre. Fibres from cereals (bran essentially), or from fruits or vegetables, are produced and may be added to various foodstuffs in order to increase their dietary fibre content. Indeed, some industries have developed programmes to obtain fibre from agricultural or agro-industrial by-products (Gelroth and Ranhotra, 2001). Sugar beet pulp and leguminous seeds are the main residues from which fibres are currently obtained and marketed, but other pulps (from fruit juice extraction for instance) may also be used. Table 16.1 shows the soluble, insoluble and total dietary fibre content of some typical wastes and by-products from fruit and vegetable processing.

Cell walls in these fruits and vegetables are almost devoid of lignin (content 1–2%), and present the typical composition of primary cell walls

Table 16.1 Soluble (SDF), insoluble (IDF) and total (TDF) dietary fibre content of some fruit and vegetable wastes (values in g/100 g dry product were obtained by the Association of Analytical Communities (AOAC) method)

Origin of the waste	SDF	IDF	TDF	Reference
Orange peel after pectin extraction	21.3	62.9	97.6	Aravantinos-Zafiris *et al.* (1994)
Apple pomace	18.6	69.9	88.5	Renard and Thibault (1991)
Apple pomace after pectin extraction	10.3	78.3	88.6	Renard and Thibault (1991)
Grape pomace	9.5	68.4	77.9	Valiente *et al.* (1995)
Beet pulp	12.5	60.3	72.8	Thibault *et al.* (1994)
Pea hulls	4.1	87.4	91.5	Ralet *et al.* (1993)

(Thibault *et al.*, 1994). They contain approximately 30% (dry matter basis) cellulose, the remaining polymeric components being polysaccharides (mostly pectins, generally highly methylated), and hemicelluloses, with minor amounts of glycoprotein (extensin). Furthermore, fibres from fruit and vegetable trimmings have an appreciable water-soluble fraction rich in pectins, the amount of which depends on the vegetable, its ripeness, its processing and especially any heat treatments – which can solubilise pectins, mainly by β-eliminative degradations (Thibault *et al.*, 1994). This is a distinctive and potentially important feature of dietary fibre from fruits and vegetables since the nutritional effects of soluble fibres are different from those of insoluble fibres. The capability to bind or hold components (water, ions, small molecules, etc.) and the granularity of the fibres are the two main physicochemical characteristics that can have an impact on the nutritional effects of the fibre as well as on the final product processing. Table 16.2 shows the characteristics of various fibre types in terms of their swelling (measure of bed volume of the fibre in an excess of solvent), water-binding capacity (amount of water bound to the fibre after an external force, e.g. centrifugation, has been applied in given conditions) and cation exchange capacity (number of cations that can bind the fibre by the anionic groups, such as carboxylate in pectins). Fibres arising from fruits and vegetables are characterised by high hydration properties: this property is due to the presence in the fibre of large amounts of hydrophilic pectins. A high water-binding capacity helps individuals to reduce their calorie intake. This property also has technological interest since a high value helps to retard staling and control ice crystal formation and water migration (Guillon *et al.*, 2000; Gelroth and Ranhotra, 2001).

16.3.2 Functional fibre

These physicochemical properties have led to proposals that plant cell wall residues could be used as water (super)absorbants and natural ion exchangers. Indeed, ion exchange resins are widely used for water depollution. The

Table 16.2 Physicochemical characteristics of dietary fibre from fruit and vegetable wastes

Fibre	Swelling (ml/g)	WBC (g/g)	CEC (meq./g)	Reference
Apple pomace	9.4	10.3		Renard and Thibault (1991)
Apple pomace after pectin extraction	8.0	7.3		Renard and Thibault (1991)
Beet pulp	19.3	32.9	0.47	Thibault *et al.* (1994)
Lettuce	37.0	3.1	0.94	Thibault *et al.* (1992)
Broccoli	8.9	3.4		Thibault *et al.* (1992)

WBC; water-binding capacity; CEC, cation exchange capacity.

available commercial resins provide a wide selection of ion selectivities and performances; because of their high cost and thanks to their high chemical stability, synthetic resins can be regenerated. Regeneration needs, however, large amounts of water while in other cases fouling or poisoning of the resins may occur. Sugar beet pulp (Dronnet *et al.*, 1997) and soybean hulls (Laszlo and Dintzis, 1994), for example, have been proposed as low-cost and single-use ion exchange resins. Furthermore, such preparations have been proposed for food or non-food uses. For instance, because of their low degree of methylation and great stability, alkali-treated citrus wastes have been suggested as thickening and gelling agents in place of the extracted and purified pectins (Speirs *et al.*, 1980). Extrusion cooking was also proposed to increase the water-soluble (pectin) fraction: the extruded citrus or apples residues may have gelling properties and could find applications in the replacement of purified pectins (Ralet *et al.*, 1994a). Wastes containing primary cell walls rich in cellulose or enriched in cellulose by different treatments have also been suggested for applications in papermaking (see Section 16.4.2).

16.4 Recovery of functional biopolymers

The polysaccharides of the fruit and vegetable trimmings (pectins, cellulose and hemicelluloses), whose relative proportions vary with respect to the plant source, can be more or less selectively recovered from fruit and vegetable wastes for multiple food and non-food uses.

16.4.1 Pectins

Chemical structure

Pectin is an extremely complex polysaccharide, composed of as many as 17 different monosaccharides which can be envisioned as a multiblock copolymer (Figure 16.1). The simplest of these blocks is homogalacturonan (HG), an unbranched polymer of (1→4)-α-D-Gal*p*A, the so-called pectic 'smooth' region. Other minor types of galacturonans (so-called 'substituted galacturonans' (Ridley *et al.*, 2001)) can be distinguished, for example, rhamnogalacturonan II (RG-II) and xylogalacturonan (XGA) (Vincken *et al.*, 2003). A second major block, rhamnogalacturonan I (RG-I), is composed of a repeating disaccharide unit [→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→]$_n$. RG-I is decorated primarily with other blocks, namely arabinan and arabinogalactan side chains. Complexes of RG-I, arabinan and arabinogalactan are often referred to as pectic 'hairy' regions in which arabinans and arabinogalactans are the 'hairs'.

'Smooth' regions

'Smooth' regions were selectively isolated and their macromolecular parameters (molar mass, degree of polymerisation and polydispersity) were

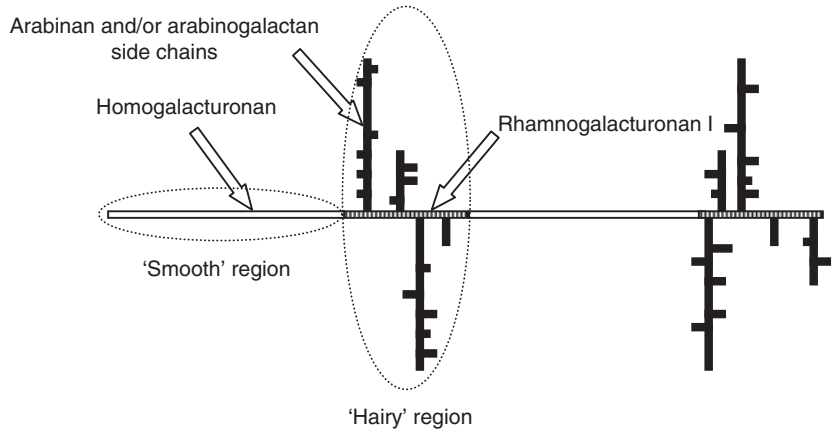

Fig. 16.1 Schematic representation of the structure of pectin.

investigated. In some studies (Powell *et al.*, 1982; Thibault *et al.*, 1993), differences in the susceptibility to acid hydrolysis of the glycosidic linkages were exploited: under mild acid conditions the linkages between adjacent GalA residues (HG domains) are much more stable than linkages between GalA and Rha (RG-I domains). Using this chemical approach, Thibault *et al.* (1993) isolated almost pure HG from apple, beet and citrus pectins. A weight-average degree of polymerisation of 108–138 was estimated by HPSEC-MALLS (high-performance size exclusion chromatography coupled on-line with multiple angle laser light scattering). Similar values were recently obtained for HG isolated by enzymatic means (Bonnin *et al.*, 2002; Hellìn *et al.*, 2005). 'Smooth' regions isolated by chemical or enzymatic means were all characterised by a very low polydispersity index, revealing a high homogeneity with respect to molar mass (Hellìn *et al.*, 2005).

Gal*p*A residue carboxylic functions are naturally partly methyl-esterified (Pilnik and Voragen, 1970). The degree of methylation (DM) is defined as the percentage of GalA units esterified with methanol. The methyl-esterification of HG has been the subject of several investigations since it determines to a large extent the industrial applicability of pectin and their interaction ability *in muro* (Rolin *et al.*, 1998; Ralet *et al.*, 2001). Not only the DM, but also the distribution of methyl groups on the HG has a deep impact on pectin gelation properties (Kohn *et al.*, 1983; Thibault and Rinaudo, 1985; Ralet *et al.*, 2001).

Secondary alcoholic functions of Gal*p*A residues can also be partly acetyl-esterified. The degree of acetylation (DAc) is defined as the number of acetyl groups for 100 GalA units. The DAc is generally low but pectins with high DAc – such as sugar beet, pear, carrot and potato – have been reported (Voragen *et al.*, 1995). Recently, the sugar beet HG acetylation pattern was assessed (Ralet *et al.*, 2005).

'Hairy' regions

As in 'smooth' regions, Gal*p*A residues in RG-I regions may be *O*-acetylated. In contrast, there is no conclusive chemical evidence that Gal*p*A residues are methyl-esterified in RG-I regions.

Depending on the plant source and method of isolation, 20–80% of the Rha*p* residues in RG-I are substituted, mainly at *C*-4 but sometimes at *C*-3, with oligomeric or polymeric side chains (O'Neill *et al.*, 1990). The predominant side chains contain linear and branched Ara*f*, and/or Gal*p* residues, although their relative proportions and chain lengths differ a lot depending on the plant source (Lerouge *et al.*, 1993). Among the side chains of neutral sugars, arabinans and arabinogalactans (type I and II) can be distinguished. Arabinans consist of a 1,5-linked α-L-Ara*f* backbone, which can be substituted α-L-Ara*f*-(1→2), α-L-Ara*f*-(1→3), and/or α-L-Ara*f*-(1→3)-α-L-Ara*f*-(1→3) side chains. Pectins with arabinans attached have been isolated from several sources including sugar beet (Guillon and Thibault, 1989), carrots (Massiot *et al.*, 1988), cabbage (Stevens and Selvendran, 1984) and onion (Ishii, 1982). In sugar beet, arabinans are present as long chains (backbone length of 60–70 residues) with 45–65% of the Ara residues substituted mainly by single Ara residues (Oosterveld *et al.*, 2002). Arabinogalactans occur in two structurally different forms. Type I arabinogalactans are composed of a 1,4-linked-β-D-Gal*p* backbone more or less substituted at *O*-3 with short 1,5-linked-α-L-Ara*f* chains (Voragen *et al.*, 1995). Pectins with type I arabinogalactans attached are commonly found in several fruits and vegetables, e.g. citrus (Labavitch *et al.*, 1976), potato (Jarvis *et al.*, 1981) and tomato (Seymour *et al.*, 1990). Type II arabinogalactan is a highly branched polysaccharide with ramified chains of β-D-Gal*p* residues joined by 1→3 and 1→6 linkages. 1,4-linked-β-D-Gal*p* are generally terminated by L-Ara*f* and to some extent by L-Ara*p* residues (Voragen *et al.*, 1995). Pectins with type II arabinogalactans attached have been found, e.g. in apple, rapeseed, lemon, beet and grape (Voragen *et al.*, 1995).

Manufacture of industrial pectins

Current raw materials

Raw materials presently employed in industrial practice are the dried press cake of apple juice manufacture (apple pomace, 10–15% extractable pectins) and the wet or dried peels and rags of citrus juice manufacture (citrus peel, 20–30% extractable pectins). Apple pomace and citrus peels are, in the wet state, very perishable commodities (May, 1990). The major part of the raw material is dried for shipment or warehousing, so that pectin can be produced outside the harvesting period and/or at another location. Washing in water is necessary prior to drying in order to minimise caramelisation (Rolin *et al.*, 1998). Other sources have been considered for the extraction of commercial pectins, e.g. sugar beet, sunflower heads and the wastes from the processing of tropical fruits. These pectin-containing

materials are potentially available in quantity and/or in logistically or economically favoured locations.

Pectin extraction

Commercially, pectin is extracted by treating the raw material with hot dilute mineral acid at ~pH 2. The hot pectin viscous extract is separated from the strongly swollen and partly disintegrated residue by a combination of centrifugation and filtration. The clarified extract is brought to pH 4 and concentrated under vacuum prior to alcoholic precipitation, pressing and drying. Throughout the process, unnecessary holding times at high temperatures are avoided in order to prevent pectin demethylation and depolymerisation. Pectins can be highly variable, depending mainly on the raw material quality, and it is usual to blend together a number of production batches and dilute them with sucrose or dextrose to a standard gelling performance. The process described above yields a pectin of around 75% methyl-esterification. To produce other types of pectins with diverse application properties, some saponification of the methyl groups is required. This is usually performed by the action of acid or alkali when pectins are in the alcoholic slurry. This process yields high-methoxy (HM) pectins (this term is used for commercial pectins of high DM (>50%)) with DM values in the range 55–75% or low-methoxy (LM) pectins (this term is used for commercial pectins of low DM (<50%)) with DM values in the range 20–45%. Alkaline treatments at low temperature to avoid β-eliminations can also be used in the presence of ammonia. In these conditions, pectins are partly de-esterified and some of the methyl groups are replaced by amides, giving LM amidated pectins (the degree of amidation must be <25%). These amidated pectins need less calcium to gel and are less prone to precipitation at high calcium concentrations; furthermore they give gels that are more thermoreversible than the non-amidated LM pectins (Voragen *et al.*, 1995).

Effluent has become a major consideration in the pectin industry; it is increasingly a major cost and sometimes a serious technical problem to the pectin manufacturer (May, 1990).

Pectin gelling properties and food applications

Pectin is first and foremost a gelling agent used to impart a gelled texture to foods, mainly fruit-based foods. The DM value is the key criterion that governs gelation conditions: HM pectins will gel with at least 55% of soluble solids and at pH ≤ 3.5 while LM pectins will gel in the presence of calcium ions.

HM pectins. About 80% of the world production of HM pectin is used in the manufacture of jams and jellies, the pectin being added to supplement the gelling power of fruits. Jams and jellies are made with a soluble solids of 65%, a final pH of 2.9–3.2 and generally contain 0.3–0.5% HM pectin.

The analytical parameter that allows prediction of gelling behaviour (setting time, pH and temperature) is the DM. This has led to a subdivision of HM pectins into four commercial categories: ultra-rapid set (DM 74–77), rapid set (DM 71–74), medium set (DM 66–69) and slow set (DM 58–65). It is the art of the jam manufacturer to choose the correct pectin type for each jam or jelly type. For example, jams with whole fruits should gel shortly after filling in order to prevent fruit flotation. To keep the fruit evenly suspended, rapid- or ultra-rapid-set pectins that gel rapidly at high temperature (80 °C at pH 3.1; May, 1990) have to be used. At the other end of the scale, clear jellies need to gel slowly to avoid trapped air bubbles. Slow-set pectins that gel at low temperature (around 40 °C at pH 3.1; May, 1990) are the most obvious choice. Jam manufacturers also make a very wide range of jams, fillings and toppings for the confectionery industry. HM pectins are mainly used for making fruit jellies and jelly centres flavoured with natural fruit constituents and/or synthetic flavours. HM pectins find further applications in fruit drink concentrates, instant fruit drink powders, fruit juice–milk combinations and sour milk products (http://www.cpkelco.com/food/index.html).

LM pectins. LM pectin is used when the soluble solids content is between 20 and 55%, typically in low- or reduced-calorie fruit spreads. An addition of calcium salt is generally needed. The type of LM pectin (slow set, DM 50; medium set, DM 40; rapid set, DM 30) must be carefully selected according to the soluble solids/pH conditions in the application medium. The heat reversibility of LM pectin gels may be utilised in bakery jams and jellies for glazing purposes. Pectin manufacturers offer ready-made blends of LM pectins and sometimes other ingredients for products of this type. LM pectins are also often used in fruit preparations for yoghurt, fruit–milk desserts, gelled milk products and confectionery products. In the latter, LM pectins are used for jellies and centres in which the low pH range necessary for HM pectin gelation is not acceptable for flavour reasons (for example in cinnamon- or peppermint-flavoured jellies) (http://www.cpkelco.com/food/index.html).

Pectin non-food applications

Sugar beet pectin suffers from several disadvantages as a competitor to apple or citrus pectin, especially its high content of acetyl groups which hinders ‘traditional’ gelation. However, in beet pectin, feruloyl groups naturally esterify neutral sugars side chains (see Section 16.5.2, Hydroxy cinnamic acids) and it is possible to take advantage of the presence of those feruloyl groups. Feruloylated pectins can indeed be oxidatively cross-linked by chemoenzymatical (peroxidase and hydrogen peroxide) or enzymatical (laccase) means (Rombouts and Thibault, 1986; Micard and Thibault, 1999). The gel formed is thermally stable and can be dehydrated and rehydrated (Rombouts and Thibault, 1986). It may thus lend itself to

applications quite different from those of current commercial pectins, such as superadsorbant systems.

The resistance of pectin to degradation in the upper gastro-intestinal tract and its complete and rapid dissolution in the colon, make pectin an ideal ingredient for colon-specific drug delivery (Rolin *et al.*, 1998). A colonic drug delivery system based on a pectin and galactomannan coating was proposed (Lee *et al.*, 1999) and the site specificity of drug release was assessed in human subjects (Yang *et al.*, 2002).

Pectins or some pectic-specific domains exhibit effects on the human immune system. The possible mechanisms of action include influence on the complement cascade, endocrinal functions, cytokine induction and the effect on chemotaxis of leucocytes (Wagner and Kraus, 2000; cited by Morra *et al.*, 2004). Furthermore, some pectins, more particularly their RG-I domains, exhibit anti-ulcer and mitogenic activities (Yamada, 2000; cited by Morra *et al.*, 2004). Finally, metastasis of some cancers depends on the cancer cells' specific recognition of galactoside epitopes, a recognition that can be inhibited by appropriately engineered pectins (Nangia-Makker *et al.*, 2002). So far, limited studies have been performed with pectins or specific pectic domains, but this molecule has a clear potential for therapeutic significance and is undergoing clinical trials (Nangia-Makker *et al.*, 2002).

Upgrading of isolated pectin domains: arabinans

Arabinans are present as pectin neutral sugar side chains in various plants (see Section 16.4.1, 'Hairy regions'). They can be extracted from isolated pectins or directly from plant by-products such as sugar beet pulp. Alkaline extraction at high temperature (70–98 °C) for 15–90 min followed by neutralisation and ultrafiltration yields a branched arabinan (molar mass of about 50 kDa) containing around 80% of L-Ara (McCleary *et al.*, 1990) (Fig. 16.2). Branched arabinan exhibits surface active properties that make it suitable for use as an emulsifying agent. Additionally, flavour oil and fragrances may be encapsulated using arabinan (McCleary *et al.*, 1990). However, the arabinan extraction and purification cost is a clear limitation for these uses.

Branched arabinan can be linearised using purified α-L-arabinofuranosidase to yield debranched arabinan (McCleary *et al.*, 1990) (Fig. 16.2). The debranched arabinan forms an aqueous gel that has the properties of a fat substitute and may be used in foods (McCleary *et al.*, 1990; Cooper *et al.*, 1992). Linearised arabinans could also find applications as texture agents in cosmetic and pharmaceutical industries (Cooper *et al.*, 1992).

16.4.2 Cellulose

Cellulose is the world's most abundant naturally occurring polymer, rivalled only by chitin. Commercial purification of cellulose is centred on

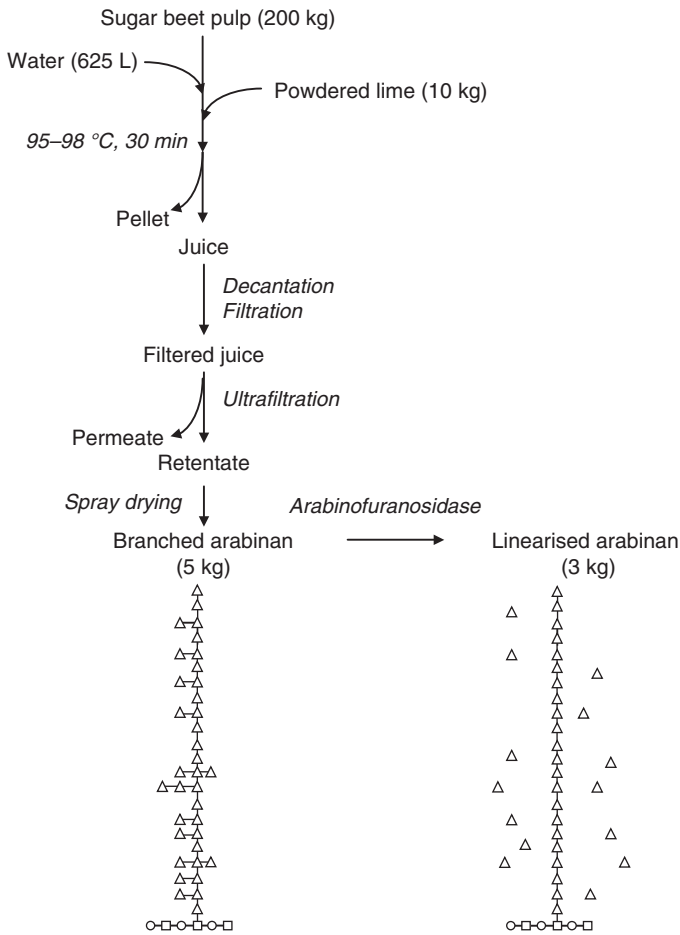

Fig. 16.2 Recovery of branched and debranched arabinan from sugar beet pulp.

wood pulp and cotton linters but fruit and vegetable wastes, which usually contain 25–30% of cellulose on a dry matter basis, could be interesting sources.

Chemical structure

Cellulose is a homopolymer of (1→4)-β-D-Glc*p*. The β-1,4 configuration results in a rigid and linear structure for cellulose. Cellulose chains exhibit a strong tendency to form intra- and intermolecular hydrogen bonds resulting in the formation of microfibrils whose length, width and crystallinity differ a lot depending on the cellulose origin. Cellulose arising from primary cell walls is particularly thin (2–3 nm width) and of low crystallinity.

Isolation and upgrading of cellulose from fruit and vegetable wastes
Following the initial work of Weibel (1986, 1989) and Weibel and Myers (1990), Dinand *et al.* (1996, 1999) purified sugar beet cellulose and evaluated the application potential. A specific alkaline purification treatment led to a partial desincrustation of the cellulose microfibrils from the other cell wall components (pectins and hemicelluloses). Aqueous suspensions of cellulose microfibrils were then defibrillated using a high-pressure homogeniser (Dinand *et al.*, 1996, 1999) (Fig. 16.3(a)). This method was also applied in order to recover defibrillated cellulose fibres from lemon peels (Rondeau-Mouro *et al.*, 2003). A similar method including an acid extraction was recently developed (Zykwinska *et al.*, 2005) (Fig. 16.3(b)). In all cases 'desincrusted' parenchymal cell cellulose (PCC) – containing roughly 90% cellulose together with residual amounts of pectin, hemicellulose and inorganics – was obtained. After high-pressure homogenisation, defibrillated PCC suspensions consisting of dispersions of cellulose microfibrils, either individual or still bundled together, were recovered (Fig. 16.3). One of the key properties of defibrillated PCC suspensions is that they do not sediment or flocculate and display liquid crystalline characteristics (Dinand *et al.*, 1996, 1999). Homogenised PCC displays rheological properties (shear thinning properties together with pseudoplasticity) that are similar to those

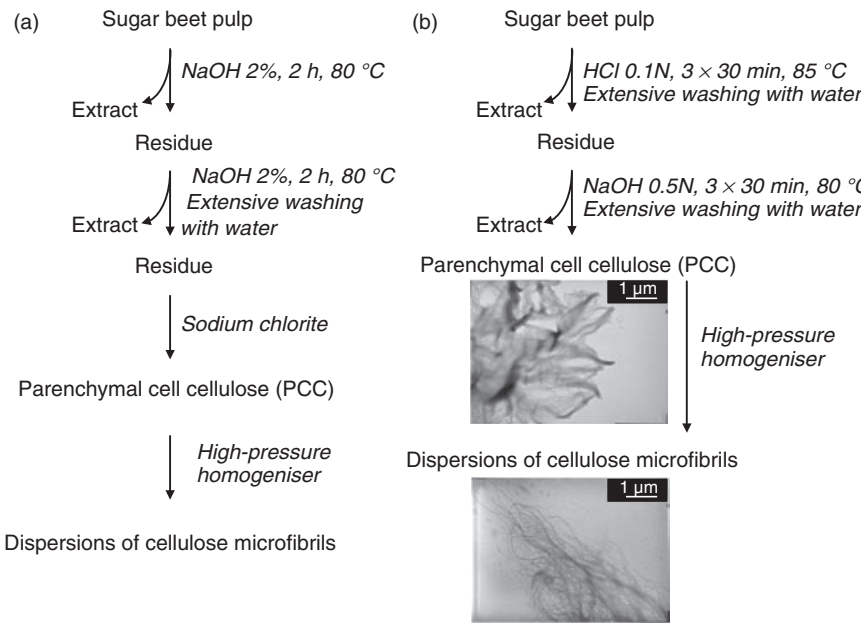

Fig. 16.3 Recovery of crude and defibrillated parenchymal cell cellulose (PCC) using the methods developed by (a) Dinand *et al.* (1996, 1999) and (b) Zykwinska *et al.* (2005).

observed for microfibrillated wood cellulose (commercialised under the trade name of Celish® by Daicel (http://www.daicel.co.jp/wsp/e/product/product-c.html)) and for bacterial cellulose (Dinand *et al.*, 1996, 1999). The interest in bacterial cellulose is reflected in a number of patents and publications devoted to this polymer (Bielecki *et al.*, 2005). Bacterial cellulose has found a multitude of commercial applications in paper, textile and food industries, and as a biomaterial in cosmetics and medicine (Bielecki *et al.*, 2005). The processing cost of bacterial cellulose is however still a pitfall to its competitive industrial production. Homogenised PCC could potentially replace bacterial cellulose in several applications: as a component of paper to reinforce paper mechanical properties (Dufresne *et al.*, 1997), in surgical drapes and gowns, in various medical applications or in food applications.

Recently, cellulose microfibrils were isolated from swede roots and homogenised as described above (Bruce *et al.*, 2005). Homogenised PCC suspensions were used to form composites with four matrix materials (polyvinylacetate, acrylic polymer, epoxy and locust bean gum). The best of the composites had a stiffness and strength significantly greater than conventional flax–epoxy composites and as good as the best glass-fibre composites (Bruce *et al.*, 2005).

Homogenised PCC suspensions were also successfully surface silylated (Goussé *et al.*, 2004). The mildly silylated microfibrils retained their morphology, but could be dispersed in a non-flocculating manner into organic solvents. This highlights the potential for the use of these microfibrils in the reinforcing of non-polar polymers, such as polyolefins or other commodity polymers.

16.5 Upgrading of the mono-/oligomeric components

As stated previously, wastes from fruit and vegetable transformation contain mainly plant cell wall which is a very complex structure. Some of the cell wall components are usable in their monomeric or oligomeric forms. Hydrolysis of the cell wall is therefore required and enzymes are often used in this purpose. However, due to the complex structure of cell wall components, many enzymes are required to achieve their degradation. These enzymes are produced by several microorganisms and are the subject of numerous studies and reviews (Kulkarni *et al.*, 1999; de Vries and Visser, 2001; Benen *et al.*, 2002).

16.5.1 Bioactive oligosaccharides

Enzymatic hydrolysis of cell-wall-rich residues releases soluble oligosaccharides, some of which, called oligosaccharins, are biologically active. Oligogalacturonides, which are linear molecules of 2 to about 20 (1→4)-α-D-Gal*p*A units arising from pectic 'smooth' regions (see Section 16.4.1,

Smooth regions), were the first plant oligosaccharins to be discovered (Bishop *et al.*, 1981 and Hahn *et al.*, 1981, reviewed by Ridley *et al.*, 2001).

Oligosaccharins exhibit a variety of regulatory effects in plants, including the elicitation of defence responses, regulation of growth and development, and induction of rapid responses at the cell surfaces (Ridley *et al.*, 2001). They could form the basis of a new non-toxic crop protection system that would be environmentally safe, would enable plants to respond to infection faster than normal and prevent potential pathogens from successfully colonising the host.

16.5.2 Antioxidants

Radical oxygen species (such as superoxide O_2^-, hydroxyl (OH), peroxyl (LOO), alkoxyl (LO), nitric oxide (NO)) and some strong oxidants (such as hypochlorous acid HOCl, hydrogen peroxide H_2O_2) can interact with a number of biomolecules (DNA, lipids, carbohydrates, proteins) causing some transient or irreversible damage which has been associated with serious diseases such as cancer or artherosclerosis.

Living cells have developed antioxidant systems to protect themselves against the reactive oxygen species. These antioxidants are defined as 'any substance that, when present at low concentration compared to the one of an oxidizable molecule, significantly delays or prevents oxidation of that molecule'. Antioxidants are also of interest in the industry as they minimise the oxidation of lipids in food and non-food matrices. Amongst the antioxidants of biological or industrial interest, plant-derived phenolic compounds are the most recently discovered and studied.

Hydroxycinnamic acids

Hydroxycinnamic acids include ferulic acid, caffeic acid and *p*-coumaric acid. Many of these compounds exhibit potential health benefits such as inhibitory effects on tumor promotion (Huang *et al.*, 1988) or cardioprotective properties (Huang *et al.*, 1998). They can also block the formation of mutagenic compounds such as nitrosamines (Kuenzig *et al.*, 1984) and may protect against photooxydative skin damage (Saija *et al.*, 1999). Their capacity to protect lipid systems is reported by several authors (Sharma, 1976; Yagi and Ohishi, 1979; Cuvelier *et al.*, 1992; Scott *et al.*, 1993; Castellucio *et al.*, 1995; Meyer *et al.*, 1998; Natella *et al.*, 1999).

The structural explanation for the antioxidant properties of these molecules is far from clear. It appears to be partly linked to the ability of hydroxycinnamic acids to donate H^+ to form stable phenoxy radicals and to the presence of substitutions on the phenolic ring. The number and position of the hydroxyl and methoxyl groups and the presence of an unsaturated conjugation system would influence the stability of the molecule (Graf, 1992).

Ferulic acid (3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid) is the most abundant hydroxycinnamic acid in the plant kingdom. It was described for the first time in 1866 as a component of *Ferula foetida* Reg (*Umbelliferae*) (von Hlasiwetz and Barth, 1866, cited by Rosazza *et al.*, 1995). In plants, ferulic acid is rarely found in the free form and it is differently located according to the source. In monocots, arabinose residues in arabinoxylans are esterified on position *O*-5 by ferulic acid (Saulnier and Thibault, 1999). In dicots, phenolic acids are generally not found except in a few plants such as the Chenopodiaceae family. In sugar beet for example, ferulic acid is present as part of the pectin. It is linked on *O*-2 of arabinose residues and on *O*-6 of galactose residues (Ralet *et al.*, 1994b). The chemical and biological properties of ferulic acid were exhaustively reviewed by Graf (1992).

Ferulic acid is also found in various dimeric forms in the plant cell wall. Indeed, the phenoxyradicals may react together to form different dehydrodimer isomers: 5-5′, 8-*O*-4′, 8-5′ and 8-8′ (Ralph *et al.*, 1994). As the monomer, ferulic acid dehydrodimers have *in vitro* antioxidant capacities (Garcia-Conesa *et al.*, 1997). Dimerisation modifies the antioxidant capacity but the final effect depends on a combination of factors such as structure (type of linkage, number of hydroxyl groups and conjugation of the molecule), lipophilicity and interaction between the test compounds and other compounds in the reaction mixture.

Removal of the ferulates may be carried out by alkalis. However, many studies have discussed the enzymatic release of ferulic acid (Graf, 1992; Cheetham, 1993; Micard *et al.*, 1994). The first publications detailing the isolation and characterisation of feruloyl esterases are now more than 15 years old (Faulds and Williamson, 1991; Tenkanen *et al.*, 1991). Since then, feruloyl esterases have been isolated from a large number of microorganisms and the related protein sequences elucidated (Crepin *et al.*, 2004). Based on substrate utilisation data and supported by primary sequence identity, four sub-classes have been characterised and termed type-A, -B, -C and -D.

At present, the lack of highly conserved sequences within the sequenced esterases does not permit further classification for the feruloyl esterases, other than that their primary amino acid sequences place them in family 1 (Coutinho and Henrissat, 1999) of the carbohydrate esterase classification (http://afmb.cnrs-mrs.fr/~cazy/CAZY/). They exhibit different specificities according to the nature of the sugar and the linkage between sugar and ferulic acid as well as according to the length of the oligosaccharide moiety (Table 16.3 from Crepin *et al.*, 2004). Two of these sub-classes release 5-5′ diferulic acid (type-A and -D). After the enzymatic release of ferulic acid, it is necessary to purify it from the reaction medium at minimal cost, for further uses. This was investigated using polystyrenic resins (Couteau and Mathaly, 1997) and activated carbon (Couteau and Mathaly, 1998).

Table 16.3 Ferulate esterase specificities (from Crepin *et al*., 2004)

Enzyme	Preferred substrate	Sequence similarity
FAE A	*O*-5 feruloylated arabinose	Lipase
FAE B	*O*-2 feruloylated arabinose *O*-6 feruloylated galactose	CE 1 acetyl xylan esterase
FAE C		Chlorogenate esterase Tannase
FAE D		Xylanase

Hydroxytyrosol: a new potent antioxidant

Olive oil contains natural antioxidants such as tocopherols, carotenoids, sterols and phenolic compounds (Boskou, 1996). The main phenolics identified in olive are: tyrosol (*p*-hydroxyphenyl ethanol); hydroxytyrosol (3,4-dihydroxyphenyl ethanol); gallic, caffeic, vanillic, *p*-coumaric, syringic, ferulic, homovanillic, *p*-hydroxybenzoic and protacateuric acids; and oleuropein (Montedoro *et al.*, 1992). They are found in olive oil but also in liquid and solid by-products generated by oil extraction. Commercial enzyme preparations, added during olive oil extraction, improve the release of phenolic compounds in olive oil and its by-products (Montedoro *et al.*, 1993). The enzymatic release of simple phenolic compounds, and especially hydroxytyrosol, from olive oil by-products was enhanced by using culture broths of *Aspergillus niger* enriched in cinnamoyl esterases. The antioxidant activity of hydroxytyrosol is higher than that of synthetic antioxidants widely used in the food industry (ascorbic acid or butylhydroxytoluene) (Bouzid *et al.*, 2005). Hydroxytyrosol is believed to be the antioxidant with the highest free radical scavenging capacity (O'Dowd *et al.*, 2004).

Olive pulp also contains polyphenols that have potentially significant antioxidant effects *in vivo* and have the same health-promoting properties as other polyphenols: prevention of atherosclerosis, promotion of intestinal and respiratory health, and prevention of cancer and heart disease (Uccella, 2001). For all these reasons, the polyphenol content in olive oil should be increased. In this way, phenolic compounds contained in olive mill wastewater are tentatively being transformed into valuable products using *Lactobacillus plantarum* during olive oil processing in order to favour their transportation to olive oil (Kachouri and Hamdi, 2004). Moreover, olive polyphenols have been demonstrated *in vitro* to inhibit or delay the rate of growth of bacteria such as *Salmonella*, *Cholerae*, *Staphylococcus*, *Pseudomonas* and *Influenza*. These data suggest a potential role for olive water polyphenol antioxidants in promoting intestinal and respiratory human and animal wellness, and as an antimicrobial food additive in pest management programmes.

16.5.3 Aromas

Flavour compounds, substances stimulating taste and smell, are extremely important for the food, animal-feed, cosmetics and pharmaceutical industries, as they represent more than 25% of the total food-additive market. In the past, they were mainly extracted from plants. However, they are most often present at very low concentrations and their extraction is thus difficult and expensive. Moreover, their availability is highly dependent on agricultural variations, plant diseases or sociopolitical stability of the producing countries. In order to bypass these difficulties, chemical synthesis of most of the flavours of industrial interest has been performed leading to cheap molecules being widely available.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most universally used flavours in the food, pharmaceutical, cosmetic and even detergent industries. Natural vanillin is extracted from the fermented pods of vanilla orchids (mainly *Vanilla planifolia*). The major producers are Mexico, Madagascar, Tahiti and Indonesia. Vanillin is absent from the green pod and is released during curing of the pods after harvest. Cured pods contain 2–3% by weight of vanillin, it occurs as vanillin-β-D-glucoside and is associated with many other flavouring compounds. Approximately 12 000 tonnes/year of vanillin are consumed, essentially as synthetic vanillin at around US$ 15/kg; in contrast the natural vanilla extract can be estimated at US$ 4000/kg (Lomascolo *et al.*, 1999).

Even though many synthetic flavours are available at very low prices, recent years have seen an increasing consumer demand for natural compounds. Fermentation and enzymatic reactions, together forming biotechnology, can be employed for the production of so-called 'natural' aromas (Krings and Berger, 1998). Several authors have described the use of enzyme preparations containing β-glucosidase to achieve vanillin release from vanilla pods, as an alternative to conventional curing (Dignum *et al.*, 2001). An enzymatic route for vanillin synthesis – from the widely available principle of red pepper, capsaicin – was also reported (van den Heuvel *et al.*, 2001). More interestingly, microorganisms can be exploited as they have rapid growth rates, they produced flavours *de novo* as secondary metabolites (i.e. not linked to cell house-keeping) and they can be genetically modified quite easily to produce new molecules from a selected precursor. In the case of vanillin, several potential precursors have been suggested – including curcumin, siam benzoin resin, phenolic stilbenes, eugenol and ferulic acid. The biotransformation of these molecules in vanillin by various microorganisms has been extensively studied, patented and/or reviewed (Falconnier *et al.*, 1994; Lesage-Meessen *et al.*, 1996; Narbad *et al.*, 1997; Rabenhorst and Hopp, 1997; Muheim *et al.*, 1998; Steinbuchel *et al.*, 1998; Thibault *et al.*, 1998; Lesage-Meessen *et al.*, 1999; Walton *et al.*, 2000; Topakas *et al.*, 2003). In any case, vanillin has posed an intriguing biosynthetic problem for many years. In vanilla, vanillin β-glucoside formation

appears to be much more complex than initially envisaged. Due to the commercial importance of vanilla-type flavourings, it is still necessary to learn more about the molecular genetic characterisation of vanillin formation that might open up the possibilities to introduce new or enhanced biosynthetic capacities in plants (Walton *et al.*, 2003).

In addition to vanillin, other aromas can be produced from other mono-/oligomeric components originating from plant co-products. This is particularly the case for sugar monomers present in cell wall polysaccharides. L-Rhamnose is mainly found in the pectic fraction of the cell wall. Commercially available rhamnose is produced by chemical hydrolysis of arabic and karaya gums, or from rutin or citrus fruits which contain by weight 10–30% rhamnose. Rhamnose is a raw material for the production of furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone), a strawberry flavour used in caramel and fruit flavour applications (Haleva-Toledo *et al.*, 1999). Arabinose, another pectic monomer, is a precursor of L-fructose and L-glucose that would be excellent as low-calorie sweeteners (Vogel, 1991).

16.5.4 Ethanol

Fruit and vegetable by-products are rich in fermentescible sugars, and as such they can be used as raw material for ethanol production. Production of ethanol from agricultural and forestry residues or other sources of lignocellulosic biomass is of both economic and environmental interest. It could be a way to counter the inevitable depletion of the world's petroleum supply and to decrease air pollution. Ethanol can be produced from glucose and xylose fermentation, both originating from cell wall polysaccharide degradation. Sugar cane bagasse is the main raw material used for this purpose but other biomass is also used, such as hardwood and grasses. Some other wastes have been studied, e.g. chicory roots (Leplus, 2004). The biomass can be treated by a concentrated acid process that uses sulphuric acid (Fig. 16.4). In that case, very efficient acid recycling is required for the process to be economically acceptable. The second possibility to recover the monomers is to degrade the wastes using enzymatic tools. This environmentally friendly process needs to overcome the natural resistance of lignocellulosic biomass to enzymatic breakdown. It was thus necessary to develop concomitant physical pretreatment to alter biomass structure and thus enhance the biodegradability of the waste, so that hydrolysis of the carbohydrate to monomeric sugars can be achieved more rapidly and with greater yields. A number of pretreatment options have been investigated, including steam-explosion, ammonia fibre explosion, organosolvents, supercritical extraction and dilute acid pretreatment (Wyman, 1994; Mosier *et al.*, 2005).

Consequently, processing of plant waste to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation and product separation/purification. Plant wastes are enzymatically degraded by a combi-

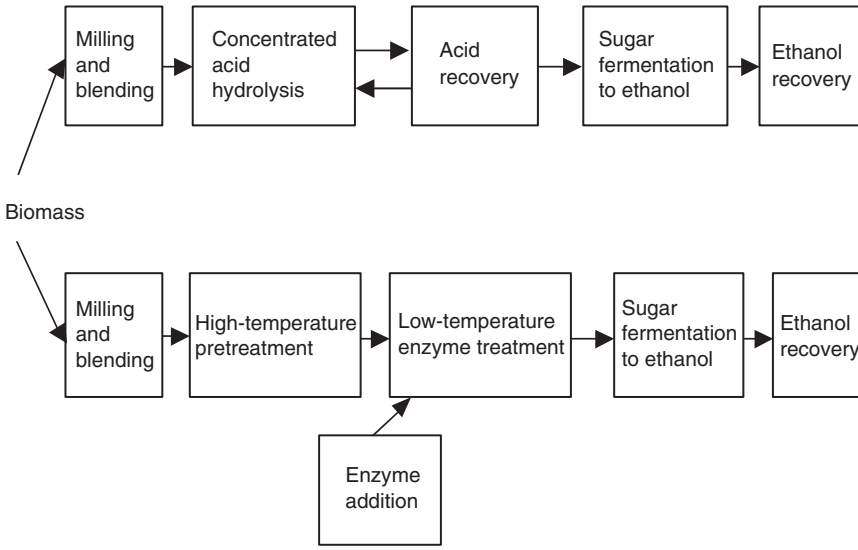

Fig. 16.4 Simplified diagram showing possible processes for ethanol production (from Mielenz, 2001). Top, concentrated acid process; bottom, enzymatic production.

nation of cellulases, hemicellulases and pectinases. The hexoses released (mainly glucose and galactose) are readily fermented to ethanol by many naturally occurring microorganisms, whereas pentoses (mainly xylose and arabinose) are fermented to ethanol by few native strains with most often relatively low yields (Mosier *et al.*, 2005). Both yeasts (such as *Saccharomyces* and *Pichia* species) and bacteria (such as *Escherichia coli*, *Klebsiella* and *Zymomonas*) have been genetically modified to ferment hexoses and pentoses (reviewed by Mielenz, 2001). The ideal bioethanol-producing strain would ferment all sugars simultaneously whilst being resistant to inhibitory by-products. This situation has been approached by cloning endoglucanase genes from *Erwinia* into *Klebsiella* species (Zhou and Ingram, 1999).

16.5.5 Organic acids

Since the early 1990s, food and food ingredient markets have been characterised by a dynamic development not seen before. The requirements for food processing are increasing. The ageing population, predominantly in Western countries, also adds to an increased demand for high-quality foods. Consequently, the demand for specific ingredients used in preparing high-quality and preserved foods also increases (BCC, 2002). Of outstanding importance in this sector are ascorbic acid (L-3-ketothreohexuronic acid lactone), isoascorbic acid (D-erythro-Hex-2-enonic acid γ-lactone) and

citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid). Ascorbic acid, in addition to its role in preservation is vitamin C, which is of increasing importance as scientific data support its beneficial effect on human health. Isoascorbic acid is the isomer of ascorbic acid and is very popular for the preservation of meat and fish products. Citric acid is an organic acid of diverse economic uses. It is a standard acidulant and the food and beverage industries use it extensively as a food additive. It is also able to complex heavy metal ions, like iron and copper, and is therefore applied in the stabilisation of oils and fats.

Citric acid is produced by fermentation and *Aspergillus niger* is the most popular strain for citric acid production on an industrial scale. The worldwide estimate of production in 2000 was 9 million tonnes (Karaffa and Kubicek, 2003), with an annual 2–3% increase. Carbon sources used should be cheap and available such as agricultural waste and waste papers. The basic substrates for citric acid fermentation using a submerged technique of fermentation are beet or cane-molasses (Pazouki *et al.*, 2000). The biochemical pathway leading to citric acid hyperproduction was discovered 50 years ago when Cleland and Johnson (1954, reviewed by Karaffa and Kubicek, 2003) demonstrated that citric acid biosynthesis involves the glycolytic catabolism of glucose to two moles of pyruvate, of which one is converted to acetyl-CoA (by releasing one mole of carbon dioxide) and the other one to oxaloacetate (by fixing this one mole of carbon dioxide onto the second pyruvate) and then finally condensing these two precursors to citric acid. Genetic engineering of *Aspergillus niger* primary metabolism is employed to improve citric acid production by this fungus (Haq *et al.*, 2001), but the nutritional status of the organism and basic fermentation parameters have also been studied (Ali *et al.*, 2002).

16.6 Conclusion and future trends

The wastes from fruit and vegetable processing mostly remain underexploited although there is a potential for bulk-scale accessible carbohydrates and phenolics. Extraction of valuable polysaccharides such as pectins, cellulose and arabinans, production of monomeric components (sugars, phenolics) and processing of dietary fibre, are up until now the main ways to upgrade these residues. New regulations, which will appear in the near future to protect our environment, as well as economic reasons for adding value to these wastes will stimulate the industry to minimise wastes and to find diversified applications for the components of wastes. Basic research on sugars and phenolics is necessary to explore new chemical and biochemical conversions using up-to-date (green) chemistry and progress in enzymology/biotechnology. Enzyme modification or bioconversion of polysaccharides and oligo-/monosaccharides or phenolics is important to produce more specific modifications in relation to the product properties.

More research, including genetic engeering, is also necessary to improve the capability of depolymerising enzymes to completely or selectively degrade cell-wall-rich wastes. These research efforts will bring progress in the use of these wastes, leading to large-scale production (for example, of ethanol); in addition, new products may emerge such as useful industrial intermediates, high-value chemicals, surfactants and cosmetics.

16.7 Sources of further information and advice

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17

High-value co-products from plant foods: nutraceuticals, micronutrients and functional ingredients

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17.1 Introduction

Epidemiology has shown that plant food products (and especially fruits and vegetables) have a positive role in protecting against cardiovascular diseases and cancer (Rimm *et al*., 1996; Doll, 1990). This effect has been associated with the content of several secondary metabolites, also named as phytochemicals or phytonutrients, that have relevant biological activities, especially when consumed regularly as part of the diet.

Clinical studies also support the role of the phytochemicals as health-promoting food constituents (Kris-Etherton *et al*., 2002; Scalbert *et al*., 2002). The role of antioxidant phytochemicals in the prevention of these diseases has been mainly attributed to the prevention of low-density lipoprotein (LDL) oxidation (Rankin *et al.*, 1993; Scalbert *et al.*, 2002) through a scavenging activity against peroxyl and hydroxyl radicals (Rankin *et al.*, 1993), although these constituents can exert their biological activity by many other mechanisms (Johnson, 2002; Finley, 2005). Extracts containing phytochemicals, and especially those obtained from food products, are actually used in the preparation of nutraceuticals and functional foods.

Secondary metabolites have a role in the plant that is related to the plant's defence against external aggressions (ultraviolet light irradiation, microbial attacks, insect feeding deterrent, etc.), they also act as visual ecological markers in flowers and fruits (attract pollinators and animals for seed dispersal); for these reasons they mainly accumulate in external tissues (Harborne, 1982). They are also responsible for different food quality characteristics – including colour, flavour and aroma of fruits and

vegetables and related products (wines, juices, etc.) (Tomás-Barberán and Espín, 2001). In addition, due to their antioxidant properties these metabolites could be used as food ingredients to prevent rancidity. Many of them occur in the vacuoles of epidermal cells, and in some instances they are excreted to the tissue surface together with waxes and resins (Wollenweber and Dietz, 1981).

During fruit and vegetable handling and processing the external tissues are usually removed and discarded, being one of the main sources of waste generation. Fruit peels and external leaves of vegetables are generally the richest in phytochemicals, and therefore the plant food industry residues constitute an excellent source for the preparation of phytochemical extracts with potential use in the preparation of functional food ingredients and nutraceuticals (Schieber *et al.*, 2001; Laufenberg *et al.*, 2003; Tomás-Barberán *et al.*, 2005).

In this chapter we evaluate the actual trends and real possibilities of using plant food residues produced in the packing houses and the food industries as a source of high-value phytochemical extracts that could be used in the preparation of functional foods, nutraceuticals and food ingredients.

17.2 Residues generation and key reasons for co-product recovery

The waste portion in the processing of some fruits and vegetables can be as large as 70% of the harvested material, as is the case of artichokes, passion fruits and some lettuce cultivars. In leaf vegetables (lettuce, spinach, Swiss chard, etc.) the external leaves are often removed as they are too hard and green and often have some defects (bruises, cuts, etc.). In other products, the edible portion is the flower (or the flower heart, as in the case of artichokes) and in this case the leaves, stems and some parts of the flower (the external and harder bracts) are discarded. In celery only the stems, especially the whiter ones, are selected, and the leaves and greener and thinner stems constitute waste product. In onions, the residues are external membranes and sometimes scales. The peels are frequently wastes, as in the case of most fruits, potatoes, tomatoes, etc. In other products the wastes are the fruit husks (banana, citrus, pomegranate, etc.) and shells (pistachio, almonds, walnuts, etc.). The fruit stones are also wastes from fruit processing (peach, apricot, etc.). After fruit pressing for juice, wine and oil production, the press-cake constitutes a relevant waste (grapes, berries, olives, etc.). In some cases the wastes generated are the waters of industrial processing (brines, blanching waters, cooking waters, etc.), and these are generally rich in secondary metabolites.

There is a need to decrease waste production as environmental legislation becomes more and more restrictive, and the trend is to take waste

generation to levels as close as possible to zero. The use of wastes as an animal feedstuff is a common practice, being the main use for plant food industry residues (Martínez-Teruel *et al.*, 1998; Iñiguez *et al.*, 2001). Bio-fuel (Hang, 1987) and compost production (Vlyssides *et al.*, 2004), as well as the preparation of dietary fibres (Femenia *et al*., 1998; Marconi *et al.*, 2000), are also alternatives already in use. The volume of waste production is increasing with agricultural production and processing. The use as animal feedstuff has some limitations as the wastes are generally very rich in water and are highly perishable, fungal development is very likely to occur with the risk of mycotoxin production (Tomás-Barberán *et al.*, 2005). These mycotoxins could then pass to the animal tissues and therefore to man. The use of waste for composting also has some applications, and composts and manures from different wastes are available on the market. These waste tissues are also rich in bioactive secondary metabolites, and therefore there is an opportunity for the extraction of high-value compounds from wastes. Once they are extracted the residues could still be used for animal feeding or for composting or fuel production.

In addition, there is a demand for functional foods (foods with specific physiological effects in humans) and nutraceutical preparations both from society and the industry (Roberfroid, 2000), and the phytochemicals present in these plant food residues can be an excellent raw material for the preparation of these extracts.

17.3 Phytochemicals present in plant food residues

The main groups of plant secondary metabolites that are suitable for use in nutraceuticals or as functional food ingredients are the terpenoids, the polyphenols and the organosulphur compounds.

The terpenoids (Fig. 17.1) are biosynthesised by the acetate–mevalonate pathway and constitute a group of lipophilic compounds with a wide range of molecular weights, biological properties and potential uses (Bartley and Scolnik, 1995). The monoterpenes (10 carbon atoms) are generally volatile compounds that are constituents of the essential oils (i.e. limonene) and are key compounds in the flavour of some fruits. Some of them have relevant biological properties. The sesquiterpenes and diterpenes (15 and 20 carbon atoms, respectively) are less relevant in food, while the triterpenes (30 carbon atoms) are relevant phytosterols (ergosterol, campesterol, sitosterol, etc.) and the tetraterpenes (40 carbon atoms) include carotenoid pigments with interesting biological properties (lycopene, β-carotene, β-cryptoxanthin, etc.).

Polyphenols (Fig. 17.2) constitute a very large group of secondary metabolites characterised by the presence of phenolic hydroxyls in the molecule. They are biosynthesised from acetate by the shikimate pathway

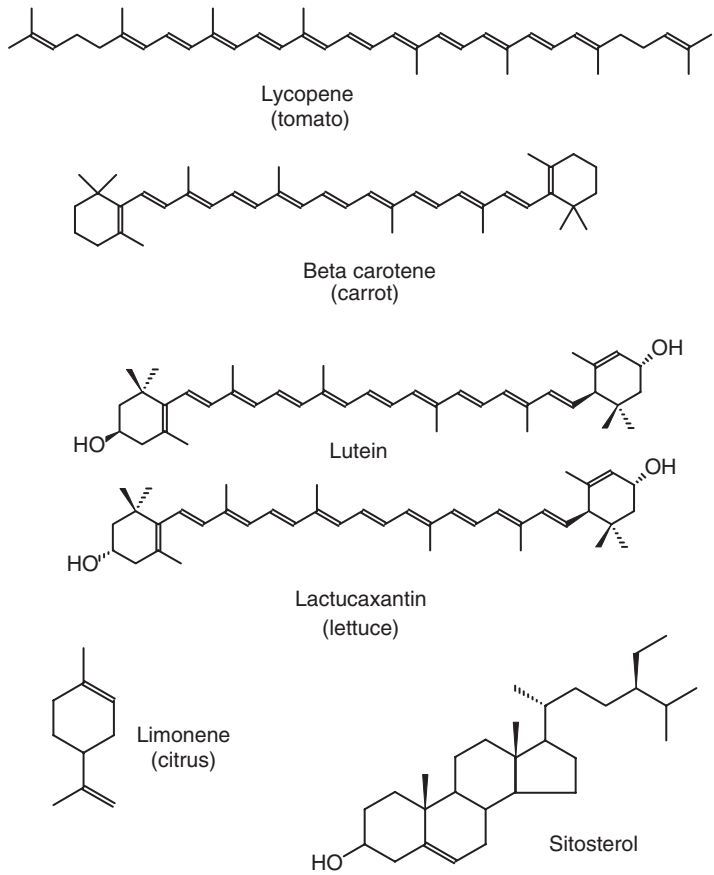

Fig. 17.1 Terpenoids from plant food wastes.

(Dixon and Paiva, 1995). They can be as simple as hydroxyl-benzoic acid and as complex as large procyanidin polymers with molecular weights of several thousand mass units. Their chemical and biological properties are also very wide. Some of them are water soluble (some anthocyanins), while others are highly lipophilic (the citrus flavone tangeretin and the stilbenoid resveratrol). They have been classified as flavonoids (C6-C3-C6) and non-flavonoid compounds. Flavonoids include anthocyanins, flavones, flavanones, chalcones, isoflavones and procyanidins (flavan-3-ols). Non-flavonoid compounds include benzoic acid derivatives, hydroxycinnamates, ellagic acid derivatives and ellagitannins, and stilbenoids.

Organosulphur compounds (Fig. 17.3) are biosynthesised from sulphur amino acids and include the compounds of the Alliaceae (alliin and allicin) and those of the Brassicaceae (glucosinolates).

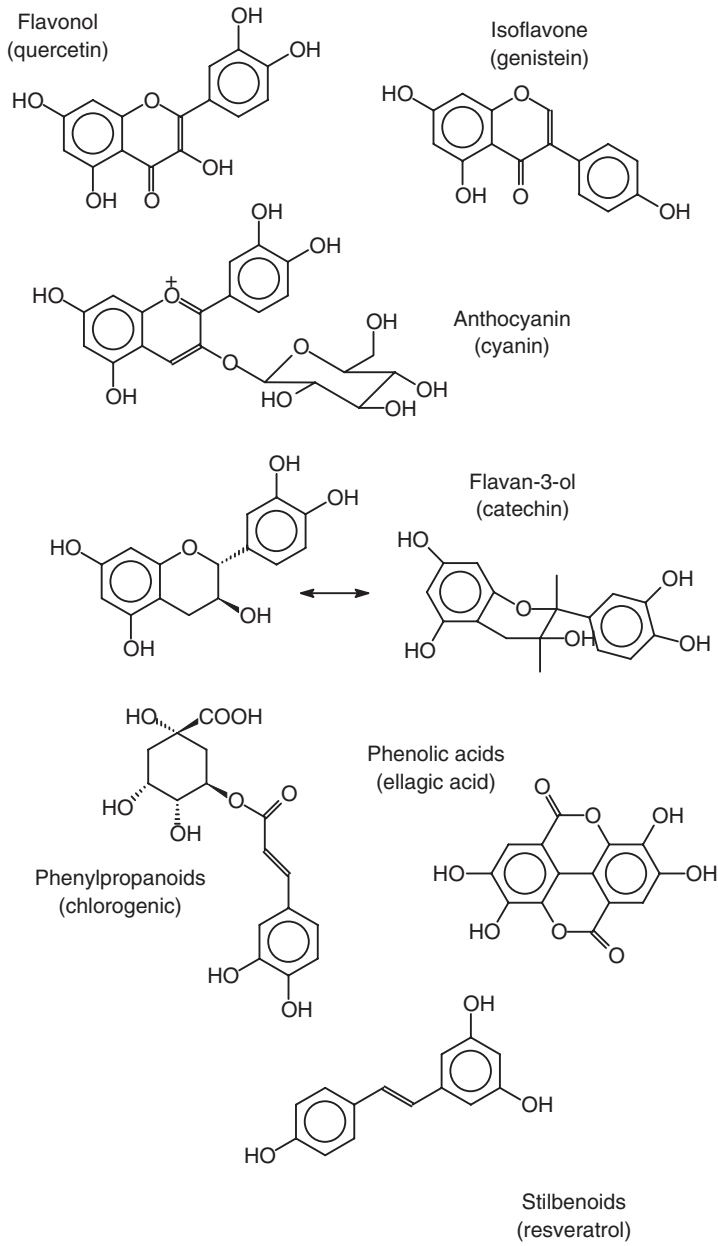

Fig. 17.2 Polyphenols from plant food residues.

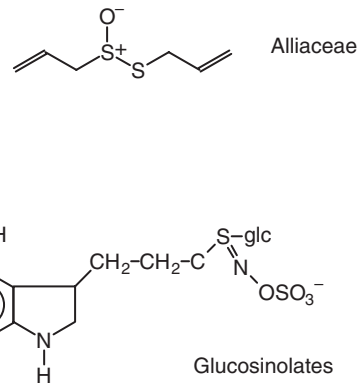

Fig. 17.3 Organosulphur compounds from plant food residues (glc) glucose.

17.4 Uses of plant food residues as sources for phytochemical extracts

Examples of the actual use of wastes from fruit processing industries to produce extracts that are available in the market include orange, grape, apple and olive residues. The residues from orange-juice extraction industries (orange albedo and flavedo, and fruit segments) have already been exploited for many years for the extraction of flavanones (hesperidin and related compounds) and pectin. Grape wastes from the wine-making industries (grape pomace and seeds) are also used industrially for the extraction of anthocyanin pigments, procyanidins and polyphenol extracts (Gabrielska *et al.*, 1997; Lu and Foo, 1999). From the olive-oil extraction industries the residues can also be used for extraction of hydroxytyrosol (Visioli *et al.*, 1999), the main phenolic antioxidant in olive oil, an efficient process for this purpose, has been patented and exploited industrially (Fernández-Bolaños *et al.*, 2002). From the cider industries, the apple pomaces are already used for extraction of pectins although phytochemical extraction has not yet been implemented. Recently research has been developed to use the tomato-juice production residues for the extraction of lycopene, a bioactive terpenoid pigment from tomato, and this has been studied by different groups using specific extraction techniques (Baysal *et al.*, 2000). The possible use of press-cake residues from berry-juice production (bilberry, blackcurrant and grapes) for the extraction of phytochemicals has already been explored (www.vtt.fi/virtual/maxfun).

The exploitation of residues from vegetable production and handling for extraction of phytochemicals is actually less developed (Tomás-Barberán *et al.*, 2005). Vegetable packing houses produce large amounts of wastes and residues (leaves, stems, etc.). These residues are very perishable products and their management is not always easy, they are also responsible for

environmental management problems in the industries. Minimising their environmental impact has been the subject of increasing concern in recent years. An interesting approach to give an added value to these materials is their use as sources of phytochemicals and natural antioxidant compounds, mainly phenolic compounds which in some cases have comparable activity to that of synthetic antioxidants (Azizah *et al.*, 1999; Lu and Foo, 2000). A number of by-products have been previously studied as potential sources of antioxidants (onion, carrot, potato peel) (Mouré *et al.*, 2001). However, as far as we know, there have been very few reports on the use of by-products from vegetables such as artichoke, cauliflower or lettuce as a possible source of antioxidant phenolics. Natural antioxidants are in great demand nowadays due to both consumer preference and health concerns associated with the use of synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxyanisole (BHA) (Oyeneho and Hettiarachchy, 1993; Azizah *et al.*, 1999).

Modern lifestyles have led to an increase in the demand for 'ready to eat' foods (canned, refrigerated, etc.) that generally contain smaller amounts of health-promoting compounds than the original fruits and vegetables (a large part of these metabolites have been removed during processing). Functional foods aim to promote healthy dietary habits by providing foodstuffs with health 'added-value'. These foods add new ingredients that increase the health-promoting properties of traditional products, or increase the bioavailability of active compounds (Roberfroid, 2000). In this context, addition of phytochemicals – enriched extracts derived from by-products – could be a feasible strategy to develop functional foods and at the same time would contribute the valorisation of these by-products (Schieber *et al.*, 2001).

17.4.1 Potential uses of high-value co-products

The phytochemical extracts can be used either for their biological properties as ingredients for nutraceutical preparations or functional foods, or for their food-quality-related properties – which include antioxidant properties, colour properties (pigments) and flavour properties (Tomás-Barberán and Espín, 2001). The health-related uses of phytochemical extracts include their use as ingredients for nutraceutical preparations or functional foods.

17.4.2 Nutraceuticals

Examples of these would be pills or capsules containing olive waste extracts or grape seed extracts. In many ways they are pharmaceutical-like forms. Although they are produced from food products, and the active phytochemicals are constituents of foods normally consumed in the diet, they are concentrated and presented in pharmaceutical forms in which the active principles are present in higher amounts. The concentration and pres-

entation as pharmaceutical forms affect their bioavailability and bioactivity. They are generally prepared as vials, capsules, pills, elixirs, solutions, etc.

17.4.3 Functional foods

In this case the extracts constitute ingredients that are added to food. Two different strategies can be followed. One related to 'food enrichment' and another to 'novel formulations'. In the 'food enrichment' strategy the extracts are added to food of the same origin. Examples include: the addition to a salad lettuce of a sauce enriched with extracts from the waste external leaves that are much richer in phytochemicals; a grape or berry juice in which the extracts obtained from the press-cake wastes are incorporated into the juice to produce a 'whole-bran' juice; olive oils in which the phenolic antioxidants remaining in the residues are incorporated into the oil, etc. In the 'novel formulation' approach extracts are added to different foods for increasing the phytochemical content (e.g. orange juice with added bilberry press-cake extracts will provide the anthocyanins and other polyphenols from berries in addition to the natural constituents of orange; milk may have extracts of soy bean isoflavones added). In some of these cases the new food products obtained should be considered 'novel foods'.

17.5 Important sources of high-value co-products

17.5.1 Fruit processing

Fruits are processed to produce fresh-cut products, an industry increasing steadily in importance, and this generates large amounts of residues (peels, stones, husks, etc.). Fruits are also used for the production of juices, wines and oils by pressing technologies, leading to press-cake residues generally very rich in bioactive secondary metabolites.

Apple

Residues are mainly generated in the juice and cider production industries. The wastes are in the form of pomaces, and include peels and seeds in addition to other solid tissue parts. The composition of these residues is very variable and depends largely on the apple cultivar processed. The phenolic content of the pomaces is very variable depending on: the content of the cultivar used for processing; the inactivation of polyphenol oxidases that lead to phenolic compound degradation and formation of brown polymers; and the use of external enzymes (pectinases) for increasing juice yield. The main use of pomaces is for pectin extraction. Other potential uses would be the extraction of phenolic compounds. Flavan-3-ols (catechins and procyanidins), hydroxycinnamates, dihydrochalcones (phloretin glycosides) and flavonols (quercetin glycosides) are the main phenolic compounds present in apple wastes. Once the oxidative enzymes are inactivated

an enhanced release of phenolics by enzymatic liquefaction with pectinases and cellulases represents an alternative that has been explored (Will *et al.*, 2000). Recently, a method for the combined recovery of pectins and polyphenols from apple pomace has been established (Carle *et al.*, 2001).

Grape

Grape pomace (from the wine and must industries) amounts to more than 9 million tons per year (Schieber *et al.*, 2001). A great range of products such as ethanol, tartrates, citric acid, grape seed oil, hydrocolloids and dietary fibre are recovered from grape pomace. Polyphenol extracts are also produced and commercialised from residues. Anthocyanins, catechins, flavonol glycosides, and phenolic acids are the principal phenolic constituents of grape pomace. The content of the stilbenoid resveratrol, although small, is relevant as the price of the extracts often depends on the content of this minor constituent due to its demand and biological activity. Grape seed extracts rich in procyanidins with different degrees of polymerisation are traded, as well as fibre with antioxidant activity due to its polyphenols and white grape skin extracts rich in flavonols in addition to procyanidins.

Peach and apricot

These fruits are used for juice production and pomaces are produced but not yet used for phytochemicals extraction. The pomaces are rich in fibre and in polyphenols (mainly procyanidins and flavonols, and hydroxycinnamates in smaller amounts), and in some cultivars they are rich in carotenoids (β-carotene). Only fibre is industrially produced today and can be found in the market although phenolic and carotenoid extracts could be alternative extracts for the future.

Citrus

The use of citrus by-products is a traditional industry and was reviewed 10 years ago by Braddock (1995). Essential oils are directly produced in the juice production plants as a co-product and have a market. The main wastes include the citrus peels and residues from segments and seeds after pressing. These residues are rich in pectins, and flavonoids (flavanones) and limonoids are present in minor amounts. Flavanones are generally extracted in alkaline water, the extracts are then acidified and the flavanones, which are slightly soluble in water, precipitate. There are differences in the flavonoid content of different citrus species and this can be used for production of specific extracts enriched in compounds with specific properties. Thus, grapefruit is rich in bitter naringin that can be further chemically transformed into the intensely sweet dihydrochalcones. Lemon is rich in eriocitrin, a flavanone with higher *in vitro* antioxidant activity than flavanones from other citrus species; mandarins and tangerine peels contain polymethoxylated flavones (tangeretin, sinensetin, nobiletin, etc.) that have a relevant anticarcinogenic activity.

Mango
The residues include peels and stones. These are produced both in the juice and fresh-cut industries. The juice kernels are rich in gallic and ellagic acids as well as gallotannins and condensed tannins. The peels are rich in flavonols and carotenoids. These extracts are not produced as yet.

Pineapple
Processing to produce juice and fresh-cut pineapple leads to wastes that contain pulp residues and external parts of the fruit. Hydroxycinnamic acid derivatives (sinapyl glutathione etc.) can be produced from pulp residues, and processes for their recovery have been established (Wrolstad and Ling, 2001) although these extracts are not available in the market.

Banana
This fruit can be processed to obtain juice. The peels are around 30% of the fruit and constitute an interesting source of procyanidins (mainly polymeric). The polyphenol oxidase activity has to be inactivated before or during extraction to avoid browning and polyphenols degradation. Other agricultural wastes are the bracts which are especially rich in anthocyanins (delphinidin, cyanidin, pelargonidin, peonidin, petunidin and malvidin) (Pazmino-Durán *et al.*, 2001). In addition, carotenoids (xanthophylls) esterified with myristate, laurate, palmitate and caprate have also been reported (Subagio *et al.*, 1996).

Guava, papaya and passion fruit
These tropical fruits also provide a good source of wastes that could be used for extraction of bioactive compounds. Some seeds contain glucosinolates. Especially relevant are the residues of passion fruit processing that constitute more than 75% of the harvested fruit (Schieber *et al.*, 2001). Additional studies are necessary to evaluate the content of phytochemicals in these residues, and their biological activity and extraction and preparation methods.

Kiwifruit
This is processed to yield juice, leading to a waste that mainly contains peels and seeds. In addition, this fruit is also prepared as fresh-cut product, and in this case the residues are the peels with part of the flesh tissue. These residues would be a reasonably good source of phenolic acids (benzoic acid derivatives), flavanol monomers and oligomers, and flavonols (Dawes and Keene, 1999).

Pomegranate
Pomegranate juice has been introduced in Western markets (especially in the USA) over the last few years, although this juice was traditionally produced in Mediterranean and Middle East countries where it is appreciated

for its aphrodisiac and health-related properties. In the USA it is traded with a label indicating its high *in vitro* antioxidant capacity, which has been reported to be higher than that of red wine and green tea (Gil *et al.*, 2000). During processing the husks, membranes and seeds constitute a residue that can account for more than 50% of the harvested fruits. This residue is rich in ellagitannins (mainly punicalagin isomers) that are water soluble and easily extracted from the residues (Gil *et al.*, 2000). In addition, the membranes are a source of procyanidins, and the seeds contain interesting oils with interesting conjugated unsaturated fatty acids with relevant biological activity (Kohno *et al.*, 2004).

Berries

Different berries are processed as juices, and the press-cake constitutes a relevant by-product rich in phytochemicals. In blueberry and bilberry (and other *Vaccinium* species) the residues are especially rich in anthocyanins and flavonols, and also procyanidins in smaller amounts, this being an evident source for extract preparation. The extracts are already traded and have a place in the dietetic and pharmaceutical markets. Raspberries and strawberries are other berries that produce phenolic-rich residues after juice production (Aaby *et al.*, 2005). These residues also contain flavonols, anthocyanins, procyanidins, and ellagic acid and ellagitannins, all of which are compounds with biological activities and a potential position in the nutraceutical and functional food market. Currants (*Ribes* species, red and black) also produce phenolic-rich residues that could be used in the same way.

17.5.2 Vegetable processing

Vegetables are processed as fresh-cut products to produce salad mixes, as ready to cook preparations (spinach, potato, etc.) and also as juices (tomato and carrot), frozen vegetables, canned products, etc. In this case the residue generation in the packing houses that trade in fresh products is also relevant as many vegetables are trimmed before despatch, packed in trays covered with plastic films.

Tomato

Tomato pomace consists of the dried and crushed skins and seeds of the fruit. This residue is rich in lycopene (from skins), and other carotenoids (β-carotene). Phenolic compounds are also present in relevant amounts, these being hydroxycinnamic acid derivatives, flavonols (quercetin derivatives), flavanones and naringenin-chalcone. Supercritical CO_2 extraction of lycopene and β-carotene from tomato paste waste resulted in recoveries of up to 50% when ethanol was added (Baysal *et al.*, 2000). Enzymatic treatment enhanced lycopene extractability (Schieber *et al.*, 2001).

Carrot
A pomace is generated after juice production. This is rich in β-carotene, but phenolic compounds are also present (hydroxycinnamates and coumarins) (Cinar, 2005).

Red beet
This is consumed as a vegetable, as juice and as a food colorant. Though still rich in betalains, the pomace from the juice industry is disposed of as feed or manure. The peel is the richest in phenolics and other compounds (betacyanins and betaxanthins); it also contains coumaric and ferulic acids as well as cyclodopa glucoside derivatives (Schieber *et al.*, 2001).

Potato
Aqueous peel extracts are rich in phenolic acids, especially chlorogenic, gallic, protocatechuic and caffeic acids. Methods for complete separation of steroidal alkaloids from phenolic compounds prior to their use in foodstuff would be desirable to avoid any risk for human health.

Lettuce
The development of the fresh-cut lettuce industries during the last few years has led to increased waste production that includes external leaves, stems and whole low-quality lettuce heads. The greener lettuce tissues (external leaves) are richer in flavonoids and phenolic acids and could be used as a source for phytochemicals extraction. By-products from lettuce (*Lactuca sativa* L.) varieties (romaine, iceberg and baby) and one chicory (*Cichorium endivia* L.) variety 'escarole' have recently been evaluated for their polyphenolic content as well as their antioxidant capacity, with interesting results (Llorach *et al.*, 2004). The phytochemical profile of lettuce by-products is composed of hydroxycinnamic acids (both caffeoylquinic and caffeoyltartaric acid derivatives) and flavonoids (both flavones and flavonols). The main hydroxycinnamic acid derivative identified was dicaffeoyltartaric acid (chicoric acid) followed by chlorogenic acid (5-*O*-caffeoylquinic acid). In addition, different isomers of isochlorogenic acid (3,5-*O*-dicaffeoylquinic acid) were identified. The flavone luteolin-7-*O*-glucuronide was identified, and – for the quercetin derivatives – quercetin-3-*O*-glucoside, quercetin 3-*O*-glucuronide and quercetin 3-*O*-(6-*O*-malonyl)-glucoside were identified. Regarding chicory by-products, the high-performance liquid chromatography (HPLC) analyses of raw extracts showed a kaempferol 3-*O*-glucoside as the main flavonol and this compound has already been reported in chicory.

Lettuce by-products have shown an interesting antioxidant capacity with both free radical scavenging activity and the capacity to reduce $Fe^{(III)}$ to $Fe^{(II)}$ (Llorach *et al.*, 2004); this has been applied to functionalise food products (Llorach *et al.*, 2005).

Artichoke

Artichoke heads are generally processed in the industry to produce artichoke hearts; this results in large quantities of waste including external bracts, stems and in some cases leaves. This waste can reach up to 70% of the harvested product depending on the processing method and the quality of the finished product. In addition, the blanching waters constitute a processing effluent that is rich in phenolic acids (hydroxycinnamic acid derivatives; cynarin, chlorogenic and isochlorogenic acids) and should be considered for its potential in the preparation of nutraceutical extracts (Tomás-Barberán *et al.*, 2005).

These by-products have also been studied concerning their application for animal feedstuffs (Martínez-Teruel *et al.*, 1998) and fibre production (Femenia *et al.*, 1998; Goñi and Saura-Calixto, 1988). The artichoke by-products are a very good source of antioxidant polyphenols with caffeic acid derivatives as the main phenolic compounds (Llorach *et al.*, 2002). The antioxidant activity has been proven with different assays, showing a special capacity to prevent the peroxidation of linoleic acid (Llorach *et al.*, 2002).

Onion

Onion handling activities produce wastes that include the external membranes. In the fresh-cut industry, scale tissues are also included in the residues. These external tissues are the richest in onion flavonoids, including different quercetin glucosides, the bioavailability of which has been demonstrated (Erlund, 2004). In addition, the residues can be a good source of organosulphur compounds that also have relevant biological activities (Tapiero *et al.*, 2004).

Brassicaceae

During the handling of broccoli, cauliflower, cabbages and Brussel sprouts for the fresh market, for fresh-cut processing or for freezing, wastes are generated that include leaves, stems, low-quality florets, etc. The wastes are a good source of highly glycosylated flavonoids, hydroxycinnamates (mainly sinapic acid derivatives) and glucosinolates (Vallejo *et al.*, 2004). The content of these metabolites is very variable depending on the nature of the tissue and the ripening stage.

Cauliflower by-products (*Brassica oleracea* L. var. *botrytis*) mainly consist of leaves and, in lesser amounts, stems. Regarding the edible portion of the cauliflower, this is rather poor in phytochemicals and only small amounts of some hydroxycinnamic acid derivatives – such as caffeic, sinapic and ferulic acids – have been identified and quantified (Llorach *et al.*, 2003a).

The HPLC analysis of cauliflower by-product extracts revealed the presence of both flavonoids and hydroxycinnamic acids (caffeic acid and sinapic acid). Different combinations of flavonols such as kaempferol and quercetin with sinapic acid and glucose have been identified with the main compounds being kaempferol-3-*O*-sophoroside-7-*O*-glucoside

and its sinapoyl derivative (kaempferol-3-*O*-(sinapoylsophoroside)-7-*O*-glucoside). Moreover, some flavonoids with an unusually high grade of glycosylation (five sugar moieties) have been isolated and tentatively identified for the first time (Llorach *et al.*, 2003a). To our knowledge, the characterisation of flavonoids with more than four sugars has not been previously reported.

The cauliflower by-products showed a relevant antioxidant capacity, estimated from their ability to reduce TPTZ(2,4,6-tripyridyl-*S*-triazine)-$Fe^{(III)}$ complex to TPTZ-$Fe^{(II)}$. In this way, 16 g (dry weight) of cauliflower by-products can provide the same antioxidant capacity as one cup of tea or one glass of red wine (Llorach *et al.*, 2003b).

Celery
This is minimally processed to be marketed as fresh sticks. The residues are composed of leaves and greener and thinner stems. The residues are very rich in celery aroma compounds, flavones (apigenin derivatives) and hydroxycinnamates.

Legume residues
During the preparation of legumes, especially in the freezing industries, the external tissues of the pods are discarded. These residues are particularly rich in flavonoids (mainly flavonols, and special mention should be given to pea flavonols (Ferreres *et al.*, 1995), beans and broad beans). The pods can also be a very good source of procyanidins and in some cases isoflavones. More research is needed on this still relatively unexplored topic (e.g. lentils, chickpeas, peanuts, etc.).

17.5.3 Other processes

Different industrial processes for plant food products yield large amounts of wastes that could also be used for phytochemicals extraction. Among them, oil extraction and nut preparation deserve a special mention due to the large volume of wastes generated and their phytochemical content. Olive-oil extraction wastes and nut hulls are of particular importance. In addition, the brines of pickles, capers and olives are also waste effluents that contain significant amounts of phytochemicals that could be used (Inocencio *et al.*, 2000).

Olive-oil extraction
In the past, the by-products resulting from olive-oil extraction were the waters (vegetation water, black water or vegetable water) and the olive husk, including skins and stones. Recently both wastes have been mixed to produce a single by-product. These wastes are rich in antioxidant compounds and particularly in hydroxytyrosol and related derivatives, and oleuropein (Fernández-Bolaños *et al.*, 2002). Flavones are also present as

well as hydroxycinnamate derivatives. Hydroxytyrosol is probably the most relevant compound in terms of its biological activity, and extracts enriched in this compound have a very promising market. The price of the extracts obtained is generally related to their hydoxytyrosol content.

Nuts and cereals preparation
Nut shells and cereal bran are also produced as wastes and can be good sources of some phytochemicals. In this case the compounds can be directly extracted or extracted after chemical treatment to release them from the cell walls. Benzoic and hydroxycinnamic acids are the main compounds, but ellagitannins can also be extracted from some by-products.

Brines
The effluents from the preparation of olives and capers and other similar products are highly contaminant and difficult to handle due to their high salt content. These brines also contain flavonoids and other phenolic compounds. In the case of caper brines, a large amount of quercetin derivatives (quercetin rutinoside and rhamnosyl-rutinoside) could be recovered from the brines (Inocencio *et al.*, 2000). In the case of olive brines, other compounds such as hydroxytyrosol, flavones and hydroxycinnamates could be recovered. Further research is needed to evaluate the potential of these by-products and to achieve salt recovery to avoid environmental contamination.

17.6 Examples of phytochemical extracts from plant food wastes

Different extracts obtained from wastes are already in the market or are potential candidates to be in the market soon. From the olive-oil industry wastes, extracts enriched in the antioxidant hydroxytyrosol are already prepared and marketed, as antioxidants for food preservation or as ingredients for functional foods or nutraceuticals (Fernández-Bolaños *et al.*, 2002). Pomegranate tannins from fruit husks (a mixture of punicalagins and other ellagitannins) can be easily prepared and introduced to the market due to the nutraceutical properties of these metabolites (Gil *et al.*, 2000). Anthocyanin-rich extracts are prepared from grape residues produced in the wine industries. Alternatively, extracts from press-cake residues from the berry-juice industries are also being marketed and these extracts provide, in addition to pigments, an excellent source of flavonols and other phenolics (www.vtt.fi/virtual/maxfun). Procyanidin extracts from grape seeds are already available in the market, but procyanidins from other residues will also be good alternatives with different profiles of polymeric forms that will give different biological properties and bioavailability. Lycopene

extracts from tomato are also available in the market. Different qualities can be found related to the lycopene content. Extracts from artichoke residues or artichoke blanching waters are rich in hydroxycinnamates; these extracts are already being produced and marketed. During the extraction and concentration of the extracts some caffeoyl-quinic derivatives are partially transformed to isomers that are only present as traces in the natural plant material (Gil-Izquierdo *et al.*, 2001). The extracts prepared from the brines of caper preparation have a high content of quercetin glycosides and in addition some quercetin aglycone (Inocencio *et al.*, 2000).

Extracts containing large amounts of citrus flavanones are obtained from the wastes of the citrus-juice industries (Braddock, 1995). Different flavanone profiles can be obtained depending on the citrus species. Sweet oranges produce extracts in which hesperidin is the main constituent, while neohesperidin is the most characteristic of sour oranges, naringin of grapefruits and eriocitrin of lemons. Many applications for these extracts have been reported, both for their biological activity and for their potential uses as food antioxidants.

From onion residues, flavonol (quercetin) extracts can be produced although these are not yet on the market (Erlund, 2004). Grapes are also a source for stilbenoids (resveratrol and piecatannol). Their content in grapes is very small but can be concentrated in the extracts. Several strategies can be used to increase the resveratrol content of grapes before extract production (Cantos *et al.*, 2001). Glucosinolate extracts could also be prepared from Brassicaceae wastes; these are not yet available in the market. A combination of glucosinolates with flavonols and hydroxycinnamates could be a reasonable approach for extract preparation from broccoli wastes (Tomás-Barberán *et al.*, 2005).

17.7 Technological processes for phytochemicals extraction from residues

Knowledge of phytochemical solubility, chemical and biochemical stability, and tissue localisation are essential in order to develop the extraction techniques and the technological processes for the preparation of high-value extracts.

As a general rule phytochemicals are poorly soluble in water, and are better extracted with organic solvents. For the more polar compounds mixtures of alcohols and water are the solvents of choice (Harborne, 1973). For the most lipophilic phytochemicals, the use of less polar solvents – such as ethyl acetate, petrol or ether – is essential. For some kinds of compounds, the solubility in water can be increased by changing the pH. Thus acidic compounds, such as polyphenols, can be extracted in water at alkaline pH

where the phenolic hydroxyls are ionised. More basic compounds, such as nitrogen-containing metabolites, can be extracted in water under acidic conditions.

Taking into consideration the nature of the residues, the extraction processes used for the preparation of these phytochemical extracts have to meet some requirements. Firstly, it is preferable to use fresh raw materials for extraction, as a drying process, although allowing storage, leads to unacceptable increases in production costs. It is also necessary to use food-compatible solvents, for example water, ethanol, methanol or a mixture of these. Thermal treatments are generally necessary to inactivate enzymes that can degrade the phytochemicals during the extraction process (Tomás-Barberán and Espín, 2001). Supercritical fluid extraction (SFE) using CO_2 in supercritical state, and frequently with organic modifiers added to enhance phytochemical solubility, has been proposed as another feasible methodology for the preparation of extracts. This technology could be especially useful for the extraction of lipophilic phytochemicals. The cost of this extraction methodology is higher than the technologies referred to above, but can have applications for the preparation of high-value phytochemicals with the advantage that it can prevent the extraction of flavour and aroma compounds, thus widening the range of applications for the obtained extracts.

In some cases, the phytochemical yield extracted from residues can be enhanced by adding enzymes such as pectinases or cellulases (Cinar, 2005; www.vtt.fi/virtual/maxfun).

The extracts obtained usually need to be concentrated before use. Spray drying and freeze drying are feasible technologies that could be applied depending on the market price of the extract produced. In some cases, extract purification through non-ionic polymeric resins (of the type Amberlite XAD) can be used to concentrate the phytochemicals before drying (Tomás-Barberán *et al.*, 1992). In these cases, the water extract (or a water solution of the ethanol–water extract) is filtered through the resin column and the phytochemicals are retained in the stationary phase. Then these compounds are eluted with ethanol, and this extract concentrated. The extracts obtained are generally prepared in dried form although preparation as liquid extracts and concentrates is another possibility. These extracts can then be used for the preparation of pills or other nutraceutical preparations (dried extracts) or to manufacture functional juices (Larrosa *et al.*, 2002) or other new foods – such as soups, sauces and margarines (Llorach *et al.*, 2005) – to which liquid or dried phytochemical extracts can be added.

Extract stabilisation or preservation is needed to prevent phytochemical degradation. Many of these compounds are antioxidants and are therefore easily oxidised by the oxygen in the atmosphere especially when stored in solution. The use of nitrogen atmospheres or addition of other antioxidants (ascorbic acid etc.) can be necessary to stabilise the extracts.

17.8 Safety issues

A topic of concern is safety. It is essential to make sure that the pesticides and other agrochemicals are not concentrated in the extracts in the same way the phytochemicals are (Tomás-Barberán *et al.*, 2005). It is therefore essential to carry out routine analysis of pesticides in all these products. It is also important to establish the risk/benefit balance of using these phytochemical extracts for health-related purposes as these are biologically active compounds and therefore potentially toxic at high doses.

In addition to the chemical risks, there are also biological risks related to these phytochemical extracts. One such risk is related to mycotoxin contamination of the extracts due to fungal growth in the residues before extraction. Another is related to the presence of microbial contamination in the extracts if thermal treatments are not used during the extraction process.

17.9 Future trends

The use of these extracts, however, presents some additional concerns. The first one is the market. Before producing these phytochemical extracts from agri-food residues it is essential to evaluate the potential market and price for these products.

In addition it is necessary to control the content of the bioactive phytochemicals in the extracts by appropriate analytical methods. It is not unusual to find pills, extracts and other preparations on the market that are based on specific bioactive compounds but in fact only contain trace amounts of these bioactive phytochemicals. This is the case of many grape extract preparations that claim a significant content of resveratrol, when the real content is very small or is even undetectable. It is necessary to give figures for the content of the main bioactive components on the product label (functional food or nutraceutical).

The biological activity of these phytochemical extracts needs to be demonstrated by *in vivo* studies and clinical assays; the bioavailability of many phytochemicals is rather low and in many cases the natural compounds are transformed into other metabolites by the gut microflora, and these metabolites, but not the original phytochemicals, are then absorbed and circulate in plasma to reach the target tissues where the biological action takes place.

7.10 Conclusions

The wastes and residues of the fruit and vegetable industries constitute an interesting source of phytochemicals that can be readily extracted by simple methods and can then be used for the preparation of different products

(functional foods, nutraceutical preparations or food ingredients). It is, however, essential to guarantee the product safety (pesticide levels and risk assessment of increasing phytochemical concentration). The preparation of these extracts is technologically feasible and they can be obtained at relatively low cost. The biological activity of these extracts needs further research. The main objective of this field of research would be the recuperation of the health-promoting metabolites and quality-related compounds from horticultural products that are currently lost during handling and processing.

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18

High-value co-products from plant foods: cosmetics and pharmaceuticals

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18.1 Introduction

In recent years, there has been a marked increase in the amount of research and publications providing scientific evidence to support the hypotheses that phytochemicals in foods and in isolated form might provide health benefits to the consumer. This fact has impacted the food, health food and pharmaceutical industries, among others (Laufenberg *et al*., 2003).

The concept of growing crops for health rather than for food is slowly changing plant biotechnology and medicine (Raskin *et al*., 2002). The rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics which may include plant-derived pharmaceuticals, multicomponent botanical drugs, dietary supplements, functional foods, plant-produced recombinant proteins and plant-based cosmetics. Many of these products will soon complement conventional pharmaceuticals in the treatment, prevention and diagnosis of diseases, while at the same time adding value to agriculture.

Plant extracts have been widely used as topical applications for wound-healing, anti-ageing and disease treatments. Examples of these include ginkgo biloba, echinacea, ginseng, grape seed, green tea, lemon, lavender, rosemary, thuja, sarsaparrilla, soy, prickly pear, sagebrush, jojoba, aloe vera, allantoin, feverwort, bloodroot, apache plume, papaya and many others (Hulse, 2004).

This chapter does not pretend to detail 'all' the plant-derived compounds that have the potential to be used in the pharmaceutical and cosmetic fields, but to focus on three important groups of plant-derived components, namely: plant polysaccharides, phenolic compounds and plant-derived oils.

Actually, the incorporation of plant-derived 'bioactive' components in pharmaceutical and cosmetic products is a great focus of interest (Montgomery, 2004). Many studies have found enough scientific evidence to confirm the health-promoting properties of plant extracts. This fact combined with the perception that a number of common synthetic preservatives might have hazardous effects has led to multiple investigations in the field of plant-based natural compounds (Aburjai and Natsheh, 2003). There are considerable amounts of data that suggest the benefits of such ingredients in pharmaceutical and/or cosmetic formulations. Thus, the objective of this chapter is to review recent published data that support the usefulness and potential benefits of incorporating plant polysaccharides, phenolic compounds and plant oils in pharmaceutical and/or cosmetic products.

18.1.1 Plant-derived polysaccharides

Over the last decades, there has been considerable scientific interest in the active components of herbal drugs and/or medicinal plants and there is increasing evidence that many polysaccharides of plant origin are responsible for their bioactive properties. A wide range of bioactivities has been identified including anti-tumour activity, anti-viral activity, anti-bacterial activity, anti-complementary activity, anti-inflammatory activity, hypoglycaemic activity, anti-coagulatory activity, phagocytotic activity, anti-thrombotic activity, anti-ulcer activity and wound-healing properties.

Although most 'bioactive' plant polysaccharides have been utilised due to their pharmacological properties, their use in cosmetics as emollient natural remedies is also common. In general, products containing plant polysaccharides are capable of relieving dryness and providing a soothing membrane that covers the human skin.

18.1.2 Natural antioxidants: phenolic compounds

Antioxidants are of interest to the food industry because they prevent rancidity. However, they are also of interest to biologists, biochemists and clinicians, because they may help to protect the human body against damage by reactive oxygen species. A broad definition of an antioxidant is 'any substance that, when present at low concentrations compared with those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate'. The term 'oxidisable substrate' includes almost everything found in foods and in living tissues including proteins, lipids, carbohydrates and DNA (Halliwell *et al.*, 1995).

Phenolic compounds are the largest group of plant antioxidants and, according to Harborne (1990), one of the most important classes of plant chemicals. In terms of pharmacological activity, it is well known that phenolics act against the oxidation of high-density lipoproteins (HDLs). Hence, they help the body retain important HDLs while helping it get rid of

problematic low-density lipoproteins (LDLs) (Hagerman *et al*., 1998). In addition, plant phenolics have also been found to have anti-ulcer, anti-carcinogenic and anti-mutagenic activities. The reason behind these activities is the strong antioxidant power of phenolic compounds, since they are able to scavenge free radicals (Shi *et al*., 2005).

18.1.3 Plant-derived oils

All plants contain oils or fats, and mainly in their seeds. In most plants storage lipids are in the form of triglycerides (Murphy, 1990). There are a very few examples of alternative forms of storage lipid in higher plants. The most well known of these is the desert shrub, jojoba, which stores its seed lipid as a liquid wax.

Triglycerides from vegetable oils can be considered as important raw and renewable materials for the preparation of products useful for foods, pharmaceuticals and cosmetics (Barrault *et al*., 2002). Currently, products claiming to be rich in polyunsaturated fatty acids (PUFAs) are marketed in many countries of the world. Interestingly, the development of these products started around 1960, when the lipid hypothesis (dietary fat composition influences blood cholesterol levels and a change from saturated to more unsaturated fats, which lowers cholesterol, is beneficial for heart health) emerged. Medical groups approached the margarine industry to see whether they could provide products that could help consumers to achieve this change. These products were developed based on linoleic acid, the fatty acid with the strongest cholesterol-lowering effect. The initial pharmacological product (sold in pharmacies) was developed into a product for the total population over the ensuing years (Korver, 1997).

The use of many plant-based oils in cosmetics is generalised. The protection of the skin hydration, and the production of skin and hair preparations with softening effects is achieved using seed oils rich in fatty acids and triglycerides that reduce transepidermal water loss.

18.2 Key reasons for exploiting plant-derived compounds from co-products

The key reasons that support the incorporation of specific plant-derived compounds in pharmaceutical and cosmetic products are summarised below.

18.2.1 Recovery of by-products from the food and food-processing industries: environmental benefits

The food and food-processing industry produces considerable amounts of wastes, residues, effluents and by-products which contain important

amounts of potentially interesting compounds (Laufenberg *et al*., 2003). The disposal of these residues often creates an enormous environmental problem. Thus, a number of by-products and/or co-products of agricultural businesses are presently returned to the land as fertilisers or soil modifiers, fed to animals or fish as nutrients, burnt for energy or applied to value-added conversions. The finding of alternative uses for these natural residues, for instance in non-food areas such as pharmaceuticals and cosmetics with a high value added, might help to alleviate this situation. With the dramatic increase in biotechnological activity comes a concomitant responsibility to increase the capacity and sophistication of waste management systems.

18.2.2 Preference for natural products versus synthetic compounds: driving force for food, cosmetics and pharmacological fields

Growing apprehensiveness about the safety of synthetic commercial antioxidants has prompted great efforts to screen active and stable antioxidants obtained from natural sources (Peschel *et al*., 2006). Until now, most attention has been paid to oral administration of natural radical scavengers as food supplements. However, protection from hazardous species is not only of nutritional relevance. Neither are oxidation reactions an exclusive concern of the food industry. In fact, the customer's awareness of 'non-chemical' ingredients in health products has also to be faced by the cosmetic and pharmaceutical industry. These three sectors are drawn together promoting products named functional foods, food supplements, nutraceuticals and/or cosmeceuticals (Schieber *et al*., 2001).

18.2.3 Economic aspects of the recovery of highly valued compounds

The development of new co-products derived from inherently low-value (or poorly characterised) raw materials might be economically beneficial for the agro-food industry. However, many different problems need to be overcome in order to assure the success of these novel products in the market. For example, the industry needs to convince investors of the potential for financial gain from investing in the production of plant-based cosmetics and pharmaceuticals. Furthermore, it will need to carefully market new products so as to capture the interest of consumers, and, perhaps most importantly, to convince them of the benefits.

18.2.4 Biotechnological advances in the extraction processes

Currently, many extraction procedures for plant phytochemicals involve the use of organic solvents. With increasingly restrictive legislation, it is imperative that safe and efficient extraction processes are developed that

guarantee an appropriately pure plant extract or plant-derived compound. Therefore, the utilisation of relatively new and safe extraction techniques such as supercritical fluid extraction (SCFE) might help to solve this problem. Furthermore, the advantages of using CO_2 as the extraction agent – being low cost, non-toxic, non-flammable and non-corrosive – make it the perfect solvent for different natural products such as phenolics and vegetable oils.

18.3 Recovery of plant-based co-products for use in cosmetics and pharmaceuticals

A wide range of plant-derived compounds with health-promoting properties and/or their technological characteristics, is available for recovery and use in cosmetic and pharmaceutical formulations.

Plant-derived polysaccharides, vegetable oils and plant-based antioxidant phenolic compounds are three of the largest groups of natural compounds that offer such potential because of their relevant beneficial properties. In this chapter, the current literature has been surveyed and the recently recognised health benefits of these specific phytochemicals are reported; their sources, extraction procedures, potential applications and bioactivities are also discussed.

18.3.1 Selected groups of plant polysaccharides: sources and bioactivity

Potentially bioactive polysaccharides have been obtained from a wide range of plant sources. Their carbohydrate composition and structural features are typical of polysaccharides of gums and mucilages, of storage polysaccharides or of cell walls from prokaryotes, fungi, and lower and higher plants (Waldron and Selvendran, 1992).

Plant polysaccharides are a complex and heterogeneous group of compounds. They might have either storage (starch, inuline, glucomannans) or structural (cell wall polysaccharides) functions within the plant. Nevertheless, their chemical composition and structure play an important role in determining the functional properties and potential bioactivity of the polymers (Diallo *et al.*, 2003; Femenia *et al.*, 2003). Thus, plant polysaccharides can be classified in different groups according to their chemical composition and the structural arrangement of the component monosaccharides (Brett and Waldron, 1996).

Glucomannans (or mannans), pectic polysaccharides and glucans are three of the most important groups of plant-derived polysaccharides that have been associated with different bioactivities. Their functional properties, such as the ability to bind water and oil, may also contribute to their potential application in the cosmetics field.

Glucomannans/mannans

Glucomannans are carbohydrate polymers widely distributed in both hardwood and softwood plants, where they have either storage or structural functions. The polymeric sequence is linear and it is composed of (1→4)-β-D-Glc*p* and (1→4)-β-D-Man*p* sugar residues. A considerable amount of work has been carried out based on the bioactivity of glucomannans from two interesting plant sources: *Aloe vera* and *Amorphhophallus konjac*.

'Acemannan' from *Aloe vera*

There are over 300 species of Aloe known, but *Aloe vera* L. (*Aloe barbadensis* Miller) is recognised as the 'true *Aloe vera*' for its widespread use and purported healing power. The plant belongs to the Liliacea family and it is not member of the cactus family as many would believe from the rosette-like arrangement of the long spiked leaves on the central stem.

Scientific investigations on *Aloe vera* have gained more attention over recent years due to its reputed medicinal properties. Some publications have appeared in respected scientific journals that have made appreciable contributions to the discovery of the functions and properties of aloe, 'nature's gift'. The use of and research on this plant up to 1998 have been well described in two well-referenced reviews (Grindlay and Reynolds, 1986; Reynolds and Dweck, 1999). Currently, the plant is most widely used in areas of skin care, cosmetics and wound-healing.

Although many physiological properties of *Aloe vera* have been described, it still remains uncertain as to which of the component(s) is responsible for these properties. The plant contains two separate juice materials, a yellow latex (exudate) and a transparent mucilaginous gel, extruded from the inner pulp. This clear pulp is widely used in various medical, cosmetic and nutraceutical applications (Eshun and He, 2004). Many biological activities (including anti-viral, anti-bacterial, laxative and anti-inflammatory properties, immunostimulation and protection against radiation) have been attributed to this gel, and in particular, its polysaccharides (Ni *et al*., 2004). The action of aloe gel as a moisturising agent is still a popular concept (Leung, 1998).

Many studies have reported the presence of polysaccharides, especially the acetylated mannan or glucomannan (acemannan, commercially known as Carrysin™), as the main component of the gel with minor amounts of various other types of polymers (t'Hart *et al*., 1989; McAnalley, 1993; Femenia *et al*., 1999; Lee *et al*., 2001; Ni *et al*., 2004; Chow *et al*., 2005).

Acemannan is a storage polysaccharide located within the protoplast of the parenchymatous cells of the *Aloe vera* parenchyma (Femenia *et al*., 1999). There is a considerable discrepancy in the literature as to the structure of the acemannan. A recent investigation suggests that the polysaccharide has a β-glucomannan backbone with a Man : Glc ratio of ~15 : 1 and the branching occurs from the O-2, O-3, and O-6 of (1→4)-β-Man*p* residues to single α-Gal*p* side chains (Chow *et al*., 2005).

The potential use of *Aloe vera* products often involves some type of processing. Thus, the main physico-chemical modifications promoted by heat treatment and dehydration at different temperatures on acemannan and cell wall polysaccharides from *Aloe vera* gel have been recently reported (Femenia *et al*., 2003; Chang *et al*., 2006). Appropriate processing techniques should be employed during the stabilisation of the gel in order to affect and extend its field of utilisation.

It seems clear that further research needs to be done to unravel the myths surrounding the biological activities and the functional properties of *Aloe vera*. Recent studies claim that the relationship between the components, including acemannan, and its overall effect has not been clarified (Choi and Chung, 2003; Eshun and He, 2004), and that some of the bioactivities – such as the prevention or minimisation of radiation-induced skin reactions in cancer patients (Richardson *et al*., 2005) or, even, the effectiveness of *Aloe vera* gel for the healing of chronic wounds – could not be demonstrated (Gallagher and Gray, 2003). A more precise understanding of the biological activities is required to develop *Aloe vera* as a pharmaceutical.

Glucomannan from *Amorphophallus konjac*

Konjac glucomannan is a food storage polysaccharide extracted in high yield from the tubers of *Amorphophallus konjac* C. Koch. This plant has been cultivated for centuries in China and Japan and used as food and as a food additive. Only recently has it found use in the West as a texture modifier and thickener. Konjac glucomannan is a β-(1→4)-linked polysaccharide composed of a D-glucosyl and D-mannosyl backbone lightly branched, possibly through β-(1→6) glucosyl units (Katsuraya *et al*., 2003). The Man : Glc ratio is typically reported to be approximately 1.6 : 1 (Cescutti *et al*., 2002), being the weight average molecular mass of this polysaccharide $9.0 \pm 1.0 \times 10^5\,\mathrm{g\,mol^{-1}}$ (Ratcliffe *et al*., 2005). The presence of about 5–10% acetyl-substituted residues confers water solubility on the glucomannan (Gao and Nishinari, 2004).

The studies on the application of konjac glucomannan have been extended greatly from food and food additives to various fields – such as the pharmaceutical, biotechnological and fine chemical industries, including cosmetics (Zhang *et al*., 2005). In the pharmaceutical area, due to its degradability and gel-forming ability, konjac glucomannan can be used in drug delivery (Wang and He, 2002; Pathak *et al*., 2003), and it can also be used to improve bio-adhesive properties. Thus, Dettmar *et al*. (2000) invented a kind of pharmaceutical composition containing an alginate, xanthan, carrageenan and glucomannan; this provided both a protecting and a healing effect on the mucosal surface for treatment of disorders of the oesophagus. Konjac glucomannan has also been used in cellular therapy (Slepian and Massia, 2001) and as a gel filler material for prosthetic implants (Ita and Clarke, 2003).

Regarding cosmetics, different products containing glucomannan have been patented in Japan: Omura *et al.* (2001) invented a hair composition containing glucomannan; Takada (2000) a kind of water-insoluble glucomannan gel as a mild scrubbing agent; Saito (2000) a product containing pigments coated with water-soluble glucomannan that exhibited good skin-moisturising effects; and Shimizu and Ohshiba (2000) a quick-drying disinfecting gel.

Pectic polysaccharides

In recent years, pectic substances have emerged as a relevant class of potentially bioactive natural products (Diallo *et al.*, 2003; Hokputsa *et al.*, 2004a, b; Inngjerdingen *et al.*, 2005; Mellinger *et al.*, 2005; Nergard *et al.*, 2005).

Native pectins are believed to consist of a backbone in which 'smooth' galacturonan regions of α-(1→4)-linked D-galacturonosyl residues are interrupted by ramified ('hairy') rhamnogalacturonan regions consisting of a backbone of alternating α-(1→2)-linked L-rhamnosyl and α-(1→4)-linked galacturonosyl residues (rhamnogalacturonan I (RG I)). Neutral side chains are predominantly attached to *O*-4 of the rhamnosyl residues, and are composed of D-galactosyl and L-arabinosyl residues. The proportion of 'smooth' and 'hairy' regions can vary greatly depending on the type of tissue or its development stage. A minor component of plant cell wall is RG II, which has an extremely complex structure (Voragen *et al.*, 2000).

Pectic substances can be found in relatively large amounts in the cell walls of fruit and vegetable tissues. However, most of the bioactivities associated with pectic polymers have mainly been reported for polysaccharides obtained from herbal and medicinal plants. The pharmacological activity of each of the pectic polysaccharides may depend on their fine chemical structure (Yamada, 1994).

Several polysaccharides isolated from plants used in phytotherapy and traditional medicine have been tested for complement modulating properties. In particular, pectins, arabinogalactans type I ((1→4)-linked Gal) and type II ((1→3,6)-linked Gal), arabinans and other heteroglycans like glucuronoarabinoxylan have the capacity to stimulate the complement system (Yamada and Kiyohara, 1999; Hokputsa *et al.*, 2004a). The complement system is an important component of the immune defence against infections, and proteolytic cleavage of the complement components leads to generation of biologically active complement activation products that may increase local vascular permeability, attract leucocytes (chemotaxis), mediate immune adherence and modulate antibody production. Agents that improve or stimulate leukocyte locomotion are of interest, as the capacity of leukocytes to respond by chemotaxis is part of an optimal host defence against infection. Very often, chronic and recurrent infections, cancer and rheumatoid arthritis are associated with diminished chemotaxis *in vitro* (Wagner and Jurcic, 1991).

Two acidic polysaccharides that exhibited intestinal immune system modulating activity were characterised by Yu *et al.* (2001) from rhizomes of *Antractylodes lancea* DC. The chemical composition of these two polysaccharides closely resembled RG I and RG II, although small differences that could affect their bioactivity were reported.

The aqueous extract of the dried leaves of *Trichilia emetica* contained different types of pectins with a rhamnogalacturonan backbone that might be responsible for the complement fixing activities observed (Diallo *et al.*, 2003). Interestingly, the removal of terminal arabinofuranosides promoted a dramatic decrease of the activity, indicating that this structural unit may be involved in the bioactive site of the molecule.

The immunomodulating activities of different fractions isolated from 50 and 100 °C water extracts from the roots of *Vernonia kotschyana* – a traditional herbal plant used for the treatment of gastritis and gastro-duodenal ulcers, and as a wound-healing remedy – were investigated by Nergard *et al.* (2004). The active principles were identified as acidic polysaccharide fractions containing pectic arabinogalactan II structures, which showed both complement fixing ability and T-cell-independent induction of B-cell proliferation *in vitro*. Some activity was also observed on macrophages.

The same research group has investigated the anti-ulcer, radical-scavenging and immunological activities of pectin-type polymers from the aqueous extract of the roots of *Cochlospermum tinctorium*, a plant of widespread occurrence in the savannah and scrub land of the drier parts of the West African Region (Nergard *et al.*, 2005). The polysaccharides extracted were shown to be of a very complex nature possibly with a highly branched RG I core with both arabinogalactan type I and type II side chains. The polysaccharides possessed both mitogenic and complement fixation activities, and may therefore, at least partly be responsible for the immunomodulating activities observed for the crude extract.

Furthermore, two pectin-type polysaccharides isolated from a 50 °C water extract from the aerial parts of *Glinus oppositifolius* were shown to exhibit dose-dependent complement fixating activities, and induced chemotaxis of macrophages, T-cells and natural killer (NK) cells (Inngjerdingen *et al.*, 2005).

Finally, Trommer and Neubert (2005) reported the protective effects of the lipids within the human skin; they used a topical application of a semi-solid formulation rich in pectic polysaccharides. The administration of lipid protective polysaccharides in cosmetic products could be helpful against ultraviolet (UV)-induced damage.

Glucans

Extensive studies have demonstrated that (1→3)-β-D-glucans, and (1→3)-linked linear and branched glucose homopolymers exhibit considerable immunomodulatory activity by binding specific macrophage receptors and activating macrophages, resulting in anti-tumour, anti-bacterial and wound-

healing activities (Ooi and Liu, 2000; da Silva and Parente, 2003; Peng *et al*., 2003; Wu *et al*., 2005). Moreover, (1→3)-β-D-glucans show other biological activities potentially exploitable in therapy as clinical immunostimulants (Liang *et al*., 1998).

Many of the bioactive (1→3)-β-D-glucans are fungi-derived polysaccharides. Thus, Madla *et al*. (2005) have recently described polymer production and some biological properties (anti-viral and anti-fungal activities and cytotoxicity) of the polymers from 16 strains of fungi from 15 different genera. Three strains contained polymers that induced the production of cytokine, which is known to stimulate the wound-healing process. These polysaccharides have been characterised by Methacanon *et al*. (2005) and they were shown to be composed of a (1→3)-β-D-glucan backbone substituted at O-6 with side chains of (1→6)-β-D-glucopyranosyl units. Polymers with similar structure have been obtained from *Ganoderma tsugae*, a mushroom used in traditional Chinese medicine. Recently these fungi have attracted much attention because their polysaccharides have been demonstrated to exhibit remarkable anti-tumour activities. These were manifested by enhancing the host-mediated mechanisms including increasing interleukin-2 (IL-2) production and stimulation of cytotoxic T-lymphocytes, NK activity and antibody production (Peng *et al*., 2003, 2005a, b).

The poor aqueous solubility of some of the glucans obtained might be a huge hindrance to their application, especially in pharmaceuticals. Biotechnological processes such as sulphation have been proposed to improve polysaccharide polarity and aqueous solubility (Wang *et al*., 2005). Interestingly, a water insoluble (1→3)-α-D-glucan isolated from the fruiting body of *Ganoderma lucidum* did not exhibit any anti-tumour activity whereas its sulphated derivative showed anti-tumour activity (Zhang *et al*., 2000).

Sulphated polysaccharides are one of the few natural compounds with excellent and promising anti-viral activities that have proven effective in reducing human immunodeficiency virus (HIV) replication and progress (Asres *et al*., 2005). It is very much hoped that anti-HIV cures and prophylactic preparations containing these natural compounds may eventually be produced.

Bioactive components in mushrooms have also been the focus of recent research on anti-tumour, anti-viral and anti-bacterial actions. Glucans and glucan–protein complexes are amongst the potential compounds that might be responsible for such bioactivities (Dikeman *et al*., 2005; Hoshi *et al*., 2005; Ou *et al*., 2005; Wong and Cheung, 2005).

Chitin and chitosan

Chitin is the second most abundant bioplolymer on Earth after cellulose; it is available largely in the exoskeletons of invertebrates, but also in the cell walls of fungi (Kumar, 2000). Chitosan is the name used for low-acetylated substituted forms of chitin. They belong to the family of the

linear copolymers of β-(1→4)-2-amino-2-deoxy-D-glucan (GlcN or glucosamine units) and β-(1→4)-2-acetamido-2-deoxy-D-glucan (GlcNAc or acetylglucosamine units) (Qin *et al*., 2004). Valuable applications for chitin and chitosan have been reported in many fields such as chemistry, biotechnology, pharmaceutics and cosmetics (Kumar, 2000; Synowiecki and Al-Khateeb, 2003; Yusof *et al*., 2003; Kumar *et al*., 2004; Muzzarelli *et al*., 2005).

18.3.2 Plant-derived antioxidants: phenolic compounds

Plant-based diets are widely suggested to contribute to reducing the risk of development of chronic diseases such as cancer, atherosclerosis, cardiac dysfunction, diabetes, hypertension and neurodegenerative disorders. Most of these functions have largely been attributed to the antioxidant effects of their bioactive components. The growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on vegetable sources and the screening of raw materials to identify new antioxidants. Oxidation reactions are not an exclusive concern of the food industry, and antioxidants are widely needed to prevent deterioration of other oxidisable goods, such as cosmetics and pharmaceuticals.

Interestingly, within the antioxidant literature, the number of residual sources studied has been augmented considerably, due mainly to the value-adding/recycling interest of the agro-food industry, and a requirement for information on the specific location of active compounds and their modification during processing (Peschel *et al*., 2006).

Several plants have been proposed as sources of potentially safe natural antioxidants for the food, pharmacological and cosmetics industries; various compounds have been isolated, many of them being phenolic compounds. A large number of low and high molecular weight plant polyphenolics presenting antioxidant properties were studied and proposed for use in preventing lipid oxidation (Hagerman *et al*., 1998).

Sources and potential applications of phenolic compounds

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants (Randhir *et al*., 2004). Their chemical structure is characterised by the presence of an aromatic ring, bearing one or more hydroxyl substituents and ranges from simple phenolic molecules to highly polymerised compounds (Bravo, 1998). Despite this structural diversity, the group of compounds are often referred to as 'polyphenols' (Balasundram *et al*., 2006).

The phenolic metabolites include: anthocyanins, anthochlors, benzofurans, chromones, coumarins, minor flavonoids, flavonones and flavonols, isoflavonoids, lignans, phenols and phenolic acids, phenolic ketones, phenylpropanoids, quinonoids, stilbenoids, tannins and xanthones (Bahorun *et al*., 2004). Flavonoids constitute the largest group of plant phenolics,

accounting for over half of the 8000 naturally occurring phenolic compounds (Heim *et al*., 2002).

The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons, and chelate metal cations (Amarowicz *et al*., 2004).

The possible health benefits derived from dietary phenolic compounds depend on their absorption and metabolism, which in turn are derived from their structure including their conjugation with other phenolics, degree of glycosylation/acylation, molecular size and solubility (Bravo, 1998).

A wide range of physiological properties such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects have been reported for phenolic compounds (Benavente-García *et al*., 1997; Samman *et al*., 1998; Middleton *et al*., 2000; Puupponen-Pimiä *et al*., 2001; Manach *et al*., 2005).

As in food products two applications of phenolic antioxidants might be of interest: as a substitute for synthetic preservatives or as active ingredients, for example as a skin-protecting additive in dermatology. Investigations on the commercial application of radical scavengers and flavonoids, the main group of phenolic compounds, as beneficial anti-ageing and photoprotection ingredients in cosmetic products have been reported by Katiyar and Elmets (2001) and Lupo (2001). Furthermore, an increase in the demand for non-toxic antioxidants that are active in hydrophilic and lipophilic systems has been observed (Peschel *et al*., 2006).

Phenolic compounds are present in almost all foods of plant origin. Fruit, vegetables and beverages such as fruit juices, tea and wines are the major sources of these compounds. Plant by-products and medicinal plants are also important sources of phenolic compounds.

Fruits and vegetables

Phenolic compounds have been closely associated with the health benefits derived from consuming high levels of fresh fruits and vegetables (Parr and Bolwell, 2000). There are wide variations between the total phenolics contents of the different fruits or vegetables, or even for the same fruits and vegetables, reported by different authors (see review of Balasundram *et al*., 2006). These differences have been attributed either to the complexity of these groups of compounds or the methods of extraction and analysis utilised (Bravo, 1998). Moreover, the total phenolics contents of plants depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental, handling and storage) factors (Tomás-Barberán and Espín, 2001).

Fruit and vegetables containing high levels of phenolic antioxidants would be attractive to health-conscious consumers, therefore optimisation of production and processing factors affecting the antioxidant capacity is desirable. Food-processing practices such as heat and aeration may decrease the antioxidant capacity of fruit phenolics (Kalt *et al*., 2001).

Flavonoids are present in many fruits and vegetables such as grapes, apples and onions. Their cardioprotective effects stem from the ability to inhibit lipid peroxidation, chelate redox-active metals and attenuate other processes involving reactive oxygen species (Heim *et al*., 2002).

Beverages

Administration of polyphenol-rich fruit juices is thought to be favourable to HIV-positive patients due to enhanced phytohaemagglutinin-induced lymphocyte proliferation, which could restore T-cell homeostasis (Winkler *et al*., 2004). Wines as natural sources of antioxidants with radical-scavenging properties are the subject of growing interest (López-Vélez *et al*., 2003; Lugasi and Hovari, 2003; Stasko *et al*., 2006). The major phenolic constituents of wines include hydroxybenzoic and hydroxycinnamic acids derivatives, as well as flavonols (Minussi *et al*., 2003). The phenolics contents and composition in wines vary widely and are determined by several factors, such as the variety of grapes used, the conditions under which they were grown, wine-making techniques, maturity and processing parameters (Villaño *et al*., 2006). Moderate wine consumption has been related to prevention of cancer, Alzheimer's disease and dementia, and it has also been implicated in decreasing the risk of coronary heart disease (Renaud *et al*., 1998).

The findings of many studies using green tea polyphenols as chromopreventive, natural healing and anti-ageing agents for human skin, and discussion of possible mechanisms of action, have recently been summarised by Hsu (2005).

Plant by-products

Processing of plant foods might result in the production of by-products that are potentially rich sources of bioactive compounds, including phenolic compounds (Schieber *et al*., 2001). The availability of phenolic compounds in industrial residues, their extraction and antioxidant activity has been the subject of a review by Moure *et al*. (2001).

The by-products of the grape/wine industry have recently attracted considerable interest as a source of phenolic compounds (Louli *et al*., 2004; Selga *et al*., 2004; Kammerer *et al*., 2005a, b). Grape skins and seeds contain flavonoids (catechin, epicatechin, procyanidins and anthocyanins), phenolic acids (gallic and ellegallic acids) and stilbenes (resveratrol and piceid). These grape seed and skin constituents have been shown to have health–functional properties comparable with those of fruits and vegetables (Yilmaz and Toledo, 2006).

In addition, citrus industry by-products, if utilised optimally, could also be major sources of phenolic compounds as the peels, in particular, have been found to contain higher amounts of total phenolics compared with the edible portions (Gorinstein *et al*., 2001; Mandalari *et al*., 2006). The peels

of several other fruits have also been found to contain higher amounts of phenolics than the edible fleshy parts. For instance, peels from apples, pears, pomegranates, mango and peaches were found to contain larger amounts of total phenolics than those found in the peeled fruits (Gorinstein *et al*., 2002; Berardini *et al*., 2005). The peels of pomegranate – which contained larger amounts of total phenolics, flavonoids and proathocyanidins than a pulp extract – have demonstrated their effectiveness in the prevention of atherosclerosis (Li *et al*., 2006).

Lu and Foo (1997) identified and quantified the major polyphenols in apple pomace. Thus, epicatechin, caffeic acid, three dihydrochalcone glycosides and five quercitin glycosides were isolated. The same authors also studied the procyanidins obtained from apple pomace. The homogeneous nature of the procyanidin fractions was demonstrated by the isolation and identification of a range of epicatechin oligomers (Foo and Lu, 1999). The antioxidant and radical-scavenging activities of all the phenolic compounds identified and isolated from apple pomace was tested. All the compounds exhibited strong antioxidant activities; their 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH)-scavenging activities were 2–3 times better, and the superoxide anion radical-scavenging activities 10–30 times better, than those of antioxidants such as vitamins C and E (Lu and Foo, 2000).

The by-products of the olive industry have also received much attention as a source of phenolic compounds, in particular hydroxytyrosol, tyrosol, oleuropein, and a variety of hydroxycinnamic acids that can be recovered from the olive mill wastes (Obied *et al*., 2005). These compounds are shown to be essentially hydrophilic, and this is the reason why they are more abundant in olive oil waste waters. Oleuropein and hydroxytyrosol exert protective effect against auto-oxidation of LDL *in vitro* (Visioli *et al*., 1995), and their free-radical-scavenging activity has also been demonstrated (Visioli and Galli, 2002). Hydroxytyrosol appeared to be the most effective phenolic compound at low concentrations to protect human erythrocytes and DNA against oxidative damage (Quiles *et al*., 2002). A new enzymatic treatment of olive oil by-product using fungal enzymes to release simple phenolic compounds, especially hydroxytyrosol, has been reported by Bouzid *et al*. (2005). The antioxidant capacity of hydroxytyrosol was higher than that of antioxidants such as ascorbic acid and butylated hydroxytoluene (BHT).

Medicinal plants

The screening of up to 70 medicinal plant extracts for antioxidant capacity and total phenolics content has recently been reported by Katalinic *et al*. (2006). The results indicate that *Melissae folium* infusions could be an important source of phenolics with high antioxidant capacity comparable with red wine or beverages like tea. The possible role that flavonoids from rosemary (*Rosemary officinalis*) and other medicinal herbs play in the

prevention of neurodegenerative diseases has been reviewed by Arouma *et al.* (2003).

Extraction procedures for plant antioxidants

So far the extraction of antioxidants, including phenolic compounds, from plant tissues has been accomplished by employing as extraction solvent, a liquid, such as methanol, ethanol, acetone, ethyl acetate, an aqueous solution of the aforementioned solvents, or even a supercritical fluid (Louli *et al.*, 2004). In any case, the composition of the extract depends not only on the solvent used, but also on the quality and the origin of the plant material, its composition, its storage conditions and its pretreatments. All these parameters should be taken into account, in order to produce a high-quality extract with antioxidant activity suitable for use in the food, cosmetic and pharmaceutical industries.

For solid–liquid extraction, an appropriate organic solvent is mixed with the plant material extracting the phenolic compounds. Then, the removal of the solvent can be achieved by means of different processes such as drying, concentration or ultrafiltration (UF). After applying any of these procedures, the extract must be dried to obtain a powderform.

Organic solvent extraction is efficient and simple, yet costly. Large amounts of organic solvents are often used. This, in turn, is also detrimental to human use because traces of the organic solvent may be present in the antioxidant extract. Phenolics separation and concentration by membrane separation is even more efficient than organic solvent extraction. Organic solvents are still used but in lower quantities, and UF ensures the purity of the polyphenol extract. The drawback is membrane fouling, which can disrupt the process, and the time needed to complete the process. The separation process has to be repeated several times.

Alternatively, SCFE can also be used, which produces the final product as a powder without any need for final drying. Furthermore, the advantages of using CO_2 – low cost, non-toxic, non-flammable, and non-corrosive – make it the perfect solvent for natural products. It is imperative to have safe and efficient extraction procedures that guarantee a pure product. According to Shi *et al.* (2005) SCFE is the extraction process of the future. However, it has been reported that the recovery of antioxidants with supercritical CO_2 often might require intense extraction conditions such as a pressure higher than 300 bar, and, in the case of phenolic compounds from grapes, a modifier in a high percentage was also required (Murga *et al.*, 2000).

In contrast to the above, SCFE has also been employed for the purification of the primary extract (Ribeiro *et al.*, 2001), in order to improve its properties without causing any thermal or chemical degradation. In this way, a high-added-value product could be obtained with only moderate conditions and equipment capacity needed, justifying the choice of a supercritical fluid both from an economic and process efficiency point of view.

18.3.3 Oils derived from plant sources

Plant oils are typically composed of triglyceride molecules (esters) composed of a 3-carbon alcohol (glycerol) plus three 18-carbon (or 16-carbon) fatty acids. Unlike the saturated fatty acids of animal fats, which are solid at room temperature, most plant-derived fatty acids are typically unsaturated, are liquid at room temperature and are often referred to as oils. Unsaturated fatty acids might contain one or more double bonds between the carbon atoms (mono-unsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs)).

Many non-food applications have been proposed for plant oils. Undoubtedly, cosmetics is one of the fields where these compounds have attracted major attention. However, some of these plant-derived oils have also found interesting applications in the pharmaceutical area (Barrault *et al*., 2002).

When polyunsaturated vegetable oils are partially hydrogenated to improve their texture, *trans* fatty acids are produced. *Trans* fatty acids tend to raise the level of LDLs and lower the level of HDLs. These changes in blood lipids (cholesterol levels) may increase the risk of heart disease (atherosclerosis). Dieticians generally recommend the use of mono-unsaturated, unhydrogenated oils such as olive oil whenever possible, and the avoidance of *trans* fatty acids found in many products such as french fries, doughnuts, chips, cookies and crackers.

Sources and applications of plant-derived oils

Most fresh fruits exhibit a very low content of fat; in contrast, dry fruits and many fruit seeds and fruit kernels contain large amounts of, often discarded, high-value oils. Selected sources of vegetable oils that are potentially useful for incorporation in cosmetics and pharmaceutical products are reviewed below.

Olive oil

Olive (*Olea europaea*) oil is currently one of the most appreciated vegetable oils and a key component of the so-called 'Mediterranean diet'. The nutritional and health aspects of olive oil have been reviewed by Harwood and Yaqoob (2002). Olive oil contains oleic acid (55–83% depending on the olive variety) and also α-linoleic acid (5–15%) as the main fatty acids. Diets high in α-linoleic acid delay and prevent diseases such as coronary heart disease, contributing to a high life expectancy (Vardavas *et al*., 2006). Apart from fatty acids and triglycerides, olive oil contains tocopherols, squalene, carotenoids, sterols, polyphenols, chlorophylls, and volatile and different flavour compounds. The extracts of mixtures of olive fruits, leaves and stems show anti-inflammatory and active oxygen-scavenging effects. The anti-inflammatory effect is exerted by both unsaponifiable and polar compounds (De la Puerta *et al*., 2000), while the free-radical-scavenging effect of virgin olive oil is mainly due to the presence of polyphenols (Perricone,

2001). Olive oil has been used to moisturise dry skin, and as a lip balm, shampoo, hand lotion, soap, massage oil and dandruff treatment (Bruneton, 1999). It is applied topically to treat skin damage, such as dermatitis, eczema, seborrhoea, psoriasis, thermal and radiation burns, other types of skin inflammation and ageing (Perricone, 2001). When the oil is topically applied after UVB exposure it can effectively reduce UVB-induced skin tumours, possibly via its antioxidant effects (Budiyanto *et al.*, 2000). Adverse cutaneous reactions to topically applied olive oil are seldom reported, but olive oil is considered in general as very weakly irritant (Kranke *et al.*, 1997). There is good evidence that olive oil is protective in cardiovascular diseases, its mechanisms may involve blood lipids, but other mechanisms – including effects on immune function, endothelial function and the coagulation pathways – remain possible and are being actively researched.

Oil from fruit kernels of the Rosaceae family

Large amounts of seeds from different fruits such as apricots, peaches or cherries are discarded annually at processing plants. This not only wastes a potentially valuable resource but also aggravates an already serious disposal problem. At present there is no systematic collection and utilisation of most of these fruit seeds; thus, valuable products with a large industrial potential remain unexploited. Some of the seeds are difficult to collect because of the direct consumption of the fresh products by consumers, but the bulk of the fruit and vegetable sources are used in food-processing plants.

The oil content of apricot kernel ranges from 40 to 56% of the dry kernel. In general, sweet kernel varieties contain larger percentages of oil than bitter kernelled varieties (Femenia *et al.*, 1995). Nevertheless, oils from both kernel varieties exhibit a fairly similar fatty acid composition, being very rich in unsaturated fatty acids; thus, oleic (~64%) and linoleic (30–31%) represent about 93–94% of total fatty acids in both oils (Femenia *et al.*, 1995; Gandhi *et al.*, 1997; Özkal *et al.*, 2005a). The total oil content and fatty acid composition of apricot kernel oil can be compared with that of almond oil (Carratala *et al.*, 1998; Sánchez-Bel *et al.*, 2005) and cherry kernel oil (Chandra and Nair, 1993). Nevertheless, although it belongs to the same family, peach kernel oil has a slightly different fatty acid profile, containing up to 21% of saturated fatty acids, mainly palmitic acid (Rahma and Abd El-Aal, 1988).

Almond oil is highly appreciated, especially in cosmetics. A typical use of this oil is the application to the skin of neonates during their early stages of life. Furthermore, a role for almond oil in the prevention of colon cancer has also been reported (Davis and Iwahashi, 2001).

One of the big constraints to the use of fruit kernels from bitter varieties of the Rosaceae, not only from a nutritional point of view but also for their utilisation in non-food areas such as cosmetics and/or pharmacology, is the presence of significant amounts (up to 5–6% of the dry kernel of almonds,

apricots and peaches) of the toxic cyanogenic glycoside amygdalin (Femenia *et al*., 1995; Gómez *et al*., 1998; Dicenta *et al*., 2002). The removal of amygdalin by biotechnological means, i.e. by using the endogenous enzymes combined with microbial fermentation (Tunçel *et al*., 1998) or by extraction with different solvents (Koo *et al*., 2005), is required in order to obtain a completely safe product.

Jojoba oil-wax

Unlike vegetable oils and animal fats, jojoba (*Simmondsia chinensis*) oil is not a triglyceride but a mixture of long chain esters (97–98%) of fatty acids and fatty alcohols, and therefore is more properly referred to as a wax; however, jojoba oil-wax is the term in general use (Canoira *et al*., 2006). Jojoba seeds contain about 50% of liquid wax which is mainly used in cosmetic preparations (Cappillino *et al*., 2003), not only acting as a humectant, but creating a protective film over the skin that keeps in moisture (Dweck, 1997b). Jojoba oil-wax provides a broad spectrum of fatty acids (such as oleic, linoleic and arachidonic), as well as triglycerides which have good compatibility with the natural sebum in the human skin (van Boven *et al*., 2000). Pharmaceutical applications of jojoba oil-wax have also been suggested (Ash *et al*., 2005). Screening of the oil revealed that it has significant analgesic, antipyretic, anti-inflammatory, antioxidant, anti-bacterial and anti-parasitic properties (Bruneton, 1999).

Other important plant-derived oils

Apart from its popular edible fruit, mangoes (*Mangifera indica*) also contain kernels that yield a valuable emollient oil rich in oleic and stearic acids, and triglycerides, and is used in cosmetics (Aikawa, 2002). Mango kernel oil has been investigated for its suitability as an ointment base, and has been observed to release drugs at a remarkably greater rate than the standard paraffin-base ointment formulations (Dweck, 1997a).

Coconut *Cocos nucifera* (Arecaceae) oil is valued as an emollient and is used as an ingredient in remedies for skin infections. Modified coconut oil containing polyunsaturated fatty acids in the form of mono-, di- and triglycerides is useful as a constituent of a barrier lipid mixture in cosmetic and pharmaceutical formulations to protect and prevent drying of the skin. However, because of its alkali laurate content some coconut oil soaps can irritate the skin (Dweck, 1997a).

Sunflower seeds from *Helianthus annuus* (Compositae), contain polyunsaturated fats, rich in triglycerides of linoleic acid, an essential fatty acid needed by the body to maintain good skin condition. Studies indicate that cutaneous application of the sunflower oil increases the linoleic acid levels of the skin, lowers transepidermal water loss and helps to eliminate scaly lesions common in patients with essential fatty acid deficiency. Sunflower oil is used for psoriasis, and on bruises (Dweck, 1997b).

Castor oil is obtained from the castor bean, *Ricinus communis* (Euphorbiaceae). The seeds contain 50% of the oil. The oil acts as a barrier agent to protect against harsh climates, and is soothing to the skin. Castor oil forms a clean, light-coloured, transparent soap, which dries and hardens well and is free from odour (Matsumura, 2001). Ricinoleic acid and its many derivatives have skin smoothing and moisturising qualities, and improve various skin conditions such as rough skin and acne (Miyahara and Sanbe, 2002). Hydrogenated castor oil and/or its esters, are useful as vehicles or carriers, emollients or solubilisers for toiletry, cosmetic, hair and skin care formulations, and are useful for cleansing and conditioning the skin (Sato, 2002).

Cocoa butter, from *Theobroma cacao* (Sterculiceae), contains triglycerides consisting mainly of oleic, stearic and palmitic acids, and about 75% of fatty acids are present as mono-unsaturates. Cocoa butter is particularly soothing after windburn or sunburn. It is used medicinally as a vehicle in suppositories and pessaries. Cocoa butter is used widely as an emollient and in various topical cosmetic preparations (Dweck, 1997a).

Other potentially interesting plant oil sources that are either currently used or have the potential to be used in the cosmetics and/or pharamaceutical areas, supported by scientific reports about their health-promoting properties, are: goldenberry oil (Ramadan and Mörsel, 2003), kukui nut oil (Ako *et al*., 2005), argan oil (Rojas *et al*., 2005), date seed oil (Besbes *et al*., 2004), *Amaranthus* oil (He and Corke, 2003; He *et al*., 2003) and *Echinacea* seed oil (Oomah *et al*., 2006).

SCFE of vegetable oils

As in the case of plant antioxidants, an attractive alternative to conventional extraction of oils using organic solvents is the application of supercritical fluids. SCFE has been widely used since it enables the recovery of valuable oils from natural matrices; high yields and better quality products with improved functional and/or nutritional characteristics are produced by operating under a wide range of conditions (Özkal *et al*., 2005b). In addition, easy and complete removal of the solvent from the final product makes it advantageous over conventional solvent extraction. CO_2 is also the most preferred supercritical solvent in oil extraction because of its low critical temperature (31 °C), non-toxic, non-explosive nature, and low price. Supercritical CO_2 (SC-CO_2) was successfully used for the extraction of oils from different plant sources including rapeseed (Eggers, 1985), peanut (Goodrum and Kilgo, 1987), canola (Temelli, 1992), almond (Marrone *et al*., 1998; Femenia *et al*., 2001), pistachio nut (Palazoglu and Balaban, 1998), sesame (Odabasi and Balaban, 2002), walnut (Oliveira *et al*., 2002), *Amaranthus* grain (He *et al*., 2003), carrot root (Ranalli *et al*., 2004) and apricot kernel (Özkal *et al*., 2005a, b).

18.4 Future trends

Over the last few years, the amount of scientific literature about the bioactivity and health-promoting properties of plant-derived compounds such as polysaccharides, phenolic compounds and vegetable oils has increased. This fact demonstrates the growing interest in the exploitation of plant-based by-products and/or co-products, for instance as potential sources of pharmaceutical and cosmetic products. However, this promising field requires interdisciplinary research of many areas such as food/plant technology, food chemistry and biochemistry, nutrition and toxicology amongst others.

Some industrial activity has already been established based on the health-promoting properties, although not always scientifically supported, of several plants and plant-derived compounds, especially in the cosmetics area (for example: *Aloe vera* derivatives, different vegetable oils and a wide variety of 'medicinal plants'). However, as it can be inferred from the vast, recent and even sometimes contradictory, scientific literature, most of the potential of many plants and specific plant-derived compounds remains almost completely unexplored.

Although the future of plant-derived co-products holds exciting opportunities for the food, pharmaceutical and cosmetics industries to create novel and high-value products, in order to achieve and optimise their exploitation the following research needs will have to be addressed. (1) Methods for complete utilisation of plant by-products (resulting mainly from food processing), on a large scale and at affordable levels, should be developed. Thus, active participation of the food and allied industries with respect to sustainable production and waste management is required. (2) The bioactivity, bioavailability and toxicology of plant-derived compounds need to be carefully assessed by *in vitro* and *in vivo* studies. The 'bioactivity' of many phytochemicals has only been tested in *in vitro* models and this may bear no relationship to the situation *in vivo* (Dillard and German, 2000). In addition, there is an urgent need to standardise the methodology applied to determine the bioactivities of different compounds, e.g. of plant polysaccharides, or the antioxidant capacity of compounds such as plant phenolics (Huang *et al*., 2005). (3) The stabilisation of many plant antioxidants, e.g. phenolics, is a clear area that requires further research. For instance, although the majority of the evidence shows remarkable benefits of the antioxidant, anti-cancer, anti-ageing and anti-inflammation effects of plant phenolics, standard delivery systems for topical application have not been fully established. This is partially because of the nature of these highly reactive compounds, which are easily oxidised in the environment and gradually lose their activity if not used immediately after preparation. Therefore, the primary goal for a topical formulation would be to maintain the stability of these antioxidants. (4) As consumers become more aware

about the use of 'questionable' organic solvents, often used for the extraction of many phytochemicals, and legislation becomes more and more restrictive, safer and more efficient extraction procedures that guarantee a pure plant extract or plant-derived compound should be investigated. Further research on the use of SCFE, either to extract or purify high-value plant compounds such as phenolics or vegetable oils, might cover this need.

Undoubtedly, pharmaceuticals and cosmetics derived from food-processing by-products might represent an important, innovative and rapidly growing part of the overall food market. However, their design, i.e. their complex matrix and their composition of bioactive principles, requires careful assessment of potential risks that might arise from isolated compounds recovered from by-products. In any case, the protection of the consumer must have priority over economic interests, and health claims need to be substantiated by standardised, scientifically sound and reliable studies.

18.5 Sources of further information and advice

To gain further insight into several aspects related to the use of plant-based compounds in the pharmaceutical and cosmetic fields, several books are recommended:

- '*Pharmaceutical Biotechnology*' (Kayser and Müller, 2004). This book reflects the combination of such pharmaceutical interests as drug delivery, drug targeting, quality and safety management, drug approval and regulation, patenting issues and biotechnology fundamentals.
- '*Pharmaceutical Chemistry: Therapeutic Aspects of Biomacromolecules*' (Bladon, 2002). The book provides a broad introduction and explores the way in which carbohydrates, amongst other phytochemicals, are used therapeutically.
- '*Encyclopaedia of Common Natural Ingredients used in Food, Drugs and Cosmetics*' (Leung and Foster, 2003). This volume provides information on the composition, processing and manner of use of around 500 of the most commonly used natural ingredients.
- '*Polysaccharide Applications: Cosmetics and Pharmaceuticals*' (El-Nokaly and Soini, 1999). The book brings together many aspects related to the use of polysaccharides in those fields.
- '*Antioxidants in food: practical applications*' (Pokorny, 2001). This book provides a review of the functional roles and the potential exploitation of plant antioxidants.
- '*Oils and Fats Handbook*' (Rossell, 1999). This book provides an authoritative text on edible vegetable oils and fats. It also describes the legislation governing their use.

In addition, two web-related pages might be of great interest:

- http://www.plantpharma.org/ – From the International Academy of Life Sciences, this web page defines itself as a science-based medically orientated public dialogue site. It has a section on plant-made pharmaceuticals and many interesting links to institutions, conferences and associations involved in this field.
- http://www.bagkf.de/sofa/ – An important database for seed oil fatty acids established by the Institute for Chemistry and Physics of Lipids in Münster is electronically searchable: the Database SOFA. This internet database allows users to search for plant species, genera and families, for individual fatty acids and combinations of fatty acids in their seed oils, and for their percentage contents. It contains literature references and numerous unpublished data. Several examples of search operations have been published (Aitzetmüller *et al.*, 2003).

Finally, although the development of biopharmaceuticals based on recombinant proteins was beyond the scope of this chapter, the reading of recent reviews such as those of Fischer *et al.* (2004), Peterson and Arntzen (2004), Walsh (2005) and Gomord *et al.* (2005) is strongly recommended.

18.6 References

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19

Natural dyes from food processing wastes

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19.1 Introduction

During the last century the textile dyeing industry developed from manufacturing procedures into fully automated technical processes. This chapter focuses on the potential for natural dyes to be used in textile dyeing, with special reference to plant-based materials found in food processing and food chain waste streams. In Section 19.2 the requirements that have to be met for successful application of natural dyes in textile dyeing operations are summarised. Natural dyes present in plant wastes have to be extracted for use in the dyebath. In Section 19.3 the extraction step is discussed with regard to energy and chemicals, which forms a basis to compare different strategies to handle natural dyes in full technical scale. Results of a screening study to identify suitable wastes, released from the food and beverage industries, as potential sources for natural dyes are shown in Section 19.4. Detailed discussion of representative examples (onion peels, walnut, pressed berries, grapes and tea) is presented in Section 19.5. Future trends and access to more detailed literature are given in Sections 19.6 and 19.7.

19.2 Natural dyes in technical textile dyeing operations

In Section 19.2.1 the general aspects to be considered in textile dyeing are discussed. Section 19.2.2 presents the requirements defined by the dyeing process, and the selection criteria for dyes and mordants are highlighted in Sections 19.2.3 and 19.2.4.

19.2.1 General aspects

The selection of an appropriate dyeing procedure and the suitability of a certain dyestuff is determined by the fibre type to be dyed and the desired final properties of the product. In 2004 the total production of textile fibres reached a volume of 68.6 million tonnes.[1] This amount is divided into different classes of material: natural fibres (e.g. cotton, flax, wool) and man-made fibres (Table 19.1). Man-made fibres hold a share of approximately 55% of the total amount of textile fibres produced. Important representatives for synthetic fibres are polyester, polyamide and polyacrylic fibres. Another class of man-made fibres is based on regenerated cellulose (for example lyocell, viscose and modal fibres).

With the exception of white textile goods and materials that are used inside a certain textile construction (e.g. fill fibres), coloration of the materials is an important step to achieve access to the customer. Figure 19.1 presents the relevant steps carried out in textile dyeing. As can be seen the most relevant difference is found in the nature of chemicals used for dyestuff fixation. For synthetic dyes – depending on the type of dyestuff – alkali, salt or reducing agents are added while in the case of natural dyes mordants are used for dyestuff fixation. Up to the end of the nineteenth century natural dyes were the main colorants available for textile dyeing procedures. The development of synthetic dyes at the beginning of the twentieth century led to a more complete level of quality and more reproducible techniques of application. As a result, a distinct lowering in the dyestuff costs per kilogram of dyed goods was achieved.[2–4]

During the twentieth century numerous groups of dyes for the various classes of fibres were developed and introduced into full-scale production. During the second half of the century a concentration on the main groups of dyes occurred and today a few classes of dyes form the major part of

Table 19.1 Production of textile fibres in 2004[1]

	Million tonnes	%
Natural fibres		
Cotton	22.0	32.1
Wool	1.2	1.7
Jute	3.2	4.7
Flax, linen	0.8	1.2
Cellulosics	3.1	4.5
Synthetics		
Polyester	24.4	35.6
Polyamide	4.1	6.0
Acrylics	2.7	3.9
Polyolefines	6.3	9.2
Others	0.8	1.1
Total	68.6	100

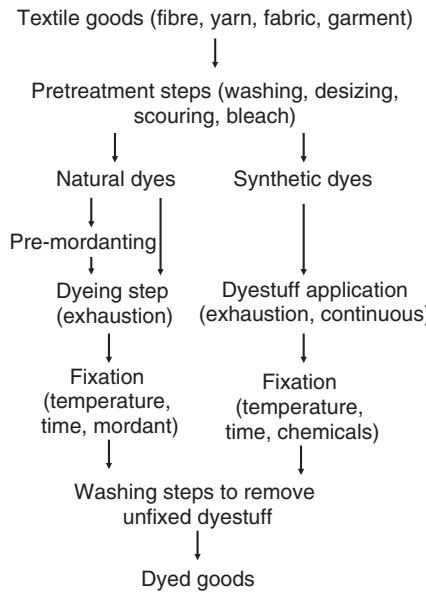

Fig. 19.1 Relevant steps in dyeing processes with synthetic dyes and natural dyes.

dyestuffs consumed; representatives are disperse dyes, reactive dyes, acid dyes, vat dyes or synthetic indigo. An estimation of the total annual dyestuff production for textile fibres can be made assuming an average colour depth of 1% of shade, which means an amount of dyestuff of 1% of the mass of the textile good is applied. The worldwide production of textile dyes thus can be estimated to reach a scale in the order of 600 000–700 000 tonnes per year. The predominance of synthetic dyes hindered a continuous development and adaptation of natural dyeing to the changing requirements of modern dyehouses. As a result, nowadays a considerable gap exists, separating the knowledge about natural dyes from the demands of commercial dyeing processes.

At present natural dyes are mainly used for the traditional or so-called 'green products'. In many cases these products are near to industrial art and require a lot of manual work, which makes them expensive and technically not suited for scale up. The expectations from the reintroduction of natural dyes into technical textile dyeing can be summarised as follows:

- improvement of the ecological situation of textile dyeing processes;
- possible use of wastes from food, beverage and forestry to obtain valuable products;
- introduction of new crops and products into modern concepts of farming;

- formation of synthetic chemistry on the basis of sustainable resources;
- replacement of 'hard products' (synthetic chemistry) by 'soft' products (natural sources).

The re-introduction of natural dyes into commercial dyehouses has to be achieved on a competitive basis, where the modern synthetic dyes define the parameters that have to be fulfilled. Representative parameters that have to be considered are summarised in Table 19.2.

Traditionally natural dyes were obtained from direct farming of crops to produce the dyestuff, typical examples are natural indigo, madder and weld. At present the production of natural dyes by direct farming results in considerably high specific costs per kilogram of plant material, or per kilogram of dyed material.[5,6] New strategies are required to establish technically and commercially competitive processes.[7–9] A promising concept for the production of natural dyes with lowered specific costs is based on the use of different plant sources for the extraction of natural dyes:

- direct farming yields rather expensive plant material that is not available from other sources;[10]

Table 19.2 Important factors in evaluating the use of a natural dyestuff

Farming	
1.	Simple farming procedures (standard equipment, easily accessible sources, e.g. forestry, food industry)
2.	Low consumption of energy, fertiliser and chemicals during farming, handling, transport and storage
Dyestuff preparation	
3.	Easy handling of plant matter
4.	High dyestuff content in plant material
5.	Standardised raw products
6.	Optimised balances (energy, water/solvents, wastes) in dyestuff extraction
7.	Database describing properties of the plants, products, dyes and dyed goods
8.	Information about toxicological properties
9.	Formation of a supplier organisation collecting the materials and providing dyehouses with standardised qualities
Dyeing processes	
10.	Application for a broad range of textile fibres (cellulose, protein, synthetic fibres)
11.	Complete colour gamut: yellow, red, blue, black
12.	Application in a set of dyes with common application (direct dye, iron- and alum-mordant)
13.	Acceptable level of fastness properties: light fastness, wet fastness, wash fastness
14.	Robust design of dyeing processes
15.	Application of existing equipment with minimal modification
16.	Optimised consumption of energy, water and chemicals

- use of cheap by-products from other agricultural activities, e.g. barks from timber industry;[11,12]
- use of dyestuff-containing wastes, e.g. those released almost free of charge from the food and beverage industries.[5,6]

Great care has to be taken to ensure that a significant improvement of the overall consumption of energy, chemicals and water is reached in relation to the state of the art processes, which have been optimised continuously for over a hundred years. The plant material has to permit simple handling and storage. The harvested crop should contain a high dyestuff content, which is easy to extract with water. The farming and the formation of a storable intermediate or dyestuff product has to be performed with a minimum consumption of energy. The dyestuff extracted from a plant source should be applicable to a maximum range of different fibres (e.g. cellulose fibres, protein fibres, polyamide fibres). A very important aspect is the possible range of colours that can be obtained from the sources. For a technical application a basic gamut containing brilliant yellow, red and blue dyes and a set of dyes for dark shades (grey/black) is needed.

In an optimum situation the dyestuff is applied as direct dye without addition of any mordant. The function of metal-salt-based mordants, e.g. iron salts or alum, can be explained by the formation of coordinative complexes that show higher affinity to the fibre and respectively exhibit lower solubility in water. Tannin-based mordants can be understood as chemicals used to increase the adsorptive properties of the textile material to be dyed.

Fastness properties

An acceptable level of fastness properties has to be achieved (Table 19.3). Fastness properties are of major interest as far as the quality of dyeing and dyestuff is concerned. The colour has to be resistant to various daily treatments, like exposure to light, contact with water and repeated washing cycles. There are several standardised tests to assess the fastness properties of dyed textiles by comparing treated samples and untreated originals.[13] Effects of light, water and detergents on coloured substrates lead to changes in colour shade and/or colour depth, 'bleeding' of dye to adjacent material and variation of physical and chemical properties of the textile fabric. While water and wash fastness properties are mainly dependent on the structure

Table 19.3 Relevant selection criteria for fastness properties of dyeings with natural dyes

Property	Method[13]	Range, poor – excellent	Acceptable lower limit
Fastness to light	DIN 54 004	1–8	3
Water fastness	DIN 54 006	1–5	3–4
Wash fastness, 40 °C	DIN 54 014	1–5	3–4

of the dye molecule, and therefore on the fixation of the dye on the substrate, light fastness is related to the photochemical properties of the colour molecule. The absorption of energy by the dye molecule causes excited states. There are different ways back to the electronic ground state: energy release with or without radiation as well as photochemical reactions that destroy the dyestuff.[14] Light-induced degradation of dyestuff results in changes of colour shade or colour depth, so-called 'fading'.[15,16]

In fact all dyes are more or less sensitive to light. However, textiles and fibres are also destroyed by irradiation. Therefore colorants require sufficient stability such that they do not fade visibly during a garment's lifetime.[17] Plant dyes predominantly show good to very good fastness values according to water and laundry variations, but light fastness seems to be the decisive factor for textile applications.[18–20]

Processes

For successful introduction of natural dyes into a modern dyehouse different dyes have to be applicable in a common procedure, which permits a mixing of different dyes and mordants in a dyeing operation. At present, classes of synthetic dyes are applied in standardised dyeing apparatus and the run of a dyeing process is adapted to the chemistry of the dyes (e.g. reactive dyes, acid dyes). Similarly dyeing processes with natural dyes have to fit on the existing range of apparatus and only minor changes in the equipment will be acceptable, for example installations required to extract the dyestuff from the plant material. Longer duration of processes or rather complex handling will lead to a considerable increase in costs and can also be identified as potential sources for deviations in the final colour of the dyed goods.

A supplier structure has to be established that is able to provide the dyehouse with standardised qualities of natural dyestuffs or plant material in a short time period (within 1–2 weeks after ordering). Similar to the presentation of modern synthetic dyes a database is required which contains:

- technical information about farming and harvesting including conditions of storage;
- information concerning dyestuff extraction and standardisation;
- toxicological properties of plants, dyes and dyed goods;
- technical recommendations for dyeing processes and high-quality relevant data about the dyeings obtained (e.g. shade, fastness properties);
- information about ecological profiles (energy, chemicals, wastes) compared with the state of the art.

19.2.2 Dyeing processes

For dyeing with natural dyes, numerous variations have been in use in traditional dyeing operations. The dyeing process is dependent on the type of goods (flax, jute, wool, silk) and in particular on the plant material to be

Table 19.4 Important groups of dyeing procedures for natural dyes

Type of process	1. Step	2. Step
Direct dye	Dyeing	–
Pre-mordant	Mordanting	Dyeing
Meta-mordant	Dyeing-mordanting	–
After-mordant	Dyeing	Mordanting
Vat dyeing	Indigo vat	–

Direct dye, the dyestuff exhausts without any addition of fixatives; pre-mordant, the mordanting step is performed in a separate bath before the dyeing; meta-mordanting, the mordant is added into the dyebath; after-mordanting, the mordanting step is carried out after dyeing in a separate bath; vat dyeing, application requires addition of reducing chemicals/alkali.

used. As a result a great variety of dyeing procedures had been in use. Dyeing operations can be classified into different groups with regard to the chemistry during the application of the dyes; important classes are direct dyes, mordant dyes and vat dyes (mainly natural indigo). In Table 19.4, a classification of relevant natural dyeing operations is shown. Wash baths have not been considered.

Processes that permit the direct addition of the mordant into the dyebath are favourable, because this technique forms the basis for a one-bath dyeing process. However, losses of dyestuff due to partial precipitation of dyestuff can occur. Dyes that do not show high affinity to the substrate require pre-mordanting. This requires the introduction of a two-bath process and in some cases the intermediate storage of mordant baths is necessary. For a dyehouse, the use of separate baths for mordanting and dyeing is quite undesirable with regard to time of dyeing, further treatment or recycling of the mordanting baths.

Indigo represents the most important blue natural dye. As indigo is a vat dye the application requires the use of a special dyeing procedure. Traditionally this was an anaerobic bacterial reduction (vatting), which is nowadays replaced by use of powerful reducing agents, e.g. $Na_2S_2O_4$/NaOH. However, the dyeing of blue and green shades needs the introduction of an additional dyeing step which makes justification of advanced ecological processing quite difficult. Introduction of an additional bath for vat dyeing with indigo and a pre-mordanting bath to dye a green shade for example would mean replacing a one-bath dyeing procedure, based on synthetic dyes, with a series of three separate treatment steps.

19.2.3 Selection requirements

In the application of dyes in the past, different techniques of mordanting and post-treatment were used to improve colour fastness properties.[2,21–24]

Today the criteria to select a certain plant source as a possible raw material are more strict, and rigorous evaluation and comparison with the state of the art dyeing technology is necessary to form a class of natural dyes that meets existing requirements.

Selection of mordant and procedures
Mordants increase the fixation of dye molecules on the substrate by building metal complexes. Whilst natural colorants themselves are generally harmless, some mordants are not environmentally acceptable. Consequently selection has to be carried out, to avoid heavy metal contaminants in waste water and to fulfil governmental limits.[25,26] Traditional mordants, like iron salt (ferrous sulphate, Fe) and alum (Al), do not cause environmental or ecological problems.[27] Dyeings using mordants such as cobalt, tin or chromium salts will cause problems with the effluents released from the dyeing process because of the wastewater limits defined for the concentrations of heavy metals.[26]

Formation of dyestuff groups
The formation of a class of dyes with compatible procedures of application is of great importance, because this enables the dyer to produce a broad variation of shades. One-bath dyeing with direct addition of the mordant in the dyebath is most favourable. In such a dyeing step, mixtures of dyes and mixtures of mordants can be applied and a broad range of variation in shade and colour depth can be achieved. Furthermore, a dyer is able to correct deviations in the desired shade during dyeing by further addition of dyestuff or mordant. Figure 19.2 shows an example of the colour shade variation achievable by systematic variation of the composition of the iron/alum mordant, using a one-bath dyeing method.

19.2.4 Ecological impact

The textile industry is one of the biggest industrial consumers of water, so extensive data about the effluents have been collected and are available from the literature.[28] Depending on the extent of other treatments performed in a dyehouse, waste water from the dyeing step is diluted to a greater or lesser extent by effluents released, for example from desizing, scouring and bleaching.[29]

Table 19.5 shows the calculated chemical load in the waste water for dyeings using natural dyes and those using selected conventional dyeing processes. Numerous dyeing methods are applied technically for a certain fibre type, so two representatives were selected for each fibre. For cellulose fibres, dyeing with reactive dyes and direct dyes has been taken into consideration, while for wool, metal complex and reactive dyeings were chosen.[30–36] The most relevant parameters of the wastewater limits for textile effluents in Austria are also given in Table 19.5.[26]

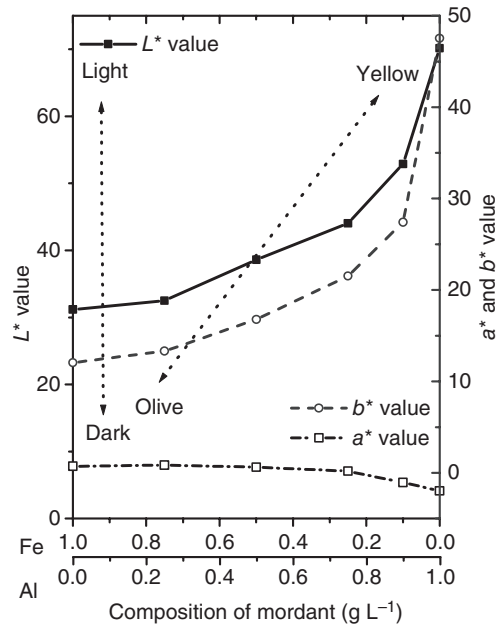

Fig. 19.2 Change of CIELab-coordinates obtained with Canadian Golden Rod (*Solidago canadensis* L.). CIELab-coordinate on wool; combination of mordants, $FeSO_4.7H_2O$ and $KAl(SO_4)_2.12H_2O$; 62 g plant material extracted with 1200 ml, dyeing liquor ratio 1:20. CIELab-coordinates: L^*, brightness (100 = white, 0 = black); a^*, red–green coordinate (positive = red, negative = green); b^*, yellow–blue coordinate (positive = yellow, negative = blue).

To facilitate a comparison of the techniques, the expected chemical load released directly into the waste water was calculated. The final concentration of chemicals was calculated for a dilution of the dyebath by a factor of four (dyebath plus three rinsing baths). Differences with regard to water consumption due to the different number of washing baths required to achieve the final fastness properties were not considered, because too many variations currently exist.

Cellulose fibres

Values for reactive dyeings on cellulose fibres were calculated from an average recipe for 1–2% of colour depth applied in exhaust dyeing at a liquor ratio of 1:20.[35] An upper limit for the sulphate concentration has been fixed, to avoid possible corrosion of concrete tubes. In the case of cellulose dyeing with reactive dyes, NaCl can be used instead of Na_2SO_4 but there is an increased risk of metal corrosion if NaCl is used. For cellulose fibres, the examples given show a distinct lowering of the chemical load in the waste water when natural dyes are used. With reactive dyes both

Table 19.5 Estimation of the chemical load released into the waste water. Natural dyes were considered as direct dyes, with iron or alum mordant; for comparison, average values for selected dyeing methods in use today are given. Limits given are valid for waste water released to a communal wastewater treatment plant in Austria[26]

Substrate	Dyestuff	Chemicals in dyebath	Final concentration in the waste water	Legal limits for textile effluents[26]
Cellulose fibre (linen)	Natural dye direct	–	–	–
	Fe mordant	1–5 g/L $FeSO_4 \cdot 7H_2O$	0.05–0.25 g/L $Fe^{2/3+}$	–
			0.086–0.43 g/L SO_4^{2-}	0.2 g/L SO_4^{2-}
	Al mordant	1–5 g/L $KAl(SO_4)_2 \cdot 12H_2O$	0.014–0.07 g/L Al^{3+}	–
			0.10–0.50 g/L SO_4^{2-}	0.2 g/L SO_4^{2-}
	Reactive dye	0.4 ml/L NaOH 50% (o.w.)	0.1 ml/L NaOH 50%	pH 6.5–9.5
		3 g/L Na_2CO_3	(o.w.)	
		40 g/L Na_2SO_4 (or NaCl)	0.75 g/L Na_2CO_3	0.2 g/L SO_4^{2-}
			10 g/L Na_2SO_4 (NaCl)	
	Direct dye	5–10 g/L Na_2SO_4	0.85–1.7 g/L SO_4^{2-}	0.2 g/L SO_4^{2-}
Protein fibre (wool)	Natural dye direct	–	–	–
	Fe mordant	1–5 g/L $FeSO_4 \cdot 7H_2O$	0.05–0.25 g/L $Fe^{2/3+}$	–
			0.086–0.43 g/L SO_4^{2-}	0.2 g/L
	Al mordant	1–5 g/L $KAl(SO_4)_2 \cdot 12H_2O$	0.014–0.07 g/L Al^{3+}	–
			0.10–0.50 g/L SO_4^{2-}	0.2 g/L
	Metal complex dyes	2.5 g/L Na_2SO_4	0.42 g/L SO_4^{2-}	0.2 g/L
		Cr content of dye		0.1 mg/L Cr^{6+}, 1 mg/L Cr^{3+}
		pH adjustment		pH 6.5–9.5
	Reactive dyes	pH adjustment		pH 6.5–9.5

o.w., of weight.

the alkalinity of the waste water and the salt content are rather high; with the use of direct dyes no alkali is required, however the salt concentration remains twice the concentration released from mordant dyeing.[36] Furthermore, iron sulphate or aluminium sulphate are compatible with the subsequent wastewater treatment, where such salts are added for flocculation and phosphate elimination.

Wool fibres
A comparison of natural dyeing processes with conventional wool dyeing methods leads to two different results: the use of metal complex dyes often requires the addition of Na_2SO_4 as a levelling agent which causes similar sulphate loads in the waste water. The bigger problem can arise from the metal content in the metal complex dyes, mainly due to the Cr content. For this element, the limits for textile waste water are fixed at 0.1 mg/L for Cr^{6+} and 1 mg/L for Cr^{3+}. The use of reactive dyes for wool causes low pollution, but careful selection of the dyestuffs is required and the method of application needs care, to obtain acceptable uniformity of the dyeing. As can be seen from Table 19.5 a lower chemical load is released from natural dyeing processes compared with technologies in use at present. From a sustainability viewpoint all the conventional techniques are based on synthetic dyes that are produced from non-regenerable sources while in the case of natural dyeings the dyestuffs are extracted from sustainable sources.

19.3 The extraction step

The extraction step is highlighted here from different points of view: raw material used (Section 19.3.1); chemical consumption (Section 19.3.2); and energy consumption (Section 19.3.3).

19.3.1 Raw materials

The successful introduction of natural dyes into commercial textile production is dependent on the formation of a standardised dye. A significant improvement in the overall consumption of energy, chemicals and water has to be reached in relation to the state of the art processes. Easy handling and storage of plant material with a minimum consumption of energy and formation of wastes has to be achieved. The material should contain a high content of extractable dyestuff. Production of commercial natural dyestuff can follow two different strategies:

- stabilised (e.g. dried) plant material, such as madder roots, green nuts and onion peels;[37]
- concentrated dyestuffs containing liquid or solid products from extraction processes.[38]

19.3.2 Chemical consumption during extraction step

In scientific investigations to identify the chemical nature of a natural dye, the use of solvents or chemicals is quite common, however for a full-scale process and commercial production of natural dyes the chemical consumption during the dyestuff extraction/stabilisation has to be thoroughly considered.[39,40] Extraction with the addition of organic solvent or chemicals requires strict calculation of chemical consumption due to the losses of chemicals with the extracted plant residues.

Detailed calculations given in the literature demonstrate the major problem of natural dye extraction. Approximately 1–5% o.w. of the plant can be expected to be extractable components useful for dyeing purposes. Considerable amounts of added solvent or chemicals will remain in the extracted residue. Figure 19.3 gives a graphic summary of model calculations for chemical losses in natural dye extraction.[18] When a rather high average dyestuff content of $P = 0.02$ is considered and 1 kg of solvent remains in 1 kg of extracted plant material, the value for the consumption of chemicals reaches 0.5 at $c = 0.01$ (10 g of chemicals added in per 1 kg of aqueous solvent) and 1.0 at $c = 0.02$. A value of 1 kg/kg for consumption indicates that 1 kg of chemicals will remain in the extracted plant wastes for each kilogram of natural dyestuff extracted. Thus great care has to be taken when chemicals or solvents other than water are used for dyestuff extraction.

19.3.3 Energy consumption

Direct use of the stabilised (e.g. dried) material in the dyehouse requires energy for stabilisation, handling and transport of large amounts of plant

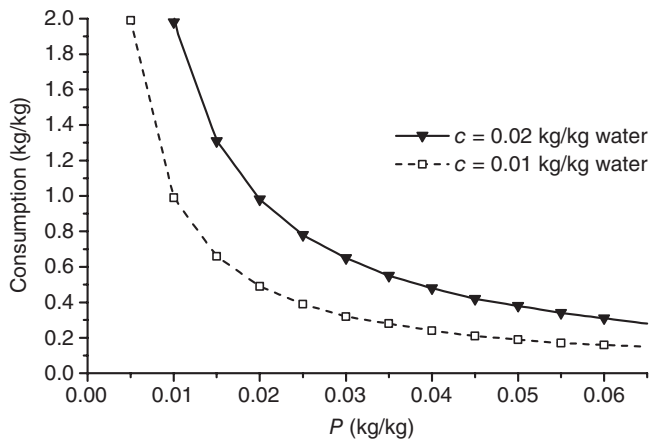

Fig. 19.3 Consumption of chemicals during dyestuff extraction, defined as mass of chemicals per mass of extracted dyestuff (kg/kg), as a function of dyestuff content, P (kg dyestuff per kg plant material), in the plant source, calculated for different concentrations, c, of chemicals used in water.

material. Formation of extracts requires energy consumption for concentration but final volumes have to be transported. A remarkable difference between these two production strategies exists, when estimated energy consumption is compared (Table 19.6). In both cases wet plant material was considered as the raw material.

In the case of extract formation and production of a concentrated product, energy is required for extraction at the site of processing, reconcentration of the product and transport to the dyehouse. In addition, heating the dyebath to 95 °C consumes energy. Besides evaporation, membrane technology has been considered as an alternative technology for reconcentration (Table 19.6). A quite low value of 1:5 has been considered as the liquor ratio for the extraction step. This value is of particular importance with regard to the total consumption of energy, because about 50% of the energy consumption will be spent removing water during the production of the concentrated dye. The energy consumption for transportation remains low for short-distance transportation (<100 km). A considerable amount of energy will be required for heating the dyebath to 95 °C, as coupling between extract formation and the dyeing step is not possible. For concentrate formation and dyeing process together, the energy consumption is estimated to range from 600 to 900 MJ for 100 kg of goods.

In cases of direct use of the wet plant material, the material is transported to the dyehouse and an aqueous extraction step is performed in the dyeing apparatus before the dyeing step starts. In this case the 95 °C hot extract can be used directly as the dyebath, thereby saving considerable amounts of energy. The main energy consumption will be for transportation and hot extraction. For stabilisation by drying, additional energy is required. The total energy consumption ranges from 300 to 500 MJ.

Independent of the strategy used to produce the natural dyestuff, in both cases approximately 200 kg of wet plant material will be released after the extraction. In the case of aqueous extraction the extracted plant material can be processed as usual, e.g. as animal feed, for composting, for use as energy crop or disposal/landfill because no chemical load has to be removed before further use.

19.4 Sources for natural dyes – results of a screening for sources in food processing

From a dyer's point of view the coloration technology itself may be more important than the source of the dyestuff,[3] particularly when total costs are at comparable levels. One main benefit of synthetically produced dyes is their tinctorial strength. For an average colour depth of 2%, 2 g of synthetic dyestuff is used per 100 g cotton, while for a similar shade 100–300 g of dry plant material is required.[3,7] Besides their different ecological profiles, natural dyes also have to compete with synthetic dyes with regard

Table 19.6 Energy balances for handling, transportation, extraction and concentration steps during dyestuff production from 100 kg wet plant material (including the dyeing process for 100 kg goods)

	Production of a concentrated dyestuff	Energy consumption for 100 kg of bark	Extraction of crop in dyehouse	Energy consumption for 100 kg of bark
Formation of commercial product	**Extraction** (LR 1:5, temperature 95 °C)	$E = 157$ MJ	**Drying** for stabilisation	$E = 180$ MJ
	Concentration (final volume 10 L product)			
	Evaporation from 400 L to 10 L (membrane concentration to 10 L)	$E = 421$ MJ $E = 140$ MJ		
	Waste (wet extracted material, approx. mass = 200 kg)			
Transportation (<100 km)	10 kg of concentrated extract	$E = 0.7$ MJ	50 kg of dried material (or 100 kg of wet material)	$E = 3.5$ MJ $E = 7$ MJ
Dyeing 100 kg goods, LR 1:10, 95 °C	Preparation of dyebath	$E = 314$ MJ	Preparation of dyebath and extraction of bark Waste: wet extracted material approx. mass = 200 kg	$E = 314$ MJ
Total energy consumption	Conc. by evaporation Conc. by membrane process	$E = 892.7$ MJ $E = 611.7$ MJ	Stabilisation by drying Without drying	$E = 497.5$ MJ $E = 321.0$ MJ

Conc., concentration; LR, liquid ratio (mass of solids per volume of liquid).

to the costs required to achieve a certain colour depth. Production of dyestuff-containing plants by direct farming results in very high specific costs. Growing and harvesting of traditional dye plants like madder, weld and woad is expensive and time consuming,[41] although recently considerable research work on the cultivation of plants for plant dyes has been carried out.[5,40,42–45] In addition to the high price, the required farmland is not available.[3,41,46,47]

To improve cost effectiveness, alternative sources of dye-containing plant material have been studied. Instead of cultivation of dye plants, some plants could be gathered from the wild, like dyer's greenweed (*Genista tinctoria* L.) and Canada goldenrod (*Solidago canadiensis* L.).[7,41] From the literature, not only traditional dye plants, but also berries, fruits, vegetables, peels, roots and barks are known as potential sources for dyestuff extraction.[2] In an extensive evaluation of new sources for colour-containing plant material, the food and beverage industries were found to release considerable amounts of cheap vegetable material as wastes.[6,7,19,48] These sources for natural dyes are less effective in terms of dyestuff content compared with the raw material, nevertheless remarkable amounts of natural dyes can be extracted.[19] Wastes from fruit and vegetable processing exhibit several advantages:[22]

- low price,
- sizeable amounts are available (however they are seasonally dependent),
- non-hazardous material, defined hygienic standards,
- high quality standards of released material.

Another important source for natural dyes was identified in the wood processing industry which releases enormous amounts of bark.[11,18,48] Based on these different sources for plant material, the calculated price for vegetable dyes can be expected to be comparable with synthetically produced colorants.[7] Screening studies showed that not all colour-containing wastes are suitable for textile dyeing. Handling and processing, application facilities, dyeability, available colour and fastness properties have to be investigated to identify suitable plant material.

While a range of brown to olive shades can be obtained from barks released as wastes from the timber industry, there is still a need for brilliant red and blue colours.[49] Brilliant red dyes could be extracted from pressed berries and grape pomace from the beverage industry.[19] For green, blue and black shades only a few sources are available – e.g. natural indigo, privet berries, hollyhock buds, extracts from barks – and further research is required. A broad study to evaluate the potential of wastes released from the food and beverage industries has led to the identification of some of the relevant aspects to be considered:

- Storage and standardisation of material differ and are dependent on the source. Stabilisation of material by drying and freezing (energy consumption!) forms a basis to overcome problems due to the seasonal release of waste and changes in product quality.
- Enormous amounts of plant material have to be handled. Due to the low content of dyestuff, approximately the same amount of plant material as textile good has to be extracted.
- The extracted material will appear after extraction with at least the same mass of waste; in the case of dried raw material, the water content will almost double the mass of waste.

A brief overview of the outcome of the screening study is given in Tables 19.7 to 19.9. A separation into three groups was performed:

- wastes from berry and grape processing (juices, wine, strong liquor, beverages);
- wastes from vegetable processing (food industry);
- wastes from tea, fruit and nut processing (beverage and food industry, and others).

Only selected representatives of the experimental results are shown in the tables. Laboratory dyeings were characterised by shade, CIELab-coordinates and selected fastness properties. The results of all experiments were summarised in a pass/fail decision, which identifies sources that exhibit sufficient potential and could serve as a source for natural dye extraction.

19.5 Natural dyes from food processing wastes – representative examples

The extract quality of selected examples is discussed in Section 19.5.1, sources presented in more detail are onion peels (19.5.2), nuts (19.5.3), berries (19.5.4), grape pomace (19.5.5) and tea residues (19.5.6).

19.5.1 Colour strength of extract

A comparison of the amount of extracted dyestuff can be made by comparison with commercial reactive dyes. In the literature, Reactive Red 4 (Cibacron Brillant Red 3B-A, Ciba, Basel) was taken as a representative dyestuff.[19] Based on the absorbance of this dye, the absorbance of plant material extracts can be used to calculate an equivalent concentration of reactive dye c_{eq}, which will show the same absorbance in solution.[29] The calculation can be performed according to equation 19.1:

$$c_{eq} = \frac{E_{extr}}{\varepsilon_{RR4} d} \qquad [19.1]$$

Table 19.7 Berries and grapes: results of screening for natural dyes, selected colour and fastness properties and assessment of properties as pass/fail

Raw material	Part of plant	Source	Representative data								Pass/fail
			Substrate	Mordant	Colour	L^*	a^*	b^*	LF	WF	
Black chokeberry	Berries	Conc. juice	Wool	–	Rose	46.22	+13.47	+6.25	2	4–5	Pass[†]
Blackberry	Berries	Pomace	Wool	–	Beige	63.83	+5.28	+14.51	–	5	[†]
Cherry	Cherries	Distiller's wash	Wool	–	Rose	56.39	+6.43	+6.48	2	4–5	Fail
Sour cherries	Berries	Pomace	Wool	Fe	Beige	55.44	–0.56	+10.27	2	4–5	Fail
Raspberry	Berries	Pomace	Wool	Fe	Grey	52.70	–2.02	+7.85	3	4–5	Pass
Raspberry	Berries	Distiller's wash	Wool	Fe	Grey	40.74	+1.86	+3.40	3–4	5	Pass
Elder	Berries	Pomace	Wool	–	Red-brown	54.36	+4.70	+5.29	2	4–5	Pass*
Elder	Berries	Distiller's wash	Wool	–	Rose	37.13	+13.69	+8.66	2	3–4	Pass[†]
Elder	Berries	Conc. juice	Wool	Al	Violet	41.60	+21.25	+3.27	3	4	Pass[†]
Blackcurrant	Berries	Pomace	Wool	Al	Grey	53.92	–0.40	–4.08	2–3	5	Pass
Blackcurrant	Berries	Conc. juice	Wool	Al	Pink	51.45	+14.94	+6.55	2–3	3–4	Pass[†]
Grapes	Berries	Pomace	Wool	–	Mauve	59.91	+6.76	+5.32	2	3–4	Pass*

LF, light fastness; WF, water fastness. Water fastness: 1 = poor; 5 = excellent. Light fastness: 1 = poor; 8 = excellent.
* Further research needed, improvement of fastness.
[†] Model tests, not real waste.

Table 19.8 Vegetables: results of screening for natural dyes, selected colour and fastness properties and assessment of properties as pass/fail

Raw material	Part of plant	Source	Representative data								Pass/fail
			Substrate	Mordant	Colour	*L**	*a**	*b**	LF	WF	
Red cabbage	Leaves of fruit	–	Wool	Fe	Olive	47.66	–1.68	+11.61	2–3	5	Pass*†
Blue potato	Potato	–	Wool	Fe	Beige	72.91	+3.27	–0.77	2	–	Fail†
Beans	Crop	–	Wool	Fe	Beige	78.28	+3.33	+13.67	3	4	Fail†
Peas	Crop	–	Wool	Fe	Brown	68.48	+4.81	+25.05	4	–	Fail†
Onion	Peels	Wastes	Wool	–	Orange	52.96	+16.17	+46.58	3	4–5	Pass
Spinach	Plant	–	Wool	Al	Light yellow	84.31	–5.63	+28.34	1–2	4–5	Fail†
Carrots	Plant	–	Wool	Fe	Beige	67.51	+5.30	+25.98	2–3	–	Fail†
Black carrots	Crop	Conc. juice	Wool	Al	Violet	44.92	+22.85	+1.67	3	4	Pass†
Beetroot	Crop	–	Wool	–	Red	47.80	+25.69	+14.64	1	4–5	Pass*†
Rhubarb	Roots	Farming	Wool	Fe	Olive	28.99	–0.29	+19.75	2–3	5	Pass†

LF, light fastness; WF, water fastness.
* Further research needed, improvement of fastness.
† Model tests, not real waste.

Table 19.9 Tea, fruits and nuts: results of screening for natural dyes, selected colour and fastness properties and assessment of properties as pass/fail

Raw material	Part of plant	Source	Representative data								Pass/fail
			Substrate	Mordant	Colour	*L**	*a**	*b**	LF	WF	
Walnut	Green shell	Processing of green nuts	Wool	Al	Brown	43.28	+9.59	+17.62	4	3–4	Pass
Walnut	Brown shell	Processing of walnut	Wool	–	Beige	68.65	+3.67	+17.35	3–4	4–5	Pass
Walnut	Young shoot	–	Wool	Fe	Beige	52.29	–0.57	+7.53	3–4	4	Pass
Tea	Leaves	Pomace from ice-tea	Wool	–	Beige	64.94	+4.13	+18.12	3	5	Pass
Hollyhock	Buds	–	Wool	Fe	Green	29.48	–1.87	+2.16	2–3	4–5	Pass[†]
Barberry	Branch, roots	–	Wool	Al	Yellow	79.97	–8.25	+43.37	1	4	Fail[†]
Pomegranate	Peels	–	Wool	Al	Yellow	66.88	+0.98	+40.55	1	4–5	Pass*

LF, light fastness; WF, water fastness.
* Further research needed, improvement of fastness.
[†] Model tests, not real waste.

where c_{eq} is the equivalent concentration of Reactive Red 4 (in g/L), E_{extr} is the absorbance of the extract, ε_{RR4} is the extinction coefficient of commercial Reactive Red 4 (9.7 L/cm g at $\lambda = 508$ nm), d is the path length of the cuvette (in cm).

The equivalent concentration c_{eq} – calculated as the mass (in g) of Reactive Red 4 dye per 1 litre of extract – obtained from different materials is given in Table 19.10. Depending on the material used for extraction the maximum absorbance obtained during extraction of the different samples ranges from 0.7 (grapes) to 28.1 (rhubarb), which corresponds to a dyestuff concentration c_{eq} of 0.62–2.9 g/L (calculated as commercial reactive dye). The theoretical equivalence between 1 kg of plant material and the commercial reactive dye Cibacron Brillant Red 3B-A is also shown in Table 19.10. Provided a comparable degree of dyestuff fixation is achieved on the textile substrate, equivalence in colour strength to commercial reactive dyes can be calculated. Extraction of 1 kg of plant waste yields solutions containing natural dyestuff, equivalent to an amount of 0.62–57.9 g of commercial Reactive Red 4. These values indicate substantial potential for application of the extracts as a source for natural dyes.

19.5.2 Flavonoid dyes – onion peels (*Allium cepa* L.)

The peels of onions, particularly red onion peels, are wildly used for egg coloration at home, but they are also applicable for textile dyeing. The outermost dry papery skins of onions contain different colouring substances. In addition to the flavonoid dyes, for example quercetin, its glucosides and cempferol, the extract also contains tannins (see Fig. 19.4 for the structure of quercetin).[2,50] As shown in Table 19.10 the high absorbance of the extracts indicates the remarkable colouristic potential of onion peels; 1 kg of dried

Table 19.10 Dyestuff extraction: maximum absorbance E obtained during extraction using a liquor ratio of 1:20, colour yield calculated as equivalent concentration of commercial reactive dye c_{eq} and equivalence mass m_{eq} given as mass of commercial reactive dyestuff Reactive Red 4 equivalent to the extract of 1 kg of plant residue

Source		Physical state	Major class of dye component	E (wavelength) (nm)	c_{eq} (g/L)	m_{eq} (g/kg)
Raspberries	Pomace	Solid	Anthocyan	2.25 (437)	0.23	4.7
Black elder	Pomace	Solid	Anthocyan	1.05 (523)	0.11	2.2
Blackcurrant	Pomace	Solid	Anthocyan	1.15 (519)	0.12	2.4
Grapes	Pomace	Solid	Anthocyan	0.7 (519)	0.07	1.4
Onions	Peels	Solid	Flavonoid	15.5 (450)	1.60	32.0
Rhubarb	Roots	Solid	Anthraquinone	28.1 (405)	2.90	57.9
Black tea	Pomace	Solid	Tannins	1.56	0.16	3.2

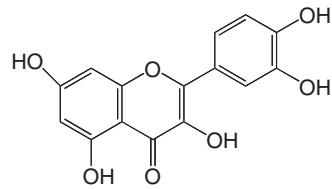

Fig. 19.4 Structure of quercetin as a representative for flavenoid dyes.

onion peels corresponds to an equivalent amount of 32 g of commercial reactive dye. The annual amount of onions harvested in Austria is about 100 000 tonnes[51] and 10% of this amount is red skin onions.[20,51] Onions lose their outermost papery skin during handling or the peels are removed before final use. Traditionally the peels are wastes that are collected and released to farming areas for composting (K. Wais, Karl Wais GmbH (large-scale vegetable merchandising company, personal communication, 2005).[20] Thus the material is available in considerable amounts and at low costs. The low specific weight of onion peels may require compression to lower volumes for transportation. Dyeing experiments showed a wide variety of orange/brown/olive shades depending on substrate, mordant and dyeing technology. The substrate's influence on the shade and fastness can be seen more clearly in Table 19.11.

For all types of mordant shown, dyeings on wool and polyamide exhibit darker shades, while for cellulose fibres (flax) lighter shades are observed. The possibility of changing the shade of a dyeing by the changing the mordant is demonstrated for all three types of fibres. Mordanting with alum results in orange shades whereas addition of iron$^{2+/3+}$ salt changes colour towards olive shades. In the case of onion peels, the use of iron mordant increases light fastness by at least one mark. Water fastness is hardly dependent on the type of substrate, however on flax a remarkable decrease in light fastness from 3 to 1–2 can be observed for direct dyeing and alum mordant. Onion peel extracts are thus only recommended on cellulose textiles when an iron mordant is used.

19.5.3 Naphtoquinone dyes – nuts (*Juglans regia* L.)

In the colour index (C.I.), natural dyes extracted from walnuts are identified as C.I. Natural Brown 7. Walnuts (*Juglans Regia* L.) have been widely used for dyeing textiles and hair since the middle ages. Some of the oldest still-available recipes are collected in the *Innsbrucker Handschrift* that were written in 1330 in Tyrol and can now be seen in the library of the University of Innsbruck.[52] The tree species was imported from Asia 100 years before Christ. Marcus Terentius Varro brought *Juglans Regia* L. to Italy where it was re-cultivated.[2,53] Traditionally the green shells and young leaves are used for dyeing purposes and beige to brown shades are obtained on wool,

Table 19.11 Onion peels – representative dyeings on different substrates; influence of substrate on colour and fastness properties

Substrate	Mordant	Colour	*L**	*a**	*b**	LF	WF
Wool	–	Orange	49.53	+19.29	+33.06	3	4
Polyamide	–	Brown	48.64	+13.91	+33.64	3	4–5
Flax	–	Brown	68.02	+8.14	+22.73	1–2	4
Wool	Al	Orange	52.96	+16.17	+46.58	3	4–5
Polyamide	Al	Orange	54.10	+15.53	+54.90	3	4–5
Flax	Al	Orange	62.73	+11.20	+54.49	1–2	5
Wool	Fe	Olive	35.57	+0.28	+12.44	4	3–4
Polyamide	Fe	Olive	29.66	+4.57	+18.49	4	4–5
Flax	Fe	Olive	43.74	+0.12	+13.91	3	5

LF, light fastness; WF, water fastness.

Table 19.12 Walnut, C.I. Natural Brown 7 – results of dyeings obtained with different parts of walnut trees

Plant material	Substrate	Mordant	*L**	*a**	*b**	DE	LF	WF
Green shells	Wool	–	43.2	+10.04	+21.18	0	4	3–4
		Al	44.82	+11.25	+23.28	2.9	3–4	3
		Fe	35.65	+3.53	+11.36	14.0	3–4	3–4
	Cotton	–	65.35	+0.79	+15.35	0	4	2–3
		Al	63.21	+2.04	+14.79	2.5	4	3–4
		Fe	57.54	+0.36	+9.66	9.7	3–4	4–5
Brown shells	Wool	–	68.65	+3.67	+17.35	0	3–4	4–5
		Al	70.05	+2.66	+21.55	4.5	4	4–5
	Flax	Fe	49.03	–0.62	+6.82	22.7	3–4	4–5
		–	80.77	+0.53	+10.41	0	4	4
		Al	72.45	+2.34	+13.52	9.1	3–4	4
		Fe	71.39	–0.89	+5.83	10.5	3–4	4
Young shoots	Wool	–	69.93	+2.87	+16.5	0	3–4	4–5
		Al	71.62	–1.2	+29.66	13.9	3–4	4–5
		Fe	39.04	–0.48	+6.7	32.6	3–4	5
	Cotton	–	86.78	–0.88	+6.75	0	4	4–5
		Al	81.74	–2.7	+14.99	9.8	3–4	4–5
		Fe	72.84	–1.66	+3.89	14.3	3–4	4–5

DE, CIELab colour difference between direct dyeing and mordant dyeing.

flax, cotton and polyamide (Table 19.12). In Table 19.12 a comparison of the different dyeings obtained from the various sources of walnut-based plant material is shown.

Walnut leaves contain juglon precursor molecules that change easily to juglon, a quinone compound (see Fig. 19.5). The relative unstable juglon polymerises and results in brown pigments. Besides flavone glycosides, 9–11% o.w. tannins have been identified. The green shells of walnut mainly contain juglon, juglon derivatives and tannin. Collection of green nuts

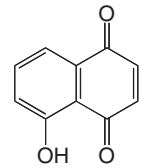

Fig. 19.5 Structure of juglon as a representative for naphthoquinone dyes.

requires harvesting the complete crop and thus green nuts cannot be said to be real waste. However, considerable numbers of green nuts fall off the trees during the ripening period, and can be seen as a waste product from walnut growth. The potential of walnuts to serve as a source for natural dyes can be estimated from the volume of walnuts harvested in Austria. In 2003, approximately 20 300 tonnes of walnuts were collected and approximately 50% of the weight can be assumed to be released as wastes (mainly dried shells).[20] Green nut shells are rather expensive. The price of 1 kg of green dried nut shells differs wildly from €3 to €10 depending on the company's location, distance of transportation and quality.[6,20]

Alternatively, brown shells from ripe walnuts can be collected and used for dyeing purposes. The material is cheaper and is released as waste, however the colour depths are much lighter (Table 19.12). Green walnut shells are preferable but young shoots and brown shells still contain enough colouring matter to give reasonable results.

Mordanting with iron salt causes remarkable colour changes due to complex formation. While colour differences between direct dyeing and alum mordanting are quite small, iron salt mordanting increases colour depth significantly. On cellulose substrates and wool, good light fastness values near 4 are observed. Results on polyamide fibres are disappointing (light fastness 1–2). Water fastness is not affected as much by the type of substrate. The distinct dependence of light fastness on substrate indicates the need to determine the fastness properties for any selected source on the chosen substrate.

There are other kinds of nut species that can serve as a source for natural dye extraction. Hazel (*Corylus avellana* L.) leaves contain the colouring substance myricitrin, a flavonoid dye. The extract of the leaves, and fixation with alum, leads to yellow colour shades. In Scotland, hazel willows have been used for dyeing using various recipes, e.g. ripe willows were extracted and dyeing was performed adding alum and ammonium hydroxide to obtain a dark yellow shade.[2]

19.5.4 Anthocyanin dyes – berries

Anthocyanin dyes are present in many intensively coloured parts of plants like fruits and blossoms. Anthocyanins are glycoside derivatives of

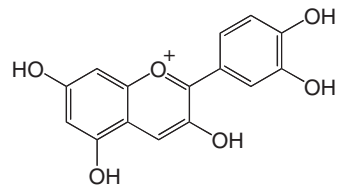

Fig. 19.6 Structure of cyanidin as a representative for anthocyanine dyes.

Table 19.13 Representative examples for berry wastes released in Austria

Berries	Latin name	Residue	Physical form
Blackberry	*Rubus fruticous* L.	Pomace	Solid
Blackcurrant	*Ribes nigrum* L.	Pomace	Solid
Elder	*Sambucus nigra* L.	Pomace	Solid
		Distiller's wash	Pulp
		Conc. juice	Liquid
Raspberry	*Rubus idaeus* L.	Pomace	Solid
		Distiller's wash	Pulp

anthocyanidins (see Fig. 19.6). The glycosidic group increases solubility in water. Depending on the position and number of glycosidic groups, shades vary from orange-red to blue. A mixture of anthocyanin compounds can be extracted from ripe berries, however cultivation of berries exclusively for dyestuff production is prohibited by the high costs of the material. Wastes released from the beverage industry and strong liquor production are available at low costs and could serve as possible sources for natural dyes.

Table 19.13 gives several examples of berry wastes released in Austrian companies.

In the case of the beverage industry, the major part of the colouring matter is intended to be transferred into the juice fraction. However, useful amounts of extractable dyestuff remain in the pressed residue, the so-called 'pomace' (Table 19.10). These residues are released in the wet state and extraction has to follow immediately or stabilisation by drying or freezing is required.[20] When the extraction is done exclusively with water, no changes in the further handling of the extracted wastes are required; the wastes can be used, for example, as animal feed.

Another source for dye-containing waste is the well-known strong liquor production in Austria. Plant material (berries, fruits and vegetables) that contains sugar or starch is fermented and high-quality spirit is obtained by distillation. After distillation the remaining plant residue, so-called 'distiller's wash', contains considerable amounts of anthocyanin dyes. Due to the high water content of the waste, immediate use is recommended or possible

microbial growth has to be hindered. Residues from the beverage industry and from spirit production are non-hazardous wastes. Thus at present there is only limited information available about amounts and classification of such wastes. Some limited data about agricultural production and processed amounts of berries are available.[51] Approximately 10–15% of the processed crop are released in the form of pressed solid wastes. In Europe, the average amount of waste released for certain types of berries can be estimated at 200–500 tonnes per year.[19]

The shade of anthocyanin dyes is very sensitive to the pH value. To avoid undesired changes of colour, the dyeing conditions, use of mordants and planned applications of the textiles have to be considered carefully. In a scientific study, concentrated juice from different berries were included in the tests to evaluate the potential of such materials for textile dyeing at optimum conditions with regard to the plant material. These results can be used to estimate the potential of a certain type of waste. Table 19.14 shows the results obtained with wastes from the processing of elder berries and with the use of the concentrated juice. Dyeings with concentrated juice exhibit violet shades while application of berry wastes predominantly leads to grey and beige shades.

While dried pomace was extracted with water, the liquid products (concentrated juice and distiller's wash) were just filtered before use. Considering the *L* values (lightness), as expected the exhaustion of dyestuff on wool is higher than on cotton. Normal standard dyeing procedures were performed at 95 °C. However in case of anthocyan-containing sources, lower dyeing temperatures were found favourable in terms of the shade of the dyeings

Table 19.14 Elder residues – results of dyeings obtained with different wastes from elder processing

Raw material	Substrate	Mordant	Colour	*L**	*a**	*b**	LF	WF
Distiller's wash	Wool	–	Brown	37.13	+13.69	+8.66	2	4–5
		Al	Olive	35.38	+2.75	+7.40	1–2	4–5
		Fe	Dark grey	22.68	–0.65	+2.77	2–3	5
	Cotton	–	Brown	58.97	+9.32	+2.37	2	3–4
		Al	Grey	55.46	+4.24	+1.24	2	4–5
		Fe	Dark grey	51.27	–0.48	–2.10	2	4–5
Pomace	Wool	–	Rose	61.06	+4.64	+8.34	2	4–5
		Al	Light grey	60.33	–0.02	+2.33	2–3	4–5
		Fe	Grey	46.78	–2.98	+2.06	1–2	4–5
	Cotton	–	Light rose	82.12	+4.11	+1.86	3	3–4
		Al	Light grey	78.13	+0.08	+0.27	2–3	3–4
		Fe	Light grey	73.77	–1.59	+0.48	2–3	4–5
Conc. juice	Wool	Al	Violet	33.07	+10.86	+4.83	2–3	4
		Fe	Brown-violet	43.14	+19.17	+4.93	1	3–4

(see Section 19.5.5). In the case of residues from strong liquor production, dyeings were performed at room temperature. Dyeing at room temperature does not change light fastness but wash fastness decreases. The shade obtained with anthocyanin dyes is promising, however light fastness and pH dependence of colour will require further improvement.

19.5.5 Anthocyan dyes – grape peels from vine production (*Vitis vinifera* L.)

Production of red vines is quite an important part of agricultural production in Austria. Various sorts of vine grapes are cultivated in the eastern regions of Austria, for example: Zweigelt, Blauer Portugieser, Blauer Burgunder and Cabernet Sauvignon. The pressed grapes contain considerable amounts of red–violet anthocyan dyes, which can be extracted and used for dyeing purposes. An estimation of the amount of grapes harvested for vine production in Austria comes near 100 000 tons per year, and approximately 20–25% of the mass will be released as waste. From these residues anthocyan dyes can be extracted with hot water. The red–violet shade of the extractable dyes in particular, makes them highly interesting for natural dyeing processes. The aqueous extract contains considerable amounts of red dyes, but dyeings obtained on cellulose or woollen samples using the standard dyeing process at 95 °C only showed disappointing colour. Satisfying results could be obtained after the introduction of pre-mordanting based on tannin mordant and lowering dyeing temperature.

Table 19.15 shows dyeing results given as CIELab-values for dyeing obtained with extracted pressed Zweigelt and Blauer Portugieser grapes using the standard process (95 °C) and the modified pre-mordant process. The considerable increase in the *a* value (red axis) and blue axis (negative *b* value) demonstrate the positive results. Dried grape pomace was used for

Table 19.15 Dyeing results obtained with extracts from pressed Zweigelt and Blauer Portugieser grapes

Material	Substrate	Mordant	T (°C)	L^*	a^*	b^*	LF	WF
Zweigelt								
	Wool	–	95	60.17	+2.97	+7.10	1	4–5
	Wool	Al (meta-)	95	64.15	+3.65	+8.78	1	4–5
	Cotton	Tannin (pre-)	RT	60.54	+15.32	–5.44	1–2	3–4
Blauer Portugieser								
	Wool	–	95	66.31	+3.40	+9.56	1–2	4–5
	Cotton	–	95	76.71	+4.62	–1.81	1	4–5
	Cotton	Tannin (pre-)	RT	63.80	+12.01	–3.53	1	3–4

RT, room temperature.

the examples given in Table 19.15. The wet residue after extraction showed an approximately three-fold increase in mass due to water uptake.

Analytical methods to determine the content of dyestuff in the extract are of importance to standardise a raw material for commercial use. In the case of grape extracts, ultraviolet spectroscopy can be applied to determine the concentration of anthocyanins in the extracted material.[54] A direct correlation between the analytically determined anthocyanin concentration calculated as monomer anthocyanin pigment and the Kubelka–Munk K/S value of the dyed fabric, measured at 550 nm, proves the suitability of the photometric method for standardising plant residues.[55]

19.5.6 Condensed tannins – tea (Theaceae)

Particularly in Far Eastern countries tea plants have been used for textile dyeing purposes and research is in progress (see Fig. 19.7). The dyeing of cotton and jute with tea as a natural dye – using alum, copper sulphate or ferrous sulphate mordants – has been studied by Deo and Desai.[22] Ice-tea holds a remarkable share in the market of soft drinks. Considerable amounts of residues from industrial production are available. In Austria the amount of wastes released from industrial tea production can be estimated at 200–300 tonnes per year. The colour strength of the residues is low, because the majority of the hot-water-soluble coloured components have been extracted during the ice-tea production. One kilogram of dried tea residues is equivalent to 3.3 g of commercial dyestuff.[19] Relevant data for colour, i.e. CIELab-coordinates, and fastness properties are summarised in Table 19.16.

The colour of dyeings based on tea residues ranges from beige to grey. The high level of colour fastness and production under quite standardised conditions make these residues very interesting for future use as a source for natural dyes.

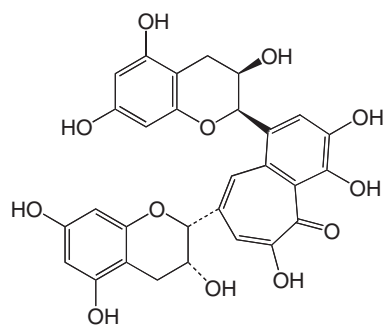

Fig. 19.7 Structure of theaflavin as a representative for condensed tannin dyes.

Table 19.16 CIELab-colour coordinates and selected fastness properties for dyeings obtained with black-tea residues from ice-tea production

Material	Mordant	L^*	a^*	b^*	LF	WF
Wool	–	64.64	+3.72	+17.60	3–4	4–5
	Fe	41.54	+0.79	+5.06	3	5
	Al	67.55	+2.84	+21.79	4	5
Cotton (bleached)	–	85.19	+0.75	+9.12	3–4	4–5
	Fe	75.38	–0.62	+2.55	3	4–5
	Al	78.07	+2.04	+12.56	3	4–5
Flax (unbleached)	Al	57.53	+3.64	+16.05	4	–

19.6 Future trends

The use of natural dyes for textile dyeing operations is of growing public interest. It is hoped that application of natural dyes could lead towards improved sustainability in parallel with reduced consumption of energy and chemicals. This requires careful consideration of each particular source for natural dyes and the corresponding application procedure is required to ensure the attempted optimum in the quality of a certain dyeing. Production of natural dyes by direct farming cannot be expected to result in a substantial replacement of synthetic dyes because the high costs of the plant material will hinder broad application. The overall properties of the dyeings (colour and fastness) are limited, and the energy consumption and mass balances are not convincing.

However, wastes from the food and beverage industries can make a substantial contribution to improving the use of natural dyes, enhancing sustainability and achieving a better cost structure. The brilliant colours of the dyes that can be obtained by extraction of wastes from fruit, berries and vegetable processing are of significant importance in the formation of a class of plant dyes that covers a broad range of colouristic shades. These wastes are released at a high quality level, in technically useful amounts and at very low costs. Standardisation of such materials could be achieved with the appropriate investment. Extraction with water will not prevent further use of the extracted waste, which is a fundamental requirement in the handling of such huge amounts of extracted plant wastes.

Further research is required to improve the quality of the dyeings to the level set at present by synthetic dyes. In particular, research activities will be needed:

- to improve the fastness properties, e.g. light fastness;
- to supply dyehouses with natural-dye-containing wastes of standardised quality;
- to supply dyehouses and textile manufacturers with the information required for marketing activities.

19.7 Sources of further information and advice

An excellent overview of natural dyes in textile dyeing is given by Schweppe.[2] A collection of the sources and chemical basis of natural dyes is presented and numerous details about procedures and results are given both on a chemical basis and from a historical view. Useful information about analytical procedures is also given in this handbook. Bhattacharya *et al*.[23] investigated the properties of selected natural dyes on jute. Nishida and Kobayashi[24] reported properties of natural dyes on silk, cotton and cashimilon using alum or ferrous sulphate mordants. Sorption behaviour of selected natural dyes on wool has also been studied and results are presented in the literature.[56] Brückner *et al*.[57] investigated the colour depth and fastness properties of selected natural dyes on wool and on synthetic fibres, e.g. polyester, polyamide and polyacrylnitrile. Lokhande *et al*.[50] presented results with selected natural dyes on polyamide using various mordants, e.g. alum, ferrous sulphate, stannous chloride and tannic acid. A number of authors describe results obtained with natural dyes using various methods and plant sources, which can be helpful in the design and scale-up of laboratory processes.[58,59]

Analytical procedures for the quantification of a certain class of extracted dye component can be helpful to determine the quality of the plant material and the concentration of dyes in the extract. A number of recent papers describe useful information about analytical procedures for anthocyanins,[54,60] phenolic components[61] and carotenoids.[62] Selected analytical procedures to characterise dyeings from plant extracts using traditional Korean methods are available in the literature.[63] Recently published papers also address the problem of the low light fastness of natural dyes.[15,16,64]

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20

Improving waste management and co-product recovery in vegetable oil processing

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20.1 Introduction

Vegetable oils and fats are principally used for human consumption but are also used in animal feed, for medicinal purposes and for certain technical applications. The vegetable oil market is strongly correlated to the protein meal market as both are largely co-products resulting from the processing of oilseeds. Supply and demand conditions in one market affect the other. For 1999–2000, world production of edible oil was 86.4 million tonnes, consisting of 85.2 million tonnes of vegetable oil and 1.2 million tonnes of marine oil (AAFC, 2000). Among vegetable oils, palm oil is the oil produced in greatest amounts. In 1998, 18.2 million tonnes of palm oil were produced worldwide (AAFC, 2000). The production in south-east Asia was 14.95 million tonnes per year, with Malaysia contributing 8.6 million, Indonesia 5.9 million, and Thailand 0.45 million tonnes per year (FAO, 1998). Due to the rising demand for plant oil for food, feed and technical applications, oil palm plantations were rapidly extended in Indonesia, which is the leading palm oil producer together with Malaysia, and led to an increased production of 7.5 million tonnes in 2000 in Indonesia (Pamin, 1998).

In the past few decades huge amounts of vegetable oil wastes have been produced worldwide. For the production of 1 tonne of crude palm oil, far more than 1 tonne of empty fruit bunches is left behind as a waste product, consisting of 16% lignin, 50% cellulose, 23% hemicellulose, 3.6% oil and 8% ash. The solid wastes are either burnt to generate steam for steaming the nuts in the fresh bunches, deposited in land depressions or composted. The latter is only executed to a minor extent, mainly due to phytohygienic

concerns (for instance, the distribution of Ganoderma, which causes stem fouling) and a lack of experience. An alternative to burning of empty fruit bunches is pyrolysis to generate charcoal (Lua and Guo, 1999).

20.1.1 Waste characteristics

In the Mediterranean countries, olivoculture and production of olive oil represents one of the most important, and oldest agricultural activities. The olive oil extraction industry produces liquid effluents termed olive oil mill wastewaters (OMWW). The disposal of OMWW is one of the main environmental problems in the Mediterranean area, where the greatest quantities of olive oil are produced, with a large volume of wastewaters within only a few months (from November to February). Nevertheless, a series of environmental issues, such as the recovery and detoxification of the effluents from olive oil mill plants, appear to be far from being resolved. A large amount of waste is produced during oil processing. Dust is generated in materials handling and in the processing of raw materials, including in the cleaning, screening and crushing operations. For palm fruit, about 2–3 m^3 of wastewater are generated per metric tonne of crude oil. The wastewater is high in organic content, resulting in a biochemical oxygen demand (BOD) of 20 000–35 000 mg L^{-1} and a chemical oxygen demand (COD) of 30 000–60 000 mg L^{-1}. In addition, the wastewaters are high in dissolved solids (10 000 mg L^{-1}), oil and fat residues (5000–10 000 mg L^{-1}), organic nitrogen (500–800 mg L^{-1}) and ash residues (4000–5000 mg L^{-1}). Seed dressing and edible fat and oil processing generate approximately 10–25 m^3 of wastewater per metric tonne of product. Most of the solid wastes (0.7–0.8 tonnes per tonne of raw material), which are mainly of vegetable origin, can be processed into by-products or used as fuel. Moulds may be found on peanut kernels, and aflatoxins may be present. The high polluting activity of OMWW is linked to their high content of organic molecules, especially polyphenolic mixtures (1–10 g L^{-1}), as well as their acidity and high concentrations of potassium, magnesium and phosphate salts. Besides aromatic compounds, OMWW contains other organic molecules – including nitrogen compounds, sugars, organic acids and pecans – that increase their organic load (COD = 80–200 g L^{-1}; BOD = 50–100 g L^{-1}). Furthermore, the physicochemical characteristics of OMWW are rather variable, depending on climatic conditions, olive cultivars, degree of fruit maturation, storage time and extraction procedure (Ielmini *et al.*, 1976).

20.1.2 Industry description and practices

Presently there are three methods for extracting the juice from the olive. They are classified according to the technology used in the phased extraction process, from which oil, solids and aqueous fractions may arise. This technology consists basically of either the press or the centrifugation system.

The press system, the traditional method, is a batch process, whereas centrifugation is usually continuous. The latter can also be further divided into two different systems depending on the number of phases produced: the two-phase and three-phase systems. The three-phase system is applied in Italy, Greece and other Mediterranean countries, whereas the two-phase is widely used in Spain, which is the main olive oil producing country. Industries working under the three-phase system generate two main residues: a solid waste (olive pomace) and an aqueous liquid (OMWW), which is a highly pollutant matrix. To reduce this pollution, the new two-phase system was developed during the past decade, and it produces only a solid by-product. It contains a higher proportion of water than the olive pomace and a great amount of lignin, cellulose, hemicellulose and phenolic compounds. Thus, in the three-phase system, oil, olive cake and OMWW are obtained, while in the two-phase system, oil and a semisolid waste made up of olive cake and concentrated OMWW are generated. Each method produces different volumes of by-products and as a consequence the effluents differ in their characteristics (Fig. 20.1 and Table 20.1). The use of the modern two-phase processing technique, in which no water is added, generates a new by-product called 'alperujo' (AL) that is a combination of liquid and solid waste. This new two-phase centrifugation process is used for the separation of the oil from the vegetable material, which includes all mineral and organic fractions (fats, proteins, sugars, organic acids, cellulose, hemicellulose, pectins, gums, tannins and polyphenols), but it produces a new contaminated wastewater as well as a solid residue. Alternatively, AL can be dried and subjected to chemical extraction with hexane, which has been a common practice for the olive pomace of the old two-phase system. This new dry olive mill residue (DOR) can be used for cogeneration of electric power. However, some problems have been raised lately such as the low residual level of oil in the unextracted solid cake, changes in cogeneration subsidies and the discovery of polycyclic aromatic hydrocarbons in olive oil pomace, which make the study of alternative uses for this solid by-product necessary. In many European countries, such as Spain, a massive change from the traditional three-phase to the new two-phase process has taken place, and large volumes of waste (3–5 million tonnes per year) are generated. An integrated approach to this waste as fertilizer or animal feed or through recovery of residual oil and/or extraction of high added value products contributes to diminish its environmental impact and will provide a way to make the wastes from the olive mill plant profitable.

20.1.3 Pollution issues

Effluents from olive production are currently one of the most serious environmental concerns in the Mediterranean basin, largely as a result of both the sheer volume of waste generated per year (around 30 million m^3 of OMMW) and their recalcitrant characteristics and toxic effects for the

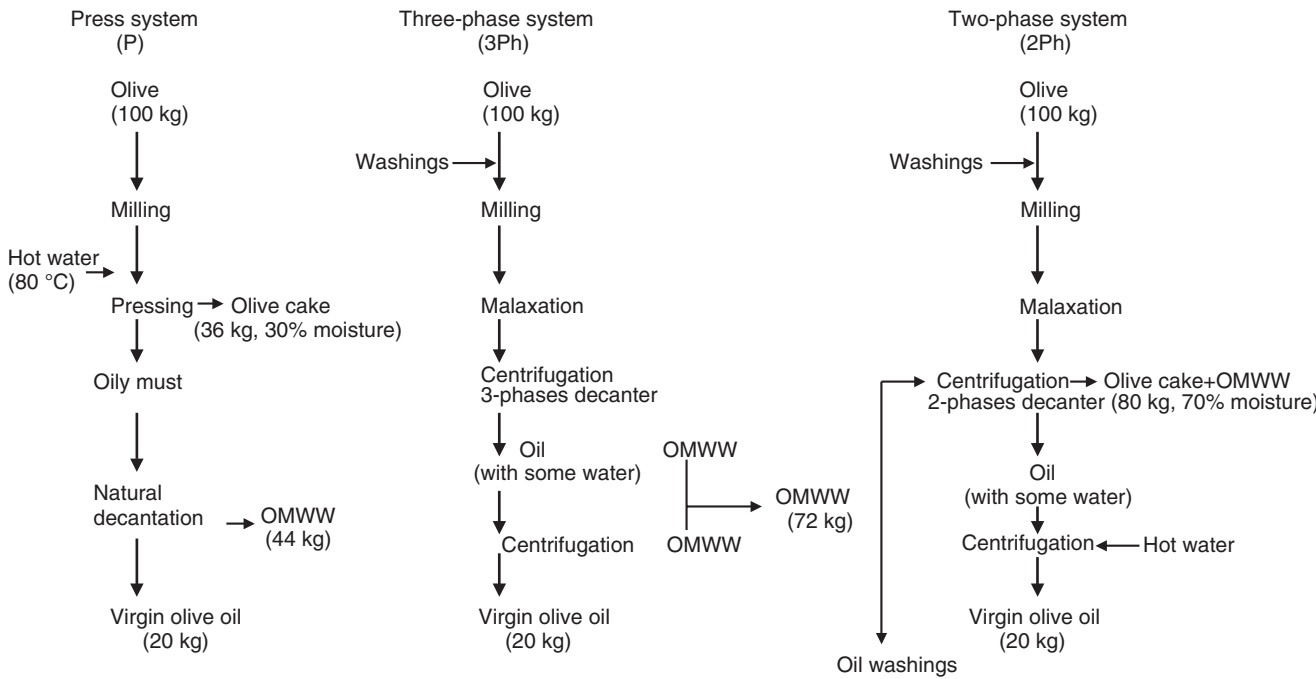

Fig. 20.1 Processes for olive oil extraction (adapted from López *et al.*, 2001).

Table 20.1 Characteristics of OMWW from different oil extraction systems (adapted from BMZ, 1995)

System	Volume generated ($L\ t^{-1}$ olive)	Total solids ($g\ L^{-1}$)	Oil ($g\ L^{-1}$)	COD ($g\ L^{-1}$)
Press	800	120	7–5	80
Three phases	1300	60	19	50
Two phases	300	5	–	20

environment (Peréz *et al*., 1990). Direct discharge of untreated waste is not allowed by law in most countries and many efforts have been made in order to either ensure waste purification or recycling (Ramos-Cormenzana *et al*., 1995). Indeed, the discharge of large quantities of this pollutant in the sewage system is not possible without any treatment. Because of the expense of new technologies for pre-treatment and the difficulty of conventional treatment methods, some regulations, such as the Italian law, allow the spreading on agricultural soil of up to $50\,m^3\,ha^{-1}$ for OMMW obtained by press and $80\,m^3\,ha^{-1}$ for OMMW obtained by centrifugation. The untreated release of OMMW on land produces potential danger for the surrounding environment, and several studies have shown that simple OMMW phenolic compounds of low molecular mass are toxic to seeds, aquatic organisms and bacteria. However, there is a controversy over what the phytotoxic components of the olive residues are. Most researchers have reported a high phytotoxicity against plant and microbial growth by low molecular mass phenols, but high molecular mass polyphenols or lignin-like polymers have also shown toxic activity and must also be considered. Furthermore, some researchers have not found a relationship between detoxification and removal of monomeric phenols from olive residues.

20.1.4 Disposal and recycling of oil wastes

Recently, Aliotta *et al*. (2002) reported the phytotoxicity of polyphenols from OMWW on seed germination, and Yesilada *et al*. (1999) reported their toxic effects on the soil bacterium *Pseudomonas aeruginosa*, but only a few studies have reported the toxic potential of this matrix on the typical organisms of the freshwater food chain. For these reasons, different biological and chemical/physical methods have been proposed to reduce the organic matter, polyphenols and tannins present in OMMW in order to avoid the toxic effects on the environment. Several pre-treatment techniques have been worked out to reduce the impact of OMWW on municipal plants and on the receiving water bodies by using microorganisms and chemical or physicochemical methods. Thus, a wide range of bioremediation processes, such as aerobic (Benitez *et al*., 1997) and anaerobic digestion (Borja *et al*., 1997) or decolorization by lignolytic microorganisms

(Peréz *et al*., 1998), have been proposed. Oxidation systems have often been used as a pre-treatment to decrease OMWW toxicity and allow biological degradation (Lopéz *et al*., 1996). Composting (Monteoliva-Sánchez *et al*., 1996) and the production of industrially useful microbial products (González-López *et al*., 1996) have been shown to be worthwhile alternatives. However, the most suitable procedures seem to be treatments involving recycling rather than detoxification. Due to these characteristics, which increase the organic load of COD (80–200 g L^{-1}) and BOD (50–100 g L^{-1}) to values 200–400 times higher than those of a typical municipal sewage, the annual disposal of several million cubic metres of OMMW is a major environmental problem for agriculture in the Mediterranean area. Another important disposal approach deals with the recovery of the organic components for use in agriculture and in industry. The olive fruits contain a wide variety of bioactive components. Among these, hydroxytyrosol stands out as a compound of high added value, due to its high antioxidant properties and beneficial properties (with regard to both nutrition and oil stability), that could be recovered from the solid by-product. Hydroxytyrosol plays a role in enhancing the oxidative stability of olive oil and also has a positive effect on human health.

Recycling of oil waste material is a major task for sustainable development as for example the use of rape for biodiesel production from rapeseed oil (Ma and Hanna, 1999). The residues of biological treatment methods may serve as fertilizers or soil conditioners, while the slugs from waste incineration could be separated into a granulated fraction replacing sand in concrete and a fine-particulate/dust fraction, which needs to be deposited in hazardous waste sites due to its content of heavy metals and other toxic components. Whenever this waste material can be recycled it must be reintroduced into production processes and the non-recyclable fractions should be used as a fuel for energy recovery. Nowadays there is an urgent need to upgrade the processes for the treatment of the organic fractions from vegetable oil production to produce more valuable re-usable products or at least to recover their energy content. The main upgrading processes of oil organic wastes include composting, biogas fermentation, production of organic acids, polyphenols, biopolymers or biosurfactant production (Bolaňos *et al*., 2004). Moreover, a variety of oil plants store a large amount of carbohydrates which can be lost in the production wastes and may serve as a raw material for a biotechnological conversion by microorganisms to new ‘value-added’ products.

20.2 Key reasons to improve waste management in vegetable oil processing

There are several environmental and economic benefits arising from the application of new waste management processes.

20.2.1 Economic and environmental reasons

In the case of olive oil, the application of the two-phase extraction process produces only a solid by-product that can be dried and extracted by solvent. The new DOR can be used for cogeneration of electric power, used in combination with saprobic fungi for removal of monomeric phenols. The by-product of the two-step olive mill process can represent an alternative to synthetic fertilizers and amendments. The development of environmentally acceptable methods for disposal of vegetable oil wastewaters still remains a problem, whereas the degradation of the toxic compounds contained in this wastewater will certainly enhance the quality of the remediated waters and of the sedimentation muds, in view of their safe utilization as fertilizers. From this view point, bioremediation of vegetable oil mill wastewater by chemical alteration induced for example by *Azotobacter vinelandii* and use of the end product as a conditioner and liquid organic fertilizer appears an economically convenient approach. A different approach regards the use of superabsorbent polymers in an innovative process that allows vegetable mill wastewaters to be used as fertilizers through the removal of molecular weight fractions of COD and phenolic compounds in an integrated treatment process of oil mill effluent. Vegetable oil effluents can be effectively pre-treated and discharged into a municipal sewerage system. Pre-treatment of effluents comprises screening and air flotation to remove fats and solids; it is normally followed by biological treatment. If space is available, land treatment or pond systems are potential treatment methods. Other possible biological treatment systems include trickling filters, rotating biological contactors and activated sludge treatment. The pre-treating process also includes proper circulation of air, using an extracting and cleaning system, to maintain dust at acceptable levels.

Vegetable oil wastewaters are rich in organic and inorganic compounds and may also be regarded as an inexpensive source of products to be recovered because of their potential economic interest and/or ability to be transformed into products for use in agriculture and industry. OMWW are rich in antioxidant compounds that could be recovered from the matrix and employed both in preservative chemistry and, following appropriate trials to evaluate their safety and efficacy, as prophylactic agents in the prevention of certain radical-induced human diseases. The possibility of isolating natural antioxidants like hydroxytyrosol from wastewater extracts is high. Hydroxytyrosol, the most active component of OMWW extracts, is of particular interest because it is amphiphilic and thus it acts at the oil–water interface and in systems where both oil and water phases are present, such as emulsions (Auroma *et al.*, 1998). Hydroxytyrosol is characterized by a high antioxidant activity, which is comparable with that of the usual synthetic antioxidants such as 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and 3-tert-butyl-6-hydroxyanisole (BHA). *In vitro* it also inhibits the oxidation of low-density lipoproteins (LDLs) and confers both cell protection and dietetic properties to virgin olive oil. Analysis of OMWW shows that their

phenolic compounds content fluctuates from 0.5 to 1.8%. Results *in vitro* demonstrate that hydroxytyrosol inhibits human LDL oxidation (a process included in the pathogenesis of atherosclerosis), scavenges free radicals, inhibits platelet aggregation and the production of leucotriene for human neutrophyls (which is indicative of anti-inflammatory properties) and confers cell protection. It has also been demonstrated that hydroxytyrosol acts *in vitro* against both Gram-positive and Gram-negative bacteria, which are causes of infections in the respiratory and intestinal tracts. Nevertheless, and despite recent bioavailability studies, more studies are required to demonstrate the antioxidant and antimicrobial effectiveness of hydroxytyrosol *in vivo*. Larger amounts of this compound are required at competitive prices, so that it can be used, for instance, as a preservative in foods. Fernández-Bolaños *et al.* (2002) showed that large quantities of phenolic compounds, especially hydroxytyrosol, can be obtained from olive cake (three-phase process) and olive stones, in both cases by means of steam treatment. Hydroxytyrosol can also be recovered from OMWW (three-phase process) and from the washing water using the Spanish-style green table olive process. Diverse synthesis procedures for the production of hydroxytyrosol have also been developed. However, the production methods so far proposed are expensive and produce low yields. Other components such as flavonoids, anthocyanins and tannins are of potential biological interest due to their antioxidant activities (Vinson *et al.*, 1995). From a commercial point of view, the most widely employed antioxidants are those indigenous to foods, the water-soluble ascorbate and the lipid-soluble butylated hydroxytoluene, butylated hydroxyanisole, the esters of 3,4,5-trihydroxybenzoic acids and vitamin E. Plant extracts are also in use, and their share of the antioxidant market is expected to grow by 15% by the year 2006 (Krishnakumar and Gordon, 1996).

Nowadays, there is growing interest in novel sources of natural antioxidants due to the recognized involvement of reactive oxygen species in the onset of several human diseases (Auroma *et al.*, 1998) and in the oxidative degradation of food, animal feed and other goods such as cosmetics. Other added-value compounds such as xylose, arabinose, glucose, oligosaccharides, mannitol, vitamin E, sterols and protein can also be isolated; waste from frying oils can be a valid economic substrate for biosurfactant production. From an economic point of view, vegetable wastewaters can also be exploited as a growth medium for the production of extracellular enzymes such as laccase and manganese peroxidase and also for the extraction of polymerins as bioamendments and metal biointegrators. Waste cooking oils can become an important alternative to conventional fossil fuels in the production of biodiesel. Fat- and oil-containing wastes from the fat or oil separators of hotels, canteens, kitchens and bakeries are considered to be an excellent supplementary substrate for biogas plants due to the high specific methane yield. It is also feasible to synthesize vegetable oil-derived esters as a diesel fuel substitute or additive using methanol and KOH as

catalyst. Biodiesel is created via transesterification, a chemical reaction between a fatty substance such as vegetable oil, an alcohol such as ethanol or methanol, and a catalyst such as lye. The two by-products of this reaction are biodiesel and glycerin. Since Rudolf Diesel's invention of the compression (diesel) engine over 100 years ago, it has been known that the engine can operate on vegetable oils. Biodiesel, the transesterified product of vegetable oil, is considered to be the most promising diesel fuel substitute. Biodiesel development can now be found in 28 countries, with Germany and France so far being the world's largest producers of biodiesel fuel. Recently, Japan started a project in Kyoto to use biodiesel at a commercial-level with municipal city-owned trucks running on 100% biodiesel fuel; this trial has been extended to 81 municipal buses with a blend of 20% biodiesel and 80% petroleum diesel fuel. Most biodiesel is produced from soybean oil, though biodiesel is also commonly produced from rapeseed (canola) and mustard seed, and any oil from crops or animal fat can be used. It is also possible to make biodiesel using waste vegetable oil from restaurant fryers. The benefits of biodiesel are as follows: it reduces all emissions except NO_x; it comes from renewable sources such as soybeans; it is carbon neutral and thus does not contribute to the greenhouse effect; it biodegrades faster than sugar and is less toxic than table salt; it has excellent lubricating properties, is an engine detergent and is sulphur-free. Biodiesel can 'gel' at temperatures below about 25 °F; this problem can be avoided by adding petroleum diesel during cold snaps.

20.3 Co-product recovery in vegetable oil processing

As stated before, vegetable wastewater can be considered as an important source of valuable products, i.e. carbohydrates, phenols, lecithin, vitamin E, sterols and proteins. Visioli *et al.* (1999), in view of the need for upgrading by-products at all stages of the olive oil industry, investigated different procedures for the recovery of the active components of OMWW and compared the antioxidant and biological activities of various extracts.

20.3.1 Sugars

Glucose is the main soluble sugar present in olive pulp together with smaller quantities of sucrose and fructose and a significant amount of the polyol called mannitol. The insoluble polysaccharides in the cell wall of olive fruit are composed of pectin, hemicellulose and cellulose. The hemicelluloses are mainly rich in acid xylan and xyloglucan. Therefore, this by-product may be utilized as a chemical feedstock for the production of fermentable sugar as a source of mannitol. Furthermore, due to cleavage of the hemicellulose by the steam treatment, a wide variety of xylo-oligomers with different molecular fragments might be obtained. Carbohydrates are not only

ideal energy substrates for the majority of microorganisms, but also the main carbon sources for biotechnological production processes. Biotransformation of hexoses to gluconic acid, itaconic acid, citric acid and lactic acid is performed on a large scale to produce basic ingredients for laundry detergents, glues, preservatives and polylactides, respectively. Polylactides are polymers of lactic acid with a molecular mass of 40 000–300 000 Da. The lactic acid is produced with lactobacilli. The recovery of lactic acid from alfalfa or soya fibres that were enzymatically digested with cellulases and pectinases, and fermented with lactobacilli, was 32–46 g per 100 g of fibres (Sreenath *et al*., 2001). The properties of polylactides depend on the proportion and the distribution of D(−) and L(+)-lactic acid isomers in the polymer. Poly-L-lactic acid and poly-D-lactic acid are crystalline polymers, whereas poly-D,L-lactic acid with a regularly alternating D- and L-isomer array is an amorphous polymer and poly-D,L-lactic acid with statistically distributed D- and L-isomers is an amorphous to crystalline polymer (Richter *et al*., 1999).

An important polysaccharide obtainable from OMWW is xanthan, which is composed of the sugars glucose, mannose and glucuronic acid. Xanthan is widely used as a thickener or viscosifier in both the food and non-food industries. Xanthan is extracellulary produced by the bacterium *Xanthomonas campestris*, and has become the focus of a great deal of interest on account of its physical properties. Xanthan production from OMWW can be achieved at reasonable levels after optimising several nutrients in culture media. López *et al*. (2001) studied the effect of OMWW variability on xanthan production. The authors determined that factors affecting wastewater composition – namely waste storage, time of olive harvesting and method for oil extraction – influenced xanthan production in shake-flask cultures. Specifically, they found a maximum xanthan production of $4\,g\,L^{-1}$ with 50% OMWW as the sole source of nutrients. OMWW storage decreased effluent quality for xanthan production. The range of effluent concentration for *Xanthomonas campestris* growth and xanthan production varied depending on the OMWW extraction method. Wastewaters from press and two-phase production varied depending on the OMWW extraction method. Wastewaters from press and two-phase extraction methods required higher dilution rates (<10%) than those from the three-phase extraction method (50%). Nitrogen supplementation improved xanthan production in press and two-phase OMWW. Conditions for xanthan production from OMWW should be optimized in accordance with the nature of the waste material. The time of olive harvesting and milling was also found to influence the quality of the substrate for xanthan production. OMWW from late harvesting were found to be more beneficial for xanthan production than OMWW from early harvesting. The main differences between the samples used are the olive ripeness and probably the storage period prior to milling. This results in changes in fruit composition that may affect effluent quality. Guillén *et al*. (1993) reported a drop in fruit sugars

during maturation. Such variations led to increases in xanthan production. Results indicated that xanthan production varied greatly depending on the type of effluent used. The main factor influencing waste quality for xanthan production was the method of extraction. Effluents with lower organic matter content such as three-phase system OMWW and two-phase system OMWW can be included in media at 50%. More concentrated effluents, such as those from press and aqueous extracts resulting from two-phase systems, should be diluted tenfold. Nitrogen supplementation results in a C/N balance that allows growth and xanthan production at higher effluent concentration levels than in media where the effluent becomes the only source of nutrients.

20.3.2 Sugar alcohol

Another important product obtainable from oil waste waters is mannitol, which is a sugar alcohol that is used as an excipient in the pharmaceutical industry and as an anti-caking and free-flow agent, lubricant, stabilizer, thickener and low-caloric sweetener in the food industry. Due to its physicochemical properties, it is predominantly used in chewing gum and in bread products for diabetics. Xylo-oligosaccharides can be used as a food additive due to their favourable effect on the intestinal flora. Non-digestible oligosaccharides are usually considered to enhance the growth of bifidobacteria and lactic acid bacteria in the human large intestine, with certain evidence of a preventive effect against colon cancer and other intestinal dysfunctions. Recently Fernández-Bolaños *et al.* (2002) optimized and integrated a process for alperujo that allows compounds of high added value to be obtained from the water-soluble fraction, leaving a solid residue enriched in cellulose, and residual oil that can be valorized by further processing. They established the operating conditions that govern the autohydrolysis process (temperature and time) in order to evaluate this by-product as a source of fermentable sugar, mannitol and oligomers. The use of sulphuric and phosphoric acids as catalysts during steam treatment and its implication on the isolation of some of these compounds can be effective and the further purification and crystallization of mannitol are also possible.

20.3.3 Polymer building blocks

Vegetable oils are a potential feedstock for polymers because their fatty acid molecules can be modified to serve as polymer building blocks (Fig. 20.2). Several projects exist to develop catalyst systems for chemistries such as hydroformylation to convert the vegetable oils to polyaldehydes and subsequent chemistry to convert the polyaldehydes to polyols, polyacids and polyamines. By adding functional groups to the vegetable oil molecules, the reactivity of the vegetable oil is increased creating the potential

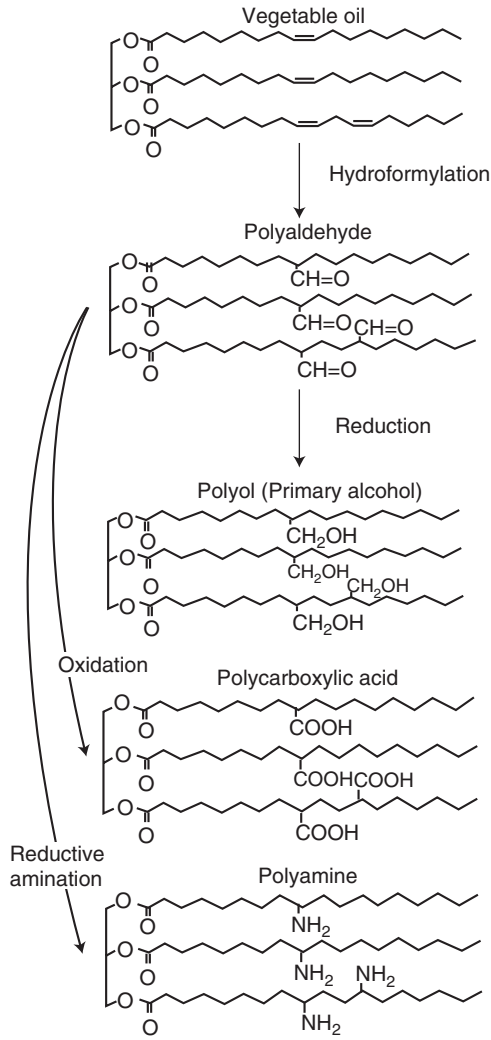

Fig. 20.2 Catalyst system for the formation of polyaldehydes, polyols, polyacids and polyamines.

for polymerizing the modified vegetable oil. These processes were originally developed for fossil feedstocks that are less viscous than vegetable oils and easier to separate from the catalyst at the end of the reaction. Researchers are developing new catalyst systems that have high efficiencies for the conversion of vegetable oils such as soy oil and allow efficient catalyst recovery and easy product separation from the vegetable oil derivatives.

20.3.4 Polymerins

Other authors have studied the possibility of obtaining useful polymeric products from OMWW. Arienzo and Capasso (2000) obtained a dark polymeric organic fraction using precipitation of the organic polymeric fraction (opf) by cold methanol. The chemical nature of the opf binding the metal cations was analyzed and its size in terms of relative molecular mass was also investigated by combining the ultraviolet (UV)-visible spectroscopic analysis with ultra-filtration experiments. The COD/BOD values of the obtained fractions were also determined to verify whether the proposed treatment (i.e. cold methanol precipitation) reduced the organic load of the initial raw material and its related environmental risks, and to evaluate the potential for use of these fractions as a soil amendment and in environmental biotechnology processes. Results indicated that most of the metal cations were bound to the OMWW opf composed of polysaccharides, phenol polymers and proteins to which K and Na are essentially bound by single electrostatic bonding, whereas all other ions are more strongly bound – even in chelated form – by means of anionic functional groups. Opf molecular mass was substantially estimated in the range 1000–30 000 Da for ~75% and in the range 30 000–100 000 Da for ~25%. Thus, the polymeric product was revealed to be rich in K and was named polymerin, in fact K was the most abundant metal, followed, in decreasing order, by Ca, Mg, Na, Zn, Fe and Cu. The free residual cations pool proved to be neutralized by the inorganic counter-anions (Tables 20.2 and 20.3). These findings were in agreement with those shown by other authors, who revealed that non-living biomass materials have a high potential in binding metals. Metal ion uptake is believed to occur through interactions with functional groups such as carboxyl, amino, sulphydryl, phenolic or hydroxyl moieties. These proved to be native to the proteins, lipids and carbohydrates that make up the cell

Table 20.2 Metal cation and inorganic anion concentrations determined in raw OMWW (adapted from Arienzo *et al.*, 2000)

	Total cations			Inorganic anions	
Cation	Concentration (g L^{-1})	Concentration (meq L^{-1})	Anion	Concentration (g L^{-1})	Concentration (meq L^{-1})
K^+	17.1	437.2	Cl^-	1.63	45.9
Mg^{2+}	2.72	223.8	$H_3PO_4^-$	1.07	11.0
Ca^{2+}	2.24	111.8	F^-	0.57	30.0
Na^+	0.40	17.4	SO_4^{2-}	0.53	10.8
Fe^{2+}	0.12	4.6	NO_3^-	0.023	0.37
Zn^{2+}	0.06	1.9			
Mn^{2+}	0.014	0.5			
Cu^{2+}	0.086	0.2			
		$\Sigma = 797.5$			$\Sigma = 98.07$

Table 20.3 Concentrations of metal cations bound to the opf in OMWW (adapted from Arienzo *et al.*, 2000)

Cation	Concentration ($g\ L^{-1}$)	Concentration ($mequiv\ L^{-1}$)
K^+	13.2	337.5
Mg^{2+}	2.51	206.4
Ca^{2+}	2.11	105.3
Na^+	0.373	16.2
Fe^{2+}	0.105	3.8
Zn^{2+}	0.059	1.8
Mn^{2+}	0.0113	0.4
Cu^{2+}	0.0058	0.2
		$\Sigma = 671.6$

walls. All cations, except K, are >90% bound to opf. K was 78% bound to opf and 22% was free in solution. Except for K and Na, cations are bivalent and possess strong chelating properties. The relative abundance of negatively charged sites of the opf explains the consistent binding of K to the opf. In the case of sodium, 99.1% of the metal was bound to opf, thus competing with K, which has a smaller ionic ray, 0.95 and 1.53 Å, respectively. This difference should make the Na more reactive towards the negative sites of opf than K. The opf that resulted was mainly formed by polysaccharides, polymeric polyphenols and proteins. In addition, the COD and BOD of this biomaterial markedly decreased in comparison with those of the raw OMWW. These findings prompted the recovery of the metal polymeric organic fraction, which was named polymerin, with the aim of studying its possible recycling in agriculture and use in environmental technology processes. The potential employment of this biomaterial in agriculture as a bioamendment and/or metal bio-integrator is motivated by its humic acid-like nature and its richness in macro- and micronutrients such as K and to a lesser extent Ca, Mg, Fe and Zn. Capasso *et al*. (2002) described the production of metal polymerins obtained separately by saturation of polymerin with various micronutrients (Cu, Zn, Mn and Fe) and other metals of interest (Na and Al). They also studied the production of the metal Cu, Zn, Mn, Fe and Al salts of deglycosylated polymerin (Me-SDpolymerins), obtained separately by saturation of a K salt of deglycosylated polymerin (K-SDpolymerin). In view of the possible application of these biomaterials, their effects on tomato cuttings were studied compared with the effect of raw OMWW. Saturated metal polymerins were characterized by diffuse reflectance infrared Fourier transform spectroscopy, and atomic absorption and atomic absorption spectrometry. Tests on tomato plants of the various polymerins showed that only the soluble polymerin, K-SDpolymerin and the insoluble Mn-SDpolymerin were significantly

toxic. The toxic effects of OMWW on tomato at the original concentration and diluted 1:10 were much stronger than those of any polymerin. The soluble polymerin and its derivative K-SDpolymerin and the insoluble Mn-SDpolymerin were only significantly toxic. The wilting effect of the soluble polymerins was attributed to a mechanical obstruction of the xylem pathways of the tomato, whereas that of Mn-SDpolymerin was related to interference with the plant metabolism. The strong phytotoxicity of OMWW on tomato observed at both original and diluted 1:1 concentrations was ascribed primarily to a synergistic effect of polyphenols, which act on plant metabolism, and secondarily to the polymeric fraction, which acts through a mechanical obstruction and is visible only at high concentrations. The authors also found that the COD and BOD of the OMWW polymeric fraction was strongly reduced with respect to the whole wastewaters. The low toxicity of polymerins, their humic acid characteristics, and the abundance of macronutrients (K, Mg and Ca) and micronutrients (Cu, Zn, Fe and Mn) suggest their promising exploitation as bio-amendments and/or metal-bio-integrators. However, the use of Me-polymerins with respect to Me-SDpolymerins is of great interest because of the simpler and cheaper production process.

The detoxification of agro-industrial effluents using superabsorbent polymers is a new and innovative process. The high metal-binding capacity of opf represents a potential industrial tool for the extraction of toxic metal ions from wastewaters and mining effluents. The single-step chemical OMWW treatment can in fact offer an opportunity to mitigate waste disposal problems, to reduce the costs of chemical by-processes and to aid in the development of a filtration system to remove and recover metal ions from contaminated waters.

Vegliò *et al.* (2003) conducted a study on olive mill residues (OMR) as a copper-adsorbing material. A rough characterization of the waste material was performed by microanalysis and scanning electron microscopy pictures. Sorption tests with suspended OMR resulted in copper removal from solution, of about 60%. The COD release in the solution was also monitored during biosorption. Before biosorption OMR were washed with water. In this case the COD release in the solution was reduced to less than $600\,mg\,L^{-1}$ after two washings, while the OMR metal sorption properties did not change. Residues regenerated by acid solutions gave a copper removal of about 40% in the same experimental conditions as the first adsorption test: regeneration with ethylenediaminetetraacetic acid (EDTA) at different concentrations suggested that it damages adsorption active sites. On the other hand, the use of HCl and $CaCl_2$ led to a complete regeneration of the biosorbent material. Tests were also performed with a column filled with 80 g of OMR and the breakpoint was demonstrated to take place after about 1 L of solution was treated. Regeneration tests demonstrated that a concentration factor of about 2 can be obtained in non-optimised conditions, highlighting the possibility of using OMR for the treatment of

metal-bearing effluents. The main advantages of the process would be the 'low-cost' biosorbing material, considering that it is a waste from olive oil production. Various parameters that characterize OMWW were evaluated after absorption in two different superabsorbent polymers (SAP1 and SAP2). The organic matter was equally distributed in both phases, while there was a concentration of protein and sodium in solution. The K:Na ratio decreased from 70:1 to 2:1. the polyphenol desorption from the gel into solution was found to follow Fick's law. The mass transfer coefficients were $0.147\,min^{-1}$ and $0.0085\,min^{-1}$ for SAP1 and SAP2, respectively. Phytotoxicity tests were carried out with SAP2. OMWW in SAP2 with polyphenol concentrations up to $200\,mg\,L^{-1}$ revealed no phytotoxicity, and even stimulated *Lepidium sativum* growth, while OMWW without the superabsorbent polymer revealed growth inhibition for all concentrations tested. Caffeic acid degradation by the immobilized biomass followed zero-order kinetics. Degradation constants of $0.087\,L^{-1}\,min^{-1}$ (g SAP2)$^{-1}$ and $1.156\,mg\,L^{-1}\,min^{-1}$ (g SAP2)$^{-1}$ were found. Fungi that developed in the plant growth medium were identified as *Aspergillus* sp. and *Penicillium* sp.

20.3.5 Phenols

Olives and olive oil contain phenolic compounds (Tsimidou *et al*., 1992; Tsimidou, 1998) that, *in vitro*, have been shown to exert potent biological activities including but not limited to antioxidant actions (Visioli *et al*., 1998). Visioli *et al*. (1999) reported that OMWW extracts contain potent antioxidants. Among these polyphenols, hydroxytyrosol has been revealed to be the most interesting because of its remarkable pharmacological and antioxidant properties. Hydroxytyrosol originates in all likelihood from the hydrolysis of oleuropein by means of an esterase during the mill process. Chikamatsu *et al*. (1996) found that hydroxytyrosol inhibits the formation of melanin and lipid peroxides and they patented its use in topical and bath preparations. Because hydroxytyrosol is commercially unavailable, chromatographic methods of purification from OMWW, virgin olive oil, olive leaves and synthetic procedures could allow a natural and non-toxic antioxidant to be obtained. Recovery of hydroxytyrosol could be a useful process for recycling OMWW, thus resolving its disposal problem, albeit partially. Capasso *et al*. (1999) synthesized hydroxytyrosol by reducing 3,4-dihydroxyphenylacetic acid with $LiAlH_4$ in tetrahydrofuran under refluxing for 2 h. The yield of the reaction was 82.8%. The spectroscopic and high-power liquid chromatography (HPLC) data of the synthesized compound proved to coincide fully with those of a pure sample obtained by chromatographic recovery from OMWW (yield = $91\,mg\,L^{-1}$). This synthetic method appears to be the most convenient compared with those reported in the literature and is more convenient than chromatographic recovery. It consists of only one step, the reaction is completed in 2 h and it gives a yield of 82.8% starting from 3,4-dihydroxyphenylacetic acid, which is a

commercially available product. It is also more convenient than the chromatographic purification methods from OMWW because they produce hydroxytyrosol in small amounts and are more expensive than the synthesis method.

The ability of OMWW extracts to scavenge superoxide, already reported for hydroxytyrosol and oleuropein (Visioli *et al.*, 1998) is suggestive of a potential use of OMWW extracts in environments in which Fenton and Haber–Weiss reactions take place and in which the concomitant production of superoxide and nitric oxide would yield the powerful oxidant peroxinitrite. It is noteworthy that the established antioxidants vitamin E and BHT do not scavenge superoxide, and thus OMWW extracts may add stability to products exposed to high superoxide levels. The protection from hypochlorous acid-induced damage of catalase is of biological significance due to the well-known protein-damaging activity of HOCl, which is produced in biological systems at the site of inflammation by activated neutrophilis through the enzyme myeloperoxidase (Aruoma and Halliwell, 1987). Also, because foods often come into contact with chlorine-based bleaches, employed as disinfectants in food plants, the use of HOCl scavengers may provide additional protection against reactive chlorine species. Finally, the potent inhibition of calcium ionophore-stimulated production of LTB4 and its metabolites by human neutrophilis suggests that OMWW extracts exert biological effects beyond their antioxidant capacities. The activity of several enzymes, including those involved in the production of eicosanoids, for example phospholipases and oxygenases, is modulated by the intracellular peroxide tone. Thus, by scavenging reactive oxygen species, OMWW extracts could lower the activity of such enzymes and, in turn, decrease the production of pro-inflammatory factors. Additional studies are needed to verify if such anti-inflammatory effects could also take place *in vivo* and to establish the exact enzymatic target of the bioactive compounds.

20.3.6 Sterols

Another important product obtainable from vegetable oil wastes is sterol. Although steroid drugs represent only a small part of the world market of pharmaceuticals, there is a great demand for new and cheaper steroid raw materials for their production (Dias *et al.*, 2002). The choice of starting material has always had a critical impact on the steroid-manufacturing industries. The most common and economical process for the production of steroid pharmaceuticals is the partial synthesis from relatively inexpensive steroid raw materials of animal and plant origins. Diosgenin and other sapogenins were the preferred starting materials until the 1970s. Soybean sterols obtained from soybean oil processing were plentiful and cheap, and included a large fraction of stigmasterol which can be easily converted to progesterone, thus being an excellent alternative to diosgenin (Dias *et al.*,

2002). Stigmasterol represented about 15% of the total precursors used in the USA, becoming, with diosgenin, one of the main raw materials for industries producing steroids of the pregnane, anderostane and estrane series. Whereas stigmasterol could be degraded chemically starting with the oxidative cleavage of the 24-double bond, preserving the steroid ring structure, sterols like sitosterol and cholesterol, with saturated side-chains resistant to selective degradation, were considered low-value or even waste products. However, since the isolation of the first mutant *Mycobacterium* sp. strain capable of degrading the side-chain of sterols giving 17-keto-steroids (Dias *et al*., 2002) and the development of methods for the chemical addition of the corticoid side-chain to these 17-intermediates, β-sitosterol, the most ubiquitous plant sterol, became a major raw material for the synthesis of corticosteroids, which represents the bulk of the steroid industry. As an alternative to purified sitosterol, mixed sterol concentrates obtained from natural sources such as soya or rape seed, or from industrial wastes (sugarcane and paper industries), have been tested as substrates for the microbial production of 17-ketosteroids (Dias *et al*., 2002). Over 1000 tonnes of the latter chemicals are produced per year. Additionally, sitosterol can be converted by other microbial mutants to 9α-hydroxy-17-ketosteroids or 20-carboxy pregnane derivatives (Dias *et al*., 2002) which are more suitable for corticosteroid synthesis.

Dias *et al*. (2002) isolated a biodegradable sterol-rich fraction from industrial wastes. Several industrial waste materials were screened for their sterol content. The authors studied the possibility of using these industrial by-products as sterol sources for the microbiological production of 4-androsten-3,17-dione (AD) and 1,4-androsta-diene-3,17-dione (ADD). Two methods of obtaining the sterol fraction from wastes were developed. Sterol-rich (96–98%) fractions were isolated in a yield above 70%, from a tall-oil effluent of the paper pulp industry and from deodorized edible oil. These fractions were subsequently used as a substrate for microbial degradation by a *Mycobacterium* sp. strain and proved to be easily converted to AD and ADD.

20.3.7 New products

The first new products obtainable from oil wastes are isoflavones, which are extracted from the waste of soy protein production. Isoflavones are structurally close to oestrogens and have been proved to aid in the fight against breast and prostate cancer. Another product is tocotrienol, which is a strong antioxidant, like vitamin E, that is extracted from palm oil processing by-products. From the palm oil waste stream there is also the possibility of extracting a natural β-carotene. Additional soy oil by-products in preparation are phosphytidyl choline and phosphytidyl serine. These products have health benefits and can be sold into the neutraceutical market.

20.3.8 Biogas

Biogas can be produced separately from collected bio-waste or from bio-waste and sewage sludge in co-digestion plants. In addition to bio-waste digestion in reactors that are built exclusively for bio-waste treatment, the bio-waste can also be co-digested together with sewage sludge or cattle manure. Co-digestion of unknown fatty and oily residues might, however, lead to failure of methanogenesis. Thus, plant operators should not risk process disturbance by feeding co-substrates of unknown composition, even though the time taken to repay the investment might be much shorter. Apart from the risk of feeding indigestible or even toxic material, the fat- and lipid-containing wastes may not be readily digestible at thermophilic temperatures, due to an absence of lipase activity or due to the inhibiting effect of long-chain fatty acid hydrolysis products. In addition, moulds on fat- and oil-containing food wastes may produce antimicrobial agents during pre-storage, and these may cause failure of acetogenesis and methanogenesis.

20.3.9 Biodiesel

Oil waste waters can be used for the production of biodiesel. Dmytryshyn *et al*. (2004) conducted the transesterification of four vegetable oils (canola oil greenseed canola oil heat-damaged seeds, processed waste fryer grease and unprocessed waste fryer grease) using methanol and KOH as catalyst. The methyl esters of the corresponding oils were separated from the crude glycerol, purified and characterized by various methods to evaluate their densities, viscosities, acid numbers, fatty acid and lipid compositions, lubricity properties and thermal properties. The fatty acid composition suggests that 80–85% of the ester was from unsaturated acids. A substantial decrease in density and viscosity of the methyl esters compared with their corresponding oils suggested that the oils were in mono- or di-glyceride form. The lubricity of the methyl esters, when blended 1 vol% treat rate with ISOPAR M reference fuel, showed that the canola ester enhanced the fuel's lubricity number. From the analyses performed, it was determined that the ester with the most potential for being an additive or substitute for diesel fuel is the canola methyl ester, whose physical and chemical characteristics are similar to diesel fuel.

Most of methods for biodiesel production use an alkaline catalyst in a batch-type processing, followed by additional effort to remove the catalyst and saponified products from free fatty acids. Recently, there has been a strong interest in developing a flow-type transesterification of vegetable oil in an effort to find a process more applicable for use on a commercial scale. Kudsiana and Saka (2001a) developed a continuous, simpler process in the absence of alkali catalyst for biodiesel fuel production. Kudsiana and Saka (2001a) conducted a series of experiments to study the optimum condition for transesterification of vegetable oil in supercritical methanol to biodiesel

fuel. The effect of temperature and pressure was quantified in terms of reaction rate constants. Results indicated that the course of the reaction is both temperature and pressure dependent. Free fatty acids are effectively converted to their fatty acid methyl esters through a methyl esterification reaction in supercritical methanol.

The basic idea of supercritical treatment is a relationship between pressure and temperature, and the thermophysical properties of the solvent (methanol) – such as dielectric constant, viscosity, specific weight and polarity. Therefore, a set of experiments was carried out to study the effect of reaction temperature and pressure on methyl ester formation. It was revealed that supercritical treatment at 350 °C, 30 MPa and 240 s in molar ratio of 42 in methanol was the best condition for transesterification of rapeseed oil to biodiesel fuel. In addition, the methyl esters produced were similar to those produced by the common catalysed process. At a sub-critical state of methanol (<239 °C, <8.1 MPa), reaction rate was low and gradually increased as either pressure or temperature rose. Furthermore, at the transition state between sub-critical and supercritical, a relatively low rate constant is apparent. The reaction rate was increased by a factor of 85 at 350 °C and 30 MPa. In addition, a considerable change in the rate constant can be seen for the pressure above 20 MPa. From this result, it is further concluded that the rate constant of the reaction was corresponding linearly to both temperature and pressure.

In the common catalysed method, direct use of crude vegetable oil as a raw material for transesterification results in an incomplete reaction because the presence of free fatty acids leads to catalyst destruction. Therefore, it is suggested that vegetable oil is refined to have the free fatty acids content as low as possible, below 0.5%. Kudsiana and Saka (2001b) reported that free fatty acids could be converted to their fatty acid methyl esters through a methyl esterification reaction. Unsaturated fatty acids (oleic, linoleic and linolenic acids) are converted effectively at the lower temperature, while for saturated fatty acids (palmitic and stearic acids), a relatively higher reaction temperature is necessary to allow the methyl esterification reaction to take place. On average over 75% of free fatty acids were converted to their fatty acid methyl esters. The optimum condition for free fatty acid conversion is similar to that of transesterification, i.e. a temperature of 350 °C. These facts suggest that free fatty acids that become wastes as saponified products in the common catalysed method can be available as biodiesel fuel.

The authors expanded the experiment to other vegetable oils. The results showed that the optimum condition for the reaction is different for different vegetable oils. For high-saturated vegetable oils such as coconut and cottonseed oils, a relatively longer treatment is needed to achieve a high conversion, whereas for soybean and corn oils, the optimum condition is similar to that of rapeseed oil, because of their similarity in fatty acid composition. A highly complete conversion of methyl esters was obtained for all of those samples. These observations suggest that supercritical methanol has a high

potential for both transesterification of tryglicerides and methyl esterification of free fatty acids to methyl esters for diesel fuel substitute production.

20.4 Reducing waste in vegetable oil production

The vegetal oil processing industry involves the extraction and processing of oils and fats from vegetable sources. The oils and fats are extracted from a variety of fruits, seeds and nuts. The preparation of raw materials includes husking, cleaning, crushing and conditioning. The extraction processes are generally mechanical (boiling for fruits, pressing for seeds and nuts) or involve the use of a solvent such as hexane. Residues are conditioned (for example, dried) and are reprocessed to yield by-products. Crude oil refining includes degumming, neutralization, bleaching, deodorization and further refining (BMZ, 1995). Continuous sampling and measuring of key production parameters allow production losses to be identified and reduced, thus reducing the waste load. Since the pollutants generated by the industry are very largely losses in production, improvements in production efficiency, as described above, are recommended to reduce pollutant loads. Under this view, pollution prevention practices in the industry focus on the following main areas:

- prevent the formation of moulds on edible materials by controlling and monitoring air humidity;
- use citric acid instead of phosphoric acid, where feasible, in degumming operations;
- where appropriate, give preference to physical refining rather than chemical refining of crude oil, as active clay has a lower environmental impact than the chemicals generally used;
- reduce product losses through better production control;
- maintain volatile organic compounds (VOCs) well below explosive limits. Hexane should be below 150 mg m^{-3} of air;
- provide dust extractors to maintain a clean workplace, recover product and control air emissions;
- recover solvent vapours to minimize losses;
- optimize the use of water and cleaning chemicals;
- recirculate cooling waters;
- collect waste product for use in by-products such as animal feed, where feasible without exceeding cattle-feed quality limits.

Wastewater loads are typically 3–5 m^3 per tonne of feedstock; plant operators should aim to achieve lower rates at the intake of the effluent treatment system. Hexane, if used, should be below 50 mg L^{-1} in wastewater. The BOD level should be less than 2.5 kg per tonne of product, with a target of 1–1.5 kg per tonne. Pre-treatment of effluents comprises screening and

air flotation to remove fats and solids; it is normally followed by biological treatment. If space is available, land treatment or pond systems are potential treatment methods. Other possible biological treatment systems include trickling filters, rotating biological contactors and activated sludge treatment. Pre-treated effluents can be discharged to a municipal sewerage system, if capacity exists, with the approval of the relevant authority. Proper circulation of air, using an extracting and cleaning system, is normally required to maintain dust at acceptable levels. Odour control is by ventilation, but scrubbing may also be required and implemented where necessary to achieve acceptable odour quality for nearby residents. Fabric filters should be used to control dust from production units to below 50 milligrams per normal cubic metre ($mg\,Nm^{-3}$). The liquid effluents should meet some important requirements. Table 20.4 indicates the effluent levels to be achieved. Monitoring of the final effluent for the parameters listed above should be carried out weekly, or more frequently if the flows vary significantly. Monitoring data should be analyzed and reviewed at regular intervals and compared with the operating standards so that any necessary corrective actions can be taken. The key production and control practices that will lead to compliance with emissions requirements can be summarized as follows:

- monitor key production parameters to reduce product losses;
- prefer citric acid to phosphoric acid in degumming operations;
- give preference to physical refining over chemical refining of crude oil, where appropriate;
- hold levels of hexane, if used, below $150\,mg\,m^{-3}$;
- design and operate the production system to achieve recommended wastewater loads;
- collect wastes for use in by-products or as fuel.

Table 20.4 Effluents from vegetable oil processing (adapted from Bressan *et al.*, 2004)

Parameter	Maximum value
pH	6–9
BOD	50
COD	250
TSS	50
Oil and grease	10
Total nitrogen	10
Temperature increase	<3 °C*

* The effluent should result in a temperature increase of no more than 3 °C at the edge of the zone where initial mixing and dilution take place.
TSS, total suspended solids.

20.5 Improving end waste management in vegetable oil production

Several methods have been proposed for OMWW and olive disposal, based on evaporation ponds, thermal concentration, physicochemical and biological treatments, as well as their application to agricultural soils as an organic fertilizer either directly or after a composting process. Various processes have been developed for the treatment of organic fractions of differently composed wastes to upgrade them to more valuable, re-usable products, organic acids and solvents, and biopolymer or bio-surfactant production, or a least to recover their energy content. Different oxidation methods exist for the abatement of the major contaminants present in oil industrial wastewaters by ozone and/or UV radiation versus solar light. The possibility of using an electrochemical treatment of OMWW to oxidize phenols and polyphenols has also been explored.

20.5.1 Anaerobic processes

Anaerobic digestion is one of the most promising technologies for disposing of OMWW as it is a process for both decontaminating and valorizing (by producing methane) such wastewaters. Furthermore, this technology produces low amounts of waste sludges and employs bacteria with very low decay rates, which allows the process to be carried out in seasonal operations without requiring to be fed with OMWW all through the year. The process is generally carried out in conventional contact bioreactors; these however are often unable to efficiently remove OMWW phenolic compounds, which therefore occur in the effluents. Bertin *et al*. (2004) explored the possibility of mitigating this problem by employing an anaerobic OMWW-digesting microbial consortium passively immobilized in column reactors packed with granular activated carbon (GAC) or 'Manville' silica beads (SB). Under batch conditions, both GAC- and SB-packed-bed biofilm reactors exhibited OMWW COD and phenolic compound removal efficiencies markedly higher (from 60% to 250%) than those attained in a parallel anaerobic dispersed growth reactor developed with the same inoculum. The GAC-reactor exhibited increased COD and phenolic compound depletion yields (by 62% and 78%, respectively) compared with those achieved using the identically configured SB-biofilm reactor. Both biofilm reactors also mediated an extensive OMWW remediation under continuous conditions, where the GAC-reactor was much more effective than the corresponding SB-reactor and showed a tolerance to high and variable organic loads along with a volumetric productivity, in terms of COD and phenolic compound removal, that was significantly higher than those averagely displayed by most of the conventional and packed-bed laboratory-scale reactors previously proposed for OMWW digestion. However, anaerobic digestion is not always a satisfactory bioremediation

process, in particular because of its inability to efficiently biodegrade low molecular weight phenolic compounds (tannins, lipids and phenolic compounds themselves exert inhibitory effects on the microflora carrying out the process, which therefore tend to persist in the digestors and to occur in the digestor effluents).

20.5.2 Biological removal of phenols

OMWW phenolic compounds, and in particular the lower molecular weight ones, are toxic molecules. Fiorentino *et al.* (2003) characterized phenolic products such as 1,2-dihydroxybenzene (catechol), derivatives of benzoic acid, phenylacetic acid, phenylethanol and cinnamic acid. The OMWW were fractioned by ultra-filtration and reverse osmosis techniques and tested for toxicity on aquatic organisms from different trophic levels: the alga *Pseudokirchneriella subcapitata*; the rotifer *Brachionus calyciflorus*; and two crustaceans, the cladoceran *Daphnia magna* and the anostracan *Thamnocephalus platyurus*. The fraction most toxic to the test organisms was that from reverse osmosis containing compounds of low molecular mass (<350 Da), and this was especially due to the presence of catechol and hydroxytyrosol, the most abundant components of the fraction. Sampedro *et al.* (2004) found that nine saprobic fungi were capable of completely removing monomeric toxic phenols from the DOR from the olive oil extraction process after 20 weeks of growth. Removal rate depended on the type of fungi and phenol. Results showed that most of the fungi tested eliminated o-diphenols and then non-o-diphenols. However, some fungi did not follow this trend. *Phanerochaete chrysosporium* first removed hydroxytyrosol and tyrosol and later their glucosides; in contrast *Paecylomyces farinosus* hydrolyzed hydroxytyrosol and tyrosol glucosides at the first stage, 2 weeks of growth, and then eliminated all monomeric phenols. The behaviour of this fungus seems of great interest for recovering phenolic antioxidants from the DOR. Similarly, differences in DOR decolorization capacity among the fungi tested were also observed. *Coriolopsis rigida* showed the highest capacity, followed by *Phebia radiata*, *Pycnoporus cinnabarinus* and *Phanerochaete chrysosporium*. Therefore, both decolorization and monomeric phenol elimination pointed out that saprobic fungi could be used to detoxify the DOR obtained from the two-phase system of the olive oil extraction process. The lignin-degrading ability of white rot fungi seems to be associated with the release of extracellular enzymes – which mainly include lignin-peroxidases, Mn-dependent peroxidases and laccase – and these enzymes could participate both in the removal of monomeric phenols and in the decolorization of olive residues. Peréz *et al.* (1987) first described decolorization of OMWW by *Phanerochaete chrysosporium*, and suggested that the decolorization occurred through the breakdown of coloured phenolic polymers into monomers, which were subsequently mineralized, but this is not a well-explained process.

Many other researchers have studied the depolymerization and dephenolization of the olive residues by saprobic fungi and a highly significant correlation was found between decolorization and laccase production; however, the same authors do not find similar correlations in other experiments. Laccase alone is able to remove monomeric phenols from OMWW, but the decolorization and dephenolization of OMWW by fungi seems to be a sequential process: monomeric phenols are first oxidized and polymerized and then the depolymerization and, therefore, decolorization occurred. Soil saprobic fungi are important and common components of the rhizosphere soil from which they obtain nutritional benefits in the form of inorganic compounds and exudates from the root. These soil fungi are important because they take part in the mobilization of nutrients and degradation of phytotoxic substances, they produce substances that promote or inhibit the growth of other rhizosphere microorganisms, they add great amounts of microbial biomass to the soil and they also contribute to the optimum use of nutrients by the plant.

20.5.3 Combining anaerobic and aerobic processes

Some biological and physicochemical OMWW pre-treatments have been proposed to reduce the occurrence of these pollutants in the wastewater sent to the digestor (Borja *et al*., 1998; Beccari *et al*., 1999, 2000); however, only limited improvements in the overall phenol removal were achieved. More promising results have been recently achieved on the laboratory scale by integrating the activity of conventional anaerobic digestors with that of an aerobic post-treatment employing activated sludge biomass (Beccari *et al*., 2002) or phenolic compound-mineralizing bacteria immobilized in packed-bed loop reactors (Bertin *et al*., 2001). A possible approach for mitigating the release of phenolic compounds from anaerobic digestors treating OMWW may consist of intensifying the bioremediation potential of biomass employed in the process upon its immobilization in dedicated packed-bed reactors (DeFilippi and Lupton, 1998; Rajeshwari *et al*., 2000). In fact, the metabolic activity and versatility of pollutant biodegrading microorganisms often significantly increases upon passive cell immobilization on porous carriers (Shreve and Vogel, 1993; Annadurai *et al*., 2002). In addition, the positive effects exerted by some carriers on the bioavailability of pollutants and/or of microbial inhibitors often contribute to further enhance the bioremediation potential of biofilm reactors (Annadurai *et al*., 2002). Furthermore, the use of such non-conventional bioreactors may allow reductions in process start-up time and the occurrence of shock loading and/or washout problems, which very often compromise the productivity of dispersed growth reactors operating with low-growth-rate biomass and high and fluctuating organic loads (Rajeshwari *et al*., 2000), as typically happens in the anaerobic digestion of OMWW (Rajeshwari *et al*., 2000; Marques, 2001). Bertin *et al*. (2004) studied the bioremediation and

biomethanization of OMWW with a GAC- and SB-packed-bed biofilm loop reactor and the conventional dispersed growth digestor operating with the same microbial inoculum and OMWW. They also determined and compared the performances and stability of the two biofilm reactors under continuous modes of operation.

20.5.4 Combining biological and chemical oxidation procedures

Studies have combined chemical oxidation and biological systems to treat biorecalcitrant pollutants, with potential advantages for wastewater treatment. The combination of chemical and biological oxidation is becoming a successful alternative to conventional treatment technologies. In this way, the quantitative degradation of the recalcitrant organics into CO_2 and H_2O by strong extensive chemical treatment is no longer necessary. The new goal is a moderate transformation by chemical means of the recalcitrant organics into more easily attackable end products, which can be subsequently submitted to conventional or advanced biological treatments.

20.5.5 Chemical oxidation

Many advanced chemical oxidation technologies are based on the production of hydroxyl radicals, possessing an extremely high oxidation potential (+2.73 V); to reduce the temperatures (and hence the pressures) required for wet oxidation, it has long been proposed that organically polluted wastewaters should be treated with Fenton reactant, i.e. hydrogen peroxide in the presence of iron salt. In the Fenton process, the completion of oxidation is dependent on the hydrogen peroxide : organic pollutant ratio (usually 2 : 1 equiv : equiv), on the catalyst : peroxide ratio (ca. 1 : 10 mol : mol) and on pH (between 3 and 4); the rate of oxidation is determined by the initial Fe^{II} concentration and the temperature. Usually, the amount of added salt is low, less than 20 mM, but there are examples of the Fenton processes running at higher concentrations, up to 60 mM. Comprehensive recent analyses of the Fenton reaction for water purification and recovery have reported that yields and rates can be enhanced by assisting the reaction photochemically or electrochemically. Given sufficient time, a long list of chemicals can be destroyed completely by the Fenton process, apart from, rather surprisingly, some common carboxylic acids such as acetic, maleic and fumaric acid, and acetone which is often found among the effluent of the Fenton treatment – being formed *in situ* by oxidation of a variety of precursors. The higher recalcitrance to the Fenton treatment of the above compounds sustains the use of biological treatments for the detoxification of OMWW. Bressan *et al.* (2004) evaluated, on a laboratory scale, the possible advantages, in terms of efficacy and depolluting effectiveness, of a combined and synergic action of a catalytic oxidation treatment in liquid phase by using an iron/hydrogen peroxide system together with advanced

microbial biotechnologies. OMWW were oxidized up to 80–90% by stoichiometric amounts of diluted hydrogen peroxide (35%) and in the presence of water soluble iron catalysts, either Fe^{II} or Fe^{III}, at concentrations up to 1% w/w and more, i.e. much larger than those reported for conventional Fenton processes. In the combined action, the mineralization activity of a selected microbial consortium was used to degrade residual volatile and non-volatile organic compounds into CO_2 and biomass (Table 20.5). Results suggested that the operational methodology was capable of reducing the potential impact of wastewaters. The chemical process is characterized by the fact that the oxidation is carried out in the presence of large amounts of iron salts, either ferric or ferrous, at over 0.05 mol L^{-1}, and therefore far beyond the amounts usually reported for conventional Fenton processes. Under these conditions, and contrary to common belief, oxidation of strongly polluted wastewater is definitively competitive with the dismutation of hydrogen peroxide, so that almost all of the added hydrogen peroxide is consumed to perform the abatement of COD, with very few organics left in the reaction mixtures. Moreover, because oxidation is very fast, no photochemical or electrochemical assistance is necessary. The marked exothermicity of the process can be controlled by gradually adding the iron salt or the oxidant to the wastewater.

The observed break in the efficiency of the Fenton treatment at iron concentrations between 30 and 50 mM (around 0.1% w/w of Fe) is the most unexpected finding of this investigation, strongly suggesting that different mechanisms are taking place when smaller or larger concentrations of iron are used. Along with the generally accepted mechanism of the Fenton reaction of hydroxyl radicals, in recent years several alternative hypotheses

Table 20.5 COD removal and chemical and microbiological parameters (CFU, total ATP, total phenol, GI) on OMWW after combined chemical and biological treatments (adapted from Bressan *et al.*, 2004)

Added H_2O_2 (%)	COD residual (COD removal %)			Parameters after both treatments		
	Chemical treatment	Biological treatment	CFU mL^{-1} (log)	Total ATP (ng mL^{-1})	Total phenol (%)	GI (%)
0	Untreated sample					
	11.4 (0)	11.4 (0)	1.7	3.5	1.24	26.0
0	Untreated sample					
	11.4 (0)	5.8 (49)	5.48	9.01	0.75	45.5
15	4.90 (59)	4.30 (62)	4.62	8.60	0.44	71.5
30	4.35 (62)	3.25 (71)	5.39	8.72	0.32	78.5
60	2.20 (81)	1.20 (90)	5.24	8.10	0.27	81.0
100	1.70 (85)	1.15 (90)	4.39	8.44	0.21	76.5

CFU, colony forming units; GI, germination index.

have been proposed, pointing to the participation of high valent oxo-iron complexes, as indeed earlier proposed by Bray and Gorin (1932). Parallel reactions can be envisaged also for Fe^{III}, leading to high valent and reactive oxo-iron species. The extreme efficiency of the reaction does not allow us to distinguish whether a radical mechanism is operating, since common hydroxyl scavengers, such as 2-proanol, disappear almost immediately upon oxidation. The Fenton system works effectively with a very unusual iron/hydrogen peroxide ratio (around 1/100, at 1:1 oxidant to COD ratio, equiv:equiv). The chemical treatment based on the described Fenton reaction indicates the possibility of rather effectively abating the polluting load of OMWW, by up to 80–90% in terms of COD. However, the treatment results in the total disappearance of the viable microflora (sterilization effect), probably as a result of the direct action of hydrogen peroxide or of the formation of toxic intermediates. Sub-stoichiometric amounts of the oxidizing reagents (modulated Fenton treatment) lead to partial removal of COD and alter the chemical composition of the OMWW in a more favourable way to the subsequent biological action. Even if longer times (15 days) are necessary, the biological treatment not only allows the attainment of further, even if less significant, demolition of the COD (up to 90%) but finally offers the immediate possibility of overcoming the intrinsic low germinability of the wastewaters. The final value of the germination index (GI) parameter is always >70%, a limit that attests the absence of chemicals capable of inhibiting the germination of seeds. These GI data must be compared not only with those of the samples treated only chemically (around 10% GI) but also with those of the samples treated only biologically, i.e. without a chemical pre-treatment, which even though it is higher (at around 40%) is still unsatisfactory. It is therefore reasonable that the chemical pre-treatment effectively removes important organic compounds that inhibit the biological oxidation.

The main component of the costs of the process is hydrogen peroxide (presently ca. €0.2 per kg of 35% solution, i.e. €0.009 /oxidation equivalent); therefore a 'soft' chemical pre-treatment (60% of the stoichiometric demand) of an average OMWW with COD of 80 000 mg L^{-1} (10 equiv L^{-1}) requires €54 mc^{-1}, in terms of hydrogen peroxide consumed. The other weak point of the treatment relates to the huge amounts of iron salts necessary, 50–100 mM, i.e. 2–5 g Fe L^{-1}, well beyond the standard accepted for iron in wastewater (2–4 mg L^{-1}). However, it should be noted that a large part of the added iron separates from the solution at the end of the treatment as insoluble Fe^{III} hydroxide, which could be recovered in high yields. This point must not be underestimated, since the addition of the iron salts to the initial OMWW sample always yields clear solutions, probably because of the complexing ability of the very concentrated organics present therein. In the course of the reaction, however, almost all of the organics are destroyed and the small residual amounts of the complexing agents are not able to maintain significant quantities of iron in solution. Fiorentino *et al.*

(2004) described the application of two oxidation methods by polymer-supported reagents and then the combination of the most effective one with an advanced biological process based on the use of selected bacteria. The use of the immobilized oxidants on the solid phase is an advantageous technique due to the simple handling of the reagents, the opportunity of recycling and the possibility of controlling the reaction and the product yields, reducing the formation of oxidation by-products. In the study, OMWW were collected in southern Italy and subjected first to a chemical oxidative procedure with $FeCl_3$ and then to a biological treatment. The latter was performed in a pilot plant where mixed, commercial, selected bacteria – suitable for polyphenols and lipid degradation – were inoculated. The effect of treatments was assessed by the extent of COD removal, reduction of total phenols and decrease of toxicity – using primary consumers of the aquatic food chain (the rotifer *Brachionus calyciflorus* and the crustacean *Daphnia magna*). Results indicated that the chemical oxidation was efficacious in reducing all the parameters analyzed. A further decrease was found by combining chemical and biological treatments (Tables 20.6 and 20.7). Beltrán *et al.* (1999) utilized ozone alone and combined with hydrogen peroxide of UV radiation for chemical OMWW oxidation as a useful pre-treatment before an aerobic biological oxidation step. These processes allowed high COD reductions, nearly complete disappearance of aromatic

Table 20.6 Phenol removal calculated by HPLC (adapted from Fiorentino *et al.*, 2004)

Compound	Removal (%)
Protocatechic acid	20
Hydroxytyrosol	72
Catechol	53
Tyrosol	10
4-Hydroxybenzoic acid	10
Vanillic acid	2

Table 20.7 Percentage removal of the chemically oxidized OMWW in the biological pilot plant after different time periods (days) (adapted from Fiorentino *et al.*, 2004)

Parameter	Starting value	Mean reduction (%)			
		1 day	2 days	3 days	4 days
COD (mg L^{-1})	4000	37.5	51.3	60.9	62.5
Total phenol (mg L^{-1})	16.2	14.8	25.0	46.1	60.2
Toxic unit *Brachionus calyciflorus*	27.3	5.6	19.4	22.2	30.6
Toxic unit *Daphnia magna*	28.2	9.8	21.4	26.8	33.0

content and colour, and moderate total carbon reductions. Other studies demonstrated the utility of photo-Fenton pre-treatment for recalcitrant wastewaters. Most of the chemical processes have proved their worth in the elimination of toxic compounds. On the other hand, these kinds of treatments have been shown to be expensive when compared with biological ones. Furthermore, the biological treatments are, at present, the most compatible with the environment. Thus, Pinto *et al*. (2003) reported the removal of low molecular mass phenols from OMWW using microalgae, and, in a recent work, OMWW were subjected to biological degradations with aerobes and facultative aerobic bacteria without strict anaerobes. However, it is important to develop efficient chemical pre-treatment processes for biologically recalcitrant compounds, processes that reduce the toxicity, increase the biodegradability of the substances and lead the pre-treated wastewaters to a biological treatment. Fernández-Bolaños *et al*. (2002) explored the possibility of obtaining hydroxytyrosol in high yield from two-phase olive waste and carried out a series of hydrothermal treatments. Usually when a lignocellulosic material is treated with water or steam to temperatures in the range of 160–240 °C, an autohydrolysis process occurs. Depending on the conditions used, there is a depolymerization and a breaking of the lignin–carbohydrate bonds, resulting in the solubilization of lignin fragments of low molecular mass. As a consequence of such treatment, the solid olive by-product was partially solubilized. Because the hydroxytyrosol is usually part of other molecules such as oleuropein, demethyloleuropein, verbascoside and hydroxytyrosol glucosides the authors defined the experimental conditions that gave the maximum concentrations of free hydroxytyrosol and also of other raw compounds (hemicellulose, cellulose, residual oil). The main operational variables governing the autohydrolysis process (temperature and the speed of the reaction) were varied. The effects of certain acidic and basic catalysts were also evaluated. Autohydrolysis plays an important role in this hydrothermal pre-treatment of alperujo, leading to liquor with a pH in the range of 2–5. Nevertheless, because the hydroxytyrosol, an orthodiphenol with important nutritional properties, seems to be strongly bound to the solid phase of alperujo, a severe hydrolytic treatment is required for its isolation. The recovery of other organic compounds present in the hydrolysate would help to reduce both the costs and the energy requirements of the process. Fernández-Bolaños *et al*. (2004) characterized three different samples of alperujo and subjected them to a hydrothermal treatment with and without acid catalyst. The main soluble compounds after the hydrolysis were represented by monosaccharides xylose, arabinose and glucose; and by the oligosaccharide mannitol; and by the products of sugar destruction. Oligosaccharides were separated by size exclusion chromatography. It was possible to obtain highly purified mannitol by applying a simple purification method. Therefore, in suitable hydrothermal operational conditions, different mixtures of soluble oligosaccharides might be obtained.

20.5.6 Composting

Lua and Guo (1999) studied the feasibility of obtaining compost and biogas from the wastewater of the palm oil production process. In addition to the empty fruit bunches, 1–2.5 tonnes of wastewater with a COD of 50–65 g L^{-1} are released per tonne of palm oil (Ibrahim *et al*., 1984; Ny *et al*., 1985). The energy content of the wastewater could be utilized if the wastewater was digested in biogas reactors. A thermophilic digestion process is the most feasible procedure, due to the high initial temperature of the effluents (70–80 °C) and the high ambient temperature in the tropics, which prevents rapid cooling to the mesophilic temperature range (Ibrahim *et al*., 1984). Anaerobic digestion in the thermophilic temperature range favours the bioavailability of oil by emulsification of palm oil residues. Since the composition and concentration of the wastewater of palm oil mills is fairly constant, the main focus for maintaining a stable digestion process lies with the temperature of the wastewater. If palm oil mill effluent (POME) is digested in fixed-bed anaerobic digestors at 46 °C and a space loading of 6.5 kg COD m^{-3} per day, equivalent to a hydraulic retention time of 3.5 days, 92% of the COD is degraded to biogas (Siller *et al*., 1998). The specific gas yield is 1.25 m^3 of biogas per kilogramme of oil, the volumetric gas yield in the reactor is 2.9 $m^3 m^{-3}$ per day and the methane content is 72%. Wastewater from olive oil or sunflower oil could be stabilized with a similar efficiency, provided that any inhibiting phenolic compounds in the wastewater of cold-pressed oil are destroyed by pre-treatment (e.g. Rivas *et al*., 2001). For anaerobic digestion, carrier-mediated reactor systems, such as polyurethane foam-bed reactors (Rozzi *et al*., 1989), have been successfully applied.

20.5.7 Biosurfactants as fermentation products from yeast

Many microorganisms (including bacteria, fungi and yeasts) excrete different types of biosurfactants (including mycolic acids, glycolipids, lipopolysaccharides, lipoproteins–lipopeptides and phospholipids) (Desai and Banat, 1997). Biosurfactants reduce the surface tension in the same manner as chemically synthesized tensides. The biological origin, low toxicity and their environmental bio-compatibility favour these fermentation products over others for application in many fields, including cosmetics, pharmaceuticals, emulsifiers, preservatives and detergents. During the microbial production of sophorose lipids by *Candida bombicola*, glucose and/or triglycerides (oil and fat from plants) are partially degraded to obtain energy and a carbon source for growth of microorganisms. Simultaneously, the tensidic glycolipids are produced via gluconeogenesis, hydroxylation of fatty acids and so on (Lang and Rau, 1999). With *Candida bombicola* as a catalyst, and glucose and fatty acids from rapeseed oil as substrates, 0.65 kg sophorose lipids per kilogramme were obtained. If glucose and canola oil were supplied as substrates, 0.78 kg sophorose lipids per kilogramme were

obtained (Lang and Rau, 1999). The production of biosurfactants from renewable resources such as vegetable oil, distillery and dairy wastes was optimized by Daniel *et al*. (1998); it is also ecologically safe and seems to be economic (Makkar and Cameotra, 2002). Biosurfactants from *Bacillus subtilis, Rhodococcus erythropolis, Candida Antarctica and Streptosporangium* sp. have been applied as anti-viral, anti-tumour and inhibiting agents for other diseases (Banat *et al*., 2000).

20.6 Future trends

Key technologies that drive new product development from vegetable oil wastes are based mainly on genetic engineering, that is the use of new organisms and new crops with good yields that can be purified to make many of the new products possible. Another important aspect is related to computer-controlled processing. The new processes can be run as a batch or continuous process with less manpower and under tighter limits that give optimum yields. The use of membrane separations with tightly controlled pore sizes, or that are functionalized to allow separation according to other properties, makes purification of natural products much easier. In addition, the use of chromatographic resins can become very important. In most cases, if the separation can be done in the laboratory liquid chromatograph, then it can be done in the plant. Furthermore, enzymes and precious metal catalyst allow for quick reactions and good yields.

The advantage of using new systems of vegetable oil processing such as the two-phase extraction olive oil system relies on the production of a solid by-product that can be dried and extracted by solvent. The new DOR can be used for cogeneration of electric power, used in combination with saprobic fungi for the removal of monomeric phenols.

A special innovative aspect of oil waste management is the biotechnological production of biopolymers. Polyhydroxyalcanoates (PHAs) can be produced with microorganisms and processed to bioplastics. Some microorganisms store biopolyesters (2–10% of their dry matter) as granular reserve material in the cells. However, if the supply of N, P, S, Mg^{2+} or oxygen for growth was limited, the biopolymers contributed up to 80% of the cell dry weight (Young Baek Kim and Lenz, 2001). Economic biotechnological plastic production depends on highly productive microorganisms and on low-cost substrates. Microorganisms with a high PHA productivity are available and the biochemical and molecular bases for PHA enrichment have been investigated in detail (Steinbuchel and Hein, 2001). To replace expensive glucose as a substrate, agroindustrial carbohydrate-rich by-products from sugar production, molasses or oily compounds such as glycerol (from biodiesel production), have been used as glucose substitutes or co-substrates for biopolymer production. If fatty acids were supplemented, the products of β-oxidation served as intermediates for PHA formation. If

saturated and unsaturated fatty acids were mixed for esterification by the microorganisms, co-polymers with elastic properties were formed by the microorganisms (Young Baek Kim and Lenz, 2001). To reduce production costs for biopolymers, fermented fruit and vegetable residues (consisting mainly of fatty acids) were supplied as a carbon source for the microorganisms (Nonato *et al*., 2001). However, 'Viopol', a co-polymer of hydroxybutyrate and hydroxyvalerate, was produced commercially by Zeneca with glucose as a sole carbon source and was marketed as packaging material.

New uses of wastes from vegetable oil processing include:

1. Vitamin C. A new two-stage fermentation-based process that produces 2-keto-gulonic acid, which is then chemically converted to vitamin C.
2. Biotin. This is a new fermentation-based product from a genetically engineered organism.
3. Isoleucine. This is the next critical amino acid for feeds. A new genetically engineered organism is nearing completion.

20.7 References

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Part V

Minimising disposal: wastewater and solid waste management in the food industry

21

Treatment of food processing wastewater

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21.1 Introduction

Whenever food, in any form, is handled, processed, packaged and stored, there will always be an inherent generation of wastewater. The quantity of processing wastewater that is generated and its general quality (i.e. pollutant strength, nature of constituents), has both economic and environmental consequences with respect to its treatment and disposal.

In fact, the cost for treating the wastewater lies in its specific characteristics and the requested standards for discharging. The pretreatment of food processing wastewaters is commonly associated with discharges to sewers and thus to a municipal wastewater treatment plant (WWTP) but sometimes treated waters are discharged to water bodies thus requiring that very high standards are met. Further, treated water can be directly reused within the food processing industry. When considering the discharging of food processing wastewaters, the degree of pretreatment required is determined by the specified discharge limitations defined in the municipal ordinances for discharging to sewers. Generally, the limitations focus on wastewater characteristics that are historically responsible for detrimental influences on the WWTP operation and pollutant-removal efficiencies. These are generally macropollutants, like chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), nutrients, etc. However, food processing wastewaters generally contain considerable amounts of other pollutants like greases, oils, proteins and salts. In addition, some specific micropollutants like pesticides, hormones, antibiotics and pharmaceuticals can be present.

This chapter deals with the application of different processes for treatment of wastewaters produced in the food industry. The first part of the chapter is devoted to the illustration of characteristics and flows coming from food processing, then the typical processes for pollutant removal and their yields are presented; the final part of the chapter presents the most innovative processes for wastewater treatment and for recovery of valuable products from these wastes.

21.2 Food wastewater production and characteristics

A typical characteristic of the food industry is the great variability in the length of the processing season and the amount of material processed. Also associated with this industry is the wide variation in both the amount of water used for processing and the waste loading from process plant to process plant. In general, wastes from the food industry contain biodegradable organic matter in the form of both dissolved and suspended solids, fats, oils and greases. Therefore, typical pollutant parameters of importance to the food industry are BOD, COD, TSS, fats, oils and greases (FOG) as well as nutrients (nitrogen and phosphorus). Other important parameters include the levels of salt or chlorine (for example in the seafood industry), or proteins (produced, for example, by the meat and dairy industries). Recently, attention has also focused on a number of micropollutants including hormones, pesticides and surfactants.

When considering the literature dealing with food wastewaters it is clear that the quantity and quality of wastewaters produced in the food industry are dramatically different from process to process. In general, processing of food from raw materials requires large volumes of high-grade water which then generate large amounts of wastewater. Table 21.1 shows some typical examples of wastewater production per tonne of treated material. Generally, as an average, some 10–20 m^3 of wastewaters are produced per tonne of product. These are also characterised by very different pollutant profiles. For example, vegetable washing generates waters with high loads of particulate matter and some dissolved organics; they may also contain surfactants. Animal slaughter and processing produces very strong organic waste from body fluids, such as blood, and gastrointestinal contents. This wastewater is frequently contaminated by significant levels of antibiotics and growth hormones from the animals and by a variety of pesticides used to control external parasites. Insecticide residues in fleeces are a particular problem in treating waters generated during processing of wool. Processing food for beer produces wastes generated from cooking which are often rich in plant organic material and may also contain salt, flavourings, colouring material and acids or alkalis. Very significant quantities of oil or fats may also be present. So, it is evident

Table 21.1 Wastewater production in the food processing industry (adapted from Johns, 1995; and Metcalf & Eddy, 2002)

Industry, activity	Range of flow (m^3/tonne of product)
Cannery	
Green beans	50–70
Peaches and pears	15–20
Fruits and vegetables (general)	4–35
Food and beverage	
Bread	2–4
Meat packing	15–20
Slaughterhouses, USA	4–17
Slaughterhouses, Europe	5–10
Milk products	10–20
Beer	10–16
Whisky	60–80
Wine	1–4

that different processes for food treatment produce a broad range of wastewaters with different characteristics. Table 21.2 summarises some of the data reported in the literature; this provides an overview of the range of wastewater compositions from the different food-industry sectors. The data also show clearly that inside any one sector the ranges of pollutant concentrations are very wide. In fact, if one examines any single kind of food processing wastewater, the situation is seen to be even more complex. Table 21.3 shows the values of the main parameters for some different kind of wastewaters produced in the dairy industry alone (from Demirel *et al*., 2005).

The variability of these wastewaters obviously affects the choice of the correct train of processes for wastewater treatment. In order to design the correct treatment process, it is imperative that a truly representative sample of the stream effluent is obtained for characterisation. Not only may samples be required for the 24-hour effluent loads, but it is necessary that peak load concentrations, the duration of peak loads and the occurrence of variation throughout the day are determined (Metcalf & Eddy, 2002). All these elements are of tremendous importance when designing the WWTP for these wastewaters and obviously orient the choices of the designer among different process options.

Table 21.2 Characteristics of food processing wastewater

Parameter	COD, (mg/l)	Soluble COD (mg/l)	BOD_5 (mg/l)	TS (mg/l)	TVS (mg/l)	TKN (mg/l)	N-NH_4 (mg/l)	N-NO_3 (mg/l)	TP (mg/l)	P-PO_4 (mg/l)	Salinity (mgNA^+/l)	References
Dairy	900–7000		500–5000	100–2500	255–830	50–270			10–300			Demirel *et al.* (2005)
Slaughter house	530–4700		400–2000	220–2100		40–230	3–70		6–34			Johns (1995)
Olive oil mill	20400–77200			16000–36000		500–800	604		5200	4000		Scioli *et al.* (1997); Becker *et al.* (1999); Inan *et al.* (2004); Ahmadi *et al.* (2005); Yuèrekli *et al.* (1999)
Winery	7130–27200	5805–12700		600–1400	130–170	25–53	0–45			0–13		Andreottola *et al.* (2005), Beck *et al.* (2005), Colin *et al.* (2005), Eusebio *et al.* (2005), Brucculeri *et al.*, 2005
Fishery	6000–66000			4600–57000	4100–6500	520	90				7000–12000	Aspé *et al.* (1997), Uttamangkabovorn *et al.* (2005),
Beverage	2500–22000	1660–6000						1280–2990				Austermann-Haun *et al.* (1997, 1999)
Vegetable	250–15000	7000	125–4700	103–3960		460–738			20–150			Mohammadi *et al.* (2004), Azbar Yonar (2004), Mishra *et al.* (2004), Burgoon *et al.* (1999), Beltrán *et al.* (1997)

TVS, total volatile solids; N-NH_4, ammonia as nitrogen; TKN, total Kjeldhal nitrogen; TP, total phosphorous.

Table 21.3 Characteristics of dairy waste effluents (adapted from Demirel *et al.*, 2005)

Effluent type	COD (mg/l)	BOD_5 (mg/l)	pH	Alkalinity ($mgCaCO_3/l$)	SS (mg/l)	VSS (mg/l)	TKN (mg/l)	TP (mg/l)
Creamery	2000–6000	1200–4000	8–11	150–300	350–1000	330–940	50–60	
Cheese whey	68814						1462	379
Cheese	1000–7500	588–5000	5.5–9.5		500–2500			
Fresh milk	4656		6.9					
Milk powder/butter	1908		5.8					
Fluid milk	950–2400	500–1300	5–9.5		90–450			
Mixed milk wastewaters	980–7500	680–4500	4.4–9.4		90–450			
Mixed dairy processing wastewaters	1150–9200		6–11	320–970	340–1730	255–830	14–272	8–68

21.3 Analysis of conventional technologies for treatment of food processing wastewater

In advance of building a wastewater treatment facility, a food industry should undertake an in-plant waste control programme in order to minimise the use of water, for example for cleaning, transportation and cooling operations, etc. (Carawan *et al.*, 1979). Within a specific food processing industry, there may be a range of approaches to achieve this; for example by substituting pneumatic transporting systems for water transporting and using nozzles that automatically shut off when released by the operator. Once appropriate in-plant control measures have been initiated, the food processor must then assess the projected strength and volume of the processing wastewater. These parameters are significantly influenced by the fluctuation in production volumes and foreseen production line expansion programmes. Hence, these factors must also be taken into account when designing the wastewater treatment system in order to ensure appropriate sizing and equipping of the system (i.e. adequate volumes, aeration systems, installed power, etc.). This again highlights the importance of properly characterising the wastewater stream to be treated. In addition, when considering the treatment of food processing wastewaters, their composition should be fully evaluated. Most wastewaters contain considerable amounts of suspended matter which can be removed by physical or chemical–physical processes. The soluble pollutants can then be removed by means of biological (both aerobic or anaerobic) treatments or chemical–physical processes (membranes or other). Figure 21.1 shows a possible, general, scheme for treatment of wastewaters from food processing. In this train of processes the following steps are considered:

1 Preliminary treatments. These generally consist of a screening step (maybe double) and eventual grit removal. The screens used could be vibrating, rotary or static. Usually the screens used have from 10 mm

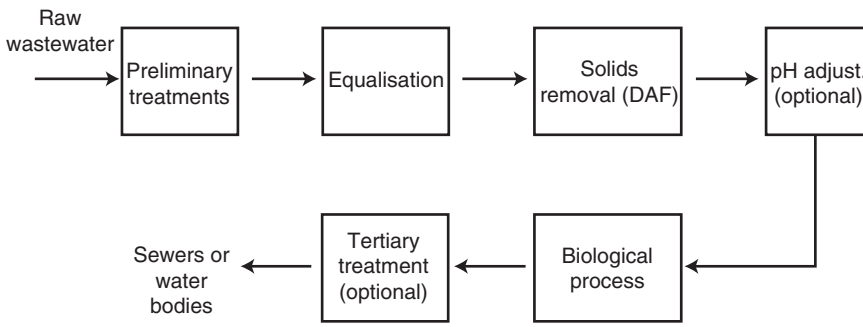

Fig. 21.1 Typical process scheme for treatment of wastewater from food processing.

down to 1 mm openings. Material of small size can be removed by high-speed circular vibratory polishing screens. Screening systems may be used in combination to maximise the efficiency of the process. Efficiencies of these systems are variable: rotary drum and disc show removal percentages up to 40–50% for suspended solids.

2 Flow equalisation. Following the screening process and preceding the unit for suspended solids removal is a flow equalization step. Flow equalization is important in reducing hydraulic and organic loading in the biological process following. Equalization facilities consist of a holding tank and pumping equipment designed to reduce the fluctuations of the waste streams. The equalizing tank will store excessive hydraulic flow and stabilize the flow rate to a uniform discharge rate over a 24-hour day.

3 Primary sedimentation or flotation for suspended solids removal. After equalisation the elimination of suspended solids is carried out. This can be obtained through the application of a typical primary sedimentation process or by applying a dissolved air flotation (DAF) system. Primary sedimentation allows for the 30% and 60% removal of COD and total suspended solids, respectively (Metcalf & Eddy, 2002). DAF is probably the most common pretreatment for food industry wastewater: it can be used to remove oil, fats, greases and fine particles. The raw wastewater is brought in contact with a recycled, clarified effluent which has been pressurized through air injection in a pressure tank. The combined flow stream enters the clarification vessel and the release of pressure causes tiny air bubbles to form which begin their ascendancy to the surface of the water, carrying the suspended particles with them in their vertical rise.

At the top of the tank there is usually a mechanical apparatus to remove the floating skimmers. To improve the efficiency of solids removal, chemicals such as ferric chloride, alum, lime and anionic polymers, and acid adjustments to pH 5, are used. Varied combinations of alum and polymer, lime and ferric chloride, and acid adjustment, alum and polymer have been demonstrated to increase the particle removal efficiency of the DAF process. For example, when considering wastewaters from the meat industry, usually 40–50% of COD is due to coarse suspended matter (1 mm mesh), insoluble in water and slowly biodegradable; thus screening, settling and DAF are widely used to remove suspended solids and also fats, oil and grease. DAF units are usually assisted by adding chemicals, which permits the removal of a large amount of nitrogen, phosphorous and BOD (75–80%). Table 21.4 shows the typical performances obtained in DAF units for treating wastewaters from meat processing (Johns, 1995). As a drawback, this system may have considerable problems due to the long retention time and low surface-overflow rate – which leads to solids settling and the production of a large volume of putrefactive material, and difficulties in

Table 21.4 DAF performances on slaughterhouse wastewater (from Johns, 1995)

Treatment efficiency	COD removal (%)	TSS removal (%)	Oil and grease removal (%)
Conventional DAF	40	60	90
Acid, pH 4–4.5	71	78	93
DAF and chemical addition	31–92	70–97	89–98

dewatering. It is also important to note that this preliminary treatment can remove the carbon that may be necessary for the removal of nutrients in a later activated sludge process for biological nutrients removal (BNR; e.g. Bolzonella *et al*., 2001).

These preliminary unit operations (stage 1–3), operating all together, will generally remove up to 85% of the total suspended solids, and 50–60% of the COD present in the wastewater.

4 pH adjustment. A pH adjustment is carried out after preliminary treatments in order to obtain a wastewater much more suitable for the biological processes following, generally in the neutral range.

5 Biological treatment processes (aerobic or anaerobic). In Europe most of the food processing plants deliver their wastewater to municipal systems after primary treatment, but in some cases the wastewaters may pass through a secondary biological (anaerobic or aerobic) treatment. In fact, to complete the treatment of the food processing wastewaters, the waste stream must be further processed by biological means. After adequate primary treatment, frequently used biological treatment systems include: anaerobic processes, extended aeration, aerated lagoons, trickling filters and land application. Recently, some new highly effective processes such as membrane bioreactors and jet loop reactors have come into operation. These processes will be presented and their performances discussed in Section 21.4.

6 Eventual tertiary treatment (membrane or other). After the biological step, treated water can be further polished by means of specific tertiary treatments such as the use of membranes (microfiltration, ultrafiltration and reverse osmosis; Cheryan, 1998), or other chemical–physical processes (activated carbon, precipitation, chelation) for the removal of specific pollutants and to further improve water characteristics.

When high standards for treated water are requested, the biological reactor is the core technology of the treatment process. The most common options for biological treatment are those already reviewed by Carawan *et al*. (1979) and reported in the following sections.

21.3.1 Anaerobic treatments

Food processing wastewaters are particularly suitable for anaerobic treatment processes, firstly because of their high organic load and secondly because they rarely contain toxicants or inhibitory compounds. Indeed, excluding equipment cleaning operations, for which chemicals and disinfectants are normally used, all the sources of wastewater are related to the preparation and processing of animal- and vegetable-derived raw material, and the wastewater characteristics are therefore mainly dependent on the nature of the organic matter processed. The upflow anaerobic sludge blanket (UASB) system has become the most widely applied reactor technology for high rate anaerobic treatment of industrial effluents (Fang and Chui, 1993; Lettinga, 1995; Driessen and Yspeert, 1999; Moletta, 2005). The main reason for the success of the UASB is its relatively high treatment capacity (up to 10 kg COD/m^3 per day) compared with the other biological systems, which permits the employment of compact and economic wastewater treatment plants. In addition, anaerobic processes enable biogas production, with associated energy recovery, and are also characterised by low sludge production: typical yields are <0.1 kg volatile solids (VS)/kilogram of COD removed. However, these processes are not suitable for removal of nutrients and only a partial removal of nitrogen can be obtained in anaerobic conditions (Strous *et al*., 1997). Therefore, when high quality standards for treated water are requested, the anaerobic processes are generally coupled with aerobic processes such as the activated sludge process (see below). In UASB reactors effective sludge retention is achieved by use of a three-phase separator on the top of the reactor, which separates biogas, sludge and treated effluents. The biomass grows in the form of granules, which are easily settled. Although the sludge has good settling characteristics, sludge retention in UASB reactors becomes critical at high upflow gas and liquid velocities. The UASB process can cope with high-strength industrial effluents having a COD concentration in the range of 3000–10 000 mg/l or more (Lettinga, 1995). The organic loading and hydraulic capacity are the most critical design criteria for the upflow sludge bed reactor. While the reactor design for treating low-strength effluents is mostly hydraulically limited, for treating high-strength effluent the system is generally limited by its organic loading capacity. UASB reactors are usually operated at maximum upflow velocities of 1–3 m/hour or for minimal hydraulic retention times of 4–5 hours (Metcalf & Eddy, 2002). To overcome these limits a new type of reactor was designed, with an increased height/diameter ratio; these systems operate with higher upflow velocities and organic loading rates. An example of such a reactor is the expanded granular sludge bed (EGSB) which contains a granulated anaerobic active biomass (Lettinga, 1995; Nunez and Martinez, 1999). The internal circulation (IC) process consists of two UASB reactors, one on the top of another, working the first at high load and the second one at low loading (Demirel *et al*., 2005). Its

Table 21.5 Efficiency of UASB reactors for different food processing wastewaters

Wastewater	COD removal (%)	BOD_5 removal (%)	Lipids removal (%)	Fat acids removal (%)	Reference
Dairy effluent	Up to 97.7	–			Demirel *et al.*, 2005
Winery	93–99	–			Brucculeri *et al.*, 2005
Slaughterhouse	57				Liu Victor *et al.*, 2004
Fruit juice	Up to 80				Austermann-Haun *et al.*, 1997
Vegetable processing	80				Rintala and Lepisto, 1997
Beer	57–80				Parawira *et al.*, 2005; Austermann-Haun and Seyfried, 1994
Confectionery	92.4	91.5			El-Gohary *et al.*, 1999
Potato-chips	82	86			El-Gohary *et al.*, 1999
Sunflower oil factory	87		70–94	81–95	Saatci *et al.*, 2003
Olive mill	80–85				Sabbah *et al.*, 2004
Clam processing	56–77	67–82			Boardman *et al.*, 1995
Coffee	70–78				Dinsdale *et al.*, 1997

special features are the separation of biogas in two stages and the internal circulation driven by the produced gas. UASB reactors are widely applied in the treatment of food processing wastewaters because of their capacity to remove BOD and COD at high levels and to recover methane, a renewable energy source that can be directly reused in the food processing plant. Table 21.5 shows the removal efficiency for BOD and COD from food processing wastewaters. As these are characterised by high levels of easily biodegradable soluble organic compounds, efficiencies are very high, generally >90%.

21.3.2 Activated sludge process treatment

In an activated sludge treatment system, an acclimatised, mixed, biological growth of microorganisms (activated sludge) is brought into contact with organic material in the wastewater in the presence of excess dissolved

oxygen and nutrients (nitrogen and phosphorus) (e.g. Metcalf & Eddy, 2002). The microorganisms convert the soluble organic compounds to carbon dioxide and cellular material, i.e. new biomass. Oxygen is obtained from applied air which also maintains adequate mixing. The bioreactor effluent is settled to separate biological sludge and a portion of the sludge is recycled; the excess is sent for further treatment such as dewatering. Activated sludge systems utilised in the food processing industry are the extended aeration types: that is, they combine long aeration times with low applied organic loadings. Typical food/microorganisms (F/M) ratios are lower than 0.1 kg COD/kg mixed liquor volatile suspended solids (MLVSS) per day while sludge age (solid retention time, SRT) should be maintained at values greater than 20 days. This practice allows for a relatively low production of waste activated sludge, typically <0.3 kg VS per kilogram of COD removed. This value is however much higher than that obtained in anaerobic processes. The detention times are typically 1–2 days. The concentration of suspended solids is maintained at moderate levels, generally some 3–6 g/l. It is usually necessary to provide a primary treatment and flow equalization prior to the activated sludge process, to ensure optimum operation. Removal of BOD_5 and suspended solids in the range of 95–98% can be achieved. One problem related to extended aeration systems can be the solid/liquid separation in the final clarifier; indeed these activated sludges are generally characterised by bad sedimentation tendencies. As this problem can drastically affect the process performances, membrane bioreactors have recently been introduced (Stephenson *et al.*, 2000). Required oxygen is generally in the range 1–1.5 kg per kilogram of treated BOD_5 for a number of food processing wastewaters (i.e. dairy, fruit and vegetable) while seafood wastes appear to require higher oxygen availability to stabilise these waste types (up to 2–3 kg per kilogram of treated BOD; Carawan *et al.*, 1979). As required oxygen can be very high, the activated sludge process is sometimes applied after an anaerobic process for the removal of excess organic compounds. Moreover, the activated sludge process enables the possibility of removing nutrients (nitrogen and phosphorous) when operated as a pre-denitrification or a BNR process (Mauret *et al.*, 2001). Temperature (for winter operations) can have a significant influence on the waste removal performance of the extended aeration system since pin-point floc can develop and loss of biological activity will decrease the performance efficiency of this system under cold-weather operating conditions.

This process, in all its different configurations, is widely applied in food wastewater treatment. Table 21.6 shows typical yields observed for this process when treating different kinds of wastewaters.

21.3.3 Aerated lagoon treatment

Aerated lagoons are generally used where there is not sufficient land available for seasonal retention or land application, and economics do not justify

Table 21.6 Efficiency of the activated sludge process for different food processing wastewaters

Wastewater	COD removal (%)	BOD_5 removal (%)	TN removal (%)	$N\text{-}NH_4$ removal (%)	TP removal (%)	Reference
Winery	89–97	96	50–71	85	88	Chudoba and Pujol, 1996 Houbron *et al.*, 1998 Brucculeri *et al.*, 2005
Egg processing	88	99	–	–	–	Olsson *et al.*, 1997
Slaughterhouse	62	90				Johnson *et al.*, 2000
Dairy effluent	86					Liu *et al.*, 2004
Pet food	70–92					Liu *et al.*, 2004
Confectionery	86.7	85.9				El-Gohary *et al.*, 1999
Potato-chips	84	86				El-Gohary *et al.*, 1999

an anaerobic or activated sludge system. Efficient biological treatment can be achieved by the use of the aerated lagoon system (Fonade *et al.*, 2000). Air is applied to these ponds by fixed or floating mechanical aerators or by compressors through air diffusers located on the bottom of the pond between 2.5 and 5 m deep. Operating with 2–10 days retention time facilitates a reduction in BOD_5 of 55–90%. Table 21.7 reports some results found in literature dealing with the application of aerated lagoons for treatment of food processing wastewaters. From the data reported one can see that a partial removal of nitrogen can be obtained because of the simultaneous nitrification/denitrification processes. There are two types of aerated lagoons in common use: completely mixed (all solids are kept in suspension and aerobic oxidation takes place) and facultative-aerated lagoons (the contents of the pond are only partially mixed and suspended solids settle to depths where anaerobic decomposition occurs). Loading rates for aerated lagoons vary considerably, however aerated lagoons achieve good BOD_5 removal. Levels of suspended solids in the effluent are

Table 21.7 Efficiency of aerated lagoons for different food processing wastewaters

Wastewater	COD removal (%)	BOD_5 removal (%)	TN removal (%)	$N\text{-}NH_4$ removal (%)	TP removal (%)	Reference
Dairy	93	97				Baick *et al.*, 1992
Meat industry	63–69	32–41	22–47	15–47		van Oostrom, 1995
Potato processing	61–72		46	19–27	0–27	Kadlec *et al.*, 1997

usually high and therefore aerated lagoons are usually followed by quiescent settling to reduce the concentration of solids.

21.3.4 Stabilisation pond treatment

A common practice for improving the effluent treated in the aerated lagoon is the use of a stabilization/polishing pond system (Costa-Pierce, 1998). This system depends on the action of aerobic bacteria on the soluble organics contained in the waste streams. The organic carbon is converted to carbon dioxide and bacterial cells. Algal growth is stimulated by incident sunlight which penetrates to a depth of 1–1.5 m. Photosynthesis results in the production of excess oxygen which is available to the aerobic bacteria; additional oxygen is provided by mass transfer at the air/water interface. The ponds are designed to provide a residence time of 2–20 days, with surface loadings of 5.5–22 g BOD_5/day per square metre.

The ponds are usually multiple cell units operated in series to eliminate the possibility of short circuiting and to permit sedimentation of dead algae and bacterial cells. The ponds are constructed with inlet and outlet structures located in positions to minimise short circuiting due to wind-induced currents; the dimensions and geometry are designed to maximise mixing. These systems can achieve 80–95% removal of BOD_5 and approximately 80% removal of suspended solids, with most of the solids discharged as algae cells. During winter periods the degree of treatment decreases markedly as temperatures decrease and ice cover eliminates algal growth.

Aerobic stabilization ponds are utilised where land is readily available. In regions where soils are permeable, it is often necessary to use plastic, asphaltic or clay liners to prevent contamination of adjacent groundwater. Although obsolete and not really 'process controlled' these systems are still in use because of their low cost and efficiency when low-standard effluents are required.

21.3.5 Trickling filters

Trickling filters, utilising plastic media in columns 4.5–6.0 m high, have been used in the treatment of high-strength fruit and vegetable wastewaters (3000–4000 mg/l BOD_5). High liquid recirculation rates and forced air circulation are used to achieve BOD_5 removals up to 90%. However, these processes tend to fail with the high organic load rates that are typical of some food processing wastewaters as, for example, in the cheese industry (Rusten *et al*., 1996).

Wastewater is distributed by rotating arms at constant rates on to the top surface of packed columns of plastic or other inert material media. A biological film is formed on the media and cellular material periodically sloughs off when the thickness becomes sufficiently great that oxygen transfer cannot occur throughout its depth and anaerobic conditions develop. Underdrains located beneath the column transport the effluent to settling tanks where the dense sludge is separated from the liquid effluent. A portion of the effluent is recycled to seed the process with biological cells and to promote consistent sloughing. When operating with loading rates up to 5 kg BOD_5/m^3 per day, filter media achieve BOD_5 reductions of 40–50% (Carawan *et al*., 1979).

21.3.6 Land disposal of wastewaters

The application of wastewater to land is a low-capital and low-operating cost method for the treatment of food processing wastes, provided sufficient land with suitable characteristics is available. This method has been the most highly used 'technology' for wastewater treatment for a long time. Obviously, it cannot be very effective and recent legislation tends to limit the possibility of spreading food wastewaters on land. However, this system still has its importance in wastewater treatment.

The ultimate disposal of wastewater applied to land is by one of the following methods:

- percolation to groundwater,
- overland runoff to surface streams,
- evaporation and evapotranspiration to the atmosphere.

The following methods are used for land application:

- irrigation,
- surface ponding,
- groundwater recharge by injection wells,
- subsurface percolation.

Generally, irrigation methods are used the most frequently. Irrigation processes can be further divided into four subcategories according to the rates of application and ultimate disposal of liquid. These subcategories are overland flow, irrigation, high-rate irrigation and infiltration–percolation.

With respect to organic carbon removal, these systems have been shown to achieve pollutant removal efficiencies of approximately 98 and 84% for the infiltration and overland flow systems, respectively. Nitrogen removal is found to be slightly more effective with the infiltration-type land application systems when compared with the overland flow application. However, the infiltration type of application has been shown to be quite effective for phosphorous and grease removal and thus offers a definite advantage over the overland flow application if phosphorous and grease removal are of prime importance.

According to Carawan *et al.* (1979), irrigation is a treatment process that consists of a number of steps:

1 Aerobic bacterial degradation of the deposited suspended materials, evaporation of water and concentration of soluble salts.
2 Filtration of small particles through the soil cover, and biological degradation of entrapped organics in the soil by aerobic and anaerobic bacteria.
3 Adsorption of organics on soil particles and uptake of nitrogen and phosphorus by plants and soil microorganisms.
4 Uptake of liquid wastes and transpiration by plants.
5 Percolation of water to groundwater.

There are both hydraulic and organic loading constraints on the use of this method for the ultimate disposal of effluent. If the maximum recommended hydraulic loadings are exceeded, the surface runoff would increase. Should the specified organic loadings be exceeded, anaerobic conditions could develop with resulting decrease in BOD_5 removal and the development of odour problems. Applied loadings of organic suspended solids average approximately $8\,g/m^2$ but loadings up to $22\,g/m^2$ have been applied successfully. A resting period between applications is important to ensure survival of the aerobic bacteria. The spray field is usually laid out in sections such that resting periods of 4–10 days can be achieved.

21.4 Future trends

In addition to the processes and technologies described above, which are well established and applied at industrial scale, some new processes and trends have recently been developed. In particular, some important aspects are receiving increasing attention: the treatment of wastewaters in 'intensified processes' like membrane bioreactors (Stephenson *et al.*, 2000) and jet loop reactors, but also the production of chemicals and biofuels through the fermentation of wastewaters rich in organic compounds. These concepts will be discussed in the following sections.

21.4.1 Membrane and membrane bioreactor process

Membranes are special devices, generally made up of synthetic polymers or inorganic material, that operate like barriers to divide a liquid from a solid stream. They are characterised by their different compositions and pore sizes. In food industry and wastewater treatment the size generally ranges between microfiltration (MF, 0.1–10 μm) and ultrafiltration (UF, 0.001–0.1 μm). However, reverse osmosis (RO, 0.0001–0.001 μm) can also be of interest if salinity is excessive. All these systems are pressure driven (Cheryan, 1998). Membranes can be used for treating high-strength wastewaters like those coming from food processing or in downstream processing to recover products, or to polish and recycle wastewater (Moresi and Lo Presti, 2003).

Raw or pretreated food processing wastewater can be treated by MF. MF can remove suspended solids, bacteria and large molecules without the need for chemicals. In addition, membranes are backwashable which allows them to be operated under low or no cross-flow and with high yield and flux. If RO is done after MF or UF, a number of chemicals can be removed. However, carbonates can precipitate and therefore some chemicals may be needed to reduce water hardness.

Although this technology is used frequently in the food industry it should be emphasised that, in this role, membranes merely serve to separate or fractionate wastewater components, hopefully into more useful and/or less polluting streams, and cannot break down or chemically alter the pollutants. At the very least, a membrane could render a permeate stream ready to discharge into the sewer or to be reused within the production process. In any case, a membrane process requires a proper pretreatment technology for removing coarse particles and suspended solids.

In order to improve the capability of removing pollutants, membranes can be coupled to activated sludge processes to form a membrane bioreactor (MBR; Stephenson *et al.*, 2000). The membrane bioreactors are systems in which a membrane is used as a liquid–solid separator and permits a high solids concentration in the bioreactor in order to intensify the treatment process. As the settling properties of the biomass are no longer driving the capacity to divide the liquid from the solid stream, the hydraulic and solid retention time are not linked, therefore the system can be operated with very high solid retention times (typically greater than 50–60 days or more) which reduces the production of excess sludge (Rosenberger *et al.*, 2000; Bae *et al.*, 2003).

Moreover, these systems are well known for their ability to completely remove suspended solids and produce virtually solids-free permeate without the presence of micropollutants like organic compounds and heavy metals (Innocenti *et al.*, 2002; Clara *et al.*, 2005; Fatone *et al.*, 2005). A typical characteristic of these processes is the production of treated waters with very high quality standards. Table 21.8 shows some typical data reported in the literature for this process: these show very high yields for COD

Table 21.8 Efficiency of the MBR for different food processing wastewaters

Wastewater	COD removal (%)	BOD_5 removal (%)	TN removal (%)	$N\text{-}NH_4$ removal (%)	TP removal (%)	Reference
Food (generic)	94		74	91		Wang *et al.*, 2005
Food (generic)	99					Katayon *et al.*, 2004
Meat industry	98.1	99.6	98.2	95	87.3	Sroka *et al.*, 2004
Winery	97					Artiga *et al.*, 2005
Vegetable oil factory	90				85	Mohammadi and Esmaeelifar, 2004
Pet food	94–98					Kurian *et al.*, 2005
Vegetable	96–99	95–96.5				Nakhla *et al.*, 2006

Table 21.9 Efficiency of the jet loop reactor for different food processing wastewaters

Wastewater	COD removal (%)	Reference
Winery	90.7–93.1	Petruccioli *et al.*, 2002
Brewery	90–97	Bloor *et al.*, 1995
Cheese whey	97	Farizoglu *et al.*, 2004

removal, always >90%, while solids removal is virtually 100%. If a biological nutrients removal process is applied, nitrogen and phosphorous can also be removed.

21.4.2 Jet loop reactors

The jet loop is an activated sludge system with a tall and deep oxidation vessel that achieves high dissolved oxygen concentrations due to a high liquid–air contact time. This allows for a high oxygen availability and the possibility of dealing with very concentrated wastewaters.

Table 21.9 reports some interesting data that can be found in literature for this process. As shown from reported data the removal efficiency for wastewaters with high organic content is generally higher than 93%.

21.4.3 Chemicals and biofuels production

Biotechnology, in particular the fermentation sector, has become more and more attractive in recent years for the production of chemicals and biofuels from organic wastes (Willke and Vorlop, 2004). In fact, there are numerous possibilities for replacing chemical techniques with biotechnological methods based on renewable resources. The most important biogenic sources of raw materials for industrial chemicals are: oil plants (oil, fat, glycerol, celluloses); starch plants (starch, inulin, carbohydrates, celluloses); sugar beets and sugar cane (sucrose); wood (ligno-cellulose, cellulose); and waste and residues from agriculture and industry (biomass, fats, oils, whey, glycerol). The food industry is probably the main source for these materials.

Fermentative processes can be used for both production of biofuels (methane, hydrogen, ethanol, biodiesel) and building blocks, such as lactic acid, succinic acid, ascorbic acid, isomalt, cyclodextrines and polyamino-acids (Wilke, 1995; Gavrilescu and Chisti, 2005). Some important examples of chemicals and energy sources or vector production are reported below.

1 Building blocks. Costs for industrial chemicals are generally in the range 2–5 US$/kg, therefore the use of innovative bioprocesses using waste as substrates and (possibly) mixed cultures is very welcome. Typical

examples of the bioproduction of building block chemicals from food waste(water) are lactic acid (anaerobic fermentation of starch, sugars or wheat) and succinic acid (fermentation of sugars by bacteria in anaerobic conditions). Lactic acid is produced at a cost of 0.5 US$/kg at 75 000 tonnes/year. Other compounds of importance are ascorbic acid (vitamine C), isomalt, cyclodextrines, 1–3 propandiol, polyaminoacids (PAA). These can be produced through the fermentation of sugar-rich waste(waters) (Wilke, 1995, 1999; Traverso *et al*., 2000; Willke and Vorlop, 2004).

2 Bulk chemicals. Bulk chemicals (production costs <1 US$/kg) produce a volume of sales of some 10 billion US$ (Wilke, 1999). These are alcohols (ethanol), organic acids, amino acids and other fermentation products. Fermentation of waste and wastewaters from the food processing industry can be the main route for the production of these compounds as the feedstocks are clearly cheaper than pure organic compounds (sugars). Traverso *et al*. (2000) and Bolzonella *et al*. (2005) have demonstrated the possibility of producing fatty acids at very high concentrations (up to 50 g/l) using vegetable and food wastes as substrates in anaerobic mesophilic (35 °C) reactors.

3 Bioenergy and biofuels. Biotechnology-based production of fuels continues to attract much attention (Kosaric and Velikonja, 1995). Bioethanol, biogas, biodiesel and biohydrogen are examples of biofuels. The use of these compounds directly reduces consumption of fossil fuels and environmental pollution (Gavrilescu and Chisti, 2005). Biodiesel, for example, can be produced through the transesterification of vegetable oils and animal fats, all by-products of food processing wastewaters (Ma and Hanna, 1999).

The biotechnological production of acetone, butanol and ethanol (ABE process; Willke and Vorlop, 2004) works on non-purified substrates like hydrolysed starch or cellulose. Ethanol can be produced by yeasts and bacteria through the fermentation of sugars, generally glucose, coming as residues from the food industry (sugar cane, beets) while hydrogen can be directly produced from mixed cultures by anaerobic fermentation of food processing wastewaters (Ginkel *et al*., 2005) or waste (Valdez-Vazquez *et al*., 2005). Obviously, at the moment, production of methane is the only well-established technology for energy recovery from food processing waste(waters) (Kosaric and Velikonja, 1995; Carucci *et al*., 2005).

Another interesting option is the possibility of co-digesting the waste(waters) from the food industry in existing anaerobic digesters operating in wastewater treatment plants. In fact, at present, some 36 000 anaerobic digesters are operating in Europe (Mata-Alvarez *et al*., 2000); these are generally under-loaded and can be conveniently used to produce methane, and thus heat and power, in co-generation units. In Treviso WWTP (Cecchi *et al*. 1994), waste from markets and canteens/restaurants is

co-digested together with waste activated sludge to produce important amounts of biogas (Bolzonella *et al.*, 2006): typical productions are in the range 0.4–0.6 m^3 of biogas (70% methane) per kilogram of solid fed into the reactor.

21.5 References

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22

Dewatering systems for solid food processing waste

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22.1 Introduction

The agri-food industry produces a large variety of wastes which must be handled in an environmental and sustainable way. Depending on the type of waste, various waste handling alternatives are available; including fermentation, separation, biofuel conversion, composting, extraction and more. Choosing the right waste handling process can help to meet environmental regulations and provide a saleable by-product for further processing, recovery or animal consumption. A lower moisture content of the waste material has benefits for transport costs with reduced volume and reduced weight.

This chapter discusses the concentration of solids from food waste using dewatering techniques. The reduction of moisture content offers flexibility in terms of handling, shelf life and subsequent use of the waste. Common dewatering processes use mechanical means of separation such as screens, screw presses, belt presses, vacuum filters and centrifuges, which can all be combined with additional forces to remove the water – such as using an electric field, ultrasonics, vibrations, chemical treatments, etc. In any dewatering application there is a definite advantage in combining multiple dewatering fields to promote the synergy of separation forces (Muralidhara, 1990).

The selection of the appropriate dewatering process depends on numerous factors such as the type and quantity of the waste product, the end use of the dewatered/dried solids, and environmental and economic considerations. With a dewatering process, the underlying advantage is that the water is removed in the liquid state. The lack of a phase change renders the

process less energy intensive and in some instances may improve the end product quality. Dewatering lowers the moisture content to a level not low enough for shelf stability and thus the dewatered material requires a finishing drying treatment or further processing.

22.2 Waste conditioning

When considering the adoption of a dewatering process for any given waste material, the initial characteristics of that sludge will govern the choice to be made and the handling process adopted. In a liquid–solid waste mixture, the water content to be expressed can be present as bulk or free water; as capillary bound water; or as adsorbed bound water. In certain cases, the sludge may be too liquid and may require a chemical pretreatment to improve the size of the solid particles through flocculation/agglomeration, or the waste may be too solid where the water is retained, for example, within the cellular structure of the plant-based waste material. In the latter case, a grinding process may help release some of that moisture, or a freezing pretreatment may help to achieve cellular breakdown. A pretreatment by freezing studied by Zhou *et al*. (2001) gave significantly enhanced water removal. In the case of mechanical dewatering of chopped alfalfa, the best water extraction results were obtained with previously macerated alfalfa which released the cell-bound moisture content (Sinha *et al*., 2000). In the case of kelp dewatering, the slurry is highly viscous and requires a chemical treatment (calcium chloride) to release the water (Lightfoot and Raghavan 1995; Orsat *et al.*, 1999).

22.3 Dewatering methods

22.3.1 Belt filter press

Belt filter press systems usually include a gravity drainage feeding section, and a mechanically applied pressure belt arrangement (Fig. 22.1). In gravity drainage, through simple screens, a large portion of free water is removed. Pressure is then applied at an increasing rate on the waste contained between supporting porous belts (Demetrakakes, 1996). The dewatered waste cake is removed from the belt with scrapers. In certain arrangements, a small vacuum must be applied (4–6 kPa) to facilitate the removal of water accumulating at the surface of the belts (Snyman *et al*., 2000). A preflocculation step is often considered to suit particular waste applications that are too liquid and to maximize dewatering efficiency right from the start of the process during gravity drainage.

In roller press dewatering, the waste material is pressed between rotating roller drums, where the single belt material only serves for conveying. The bottom rollers are perforated to allow for drainage of the pressed liquid.

Fig. 22.1 Double-belt press filter BPF S7-E (Sernagiotto Technologies SpA, 2006).

The basic system is composed of a top roller that presses down on to two bottom rollers and the drums rotate to facilitate the passage of the material on a conveyor belt (Orsat *et al*., 1999). This system is well adapted to combining with electro-osmotic dewatering. Design improvements were investigated by Kauppila *et al*. (2001) for roller groove geometries. It appears that larger groove angles can help to further reduce the moisture content during roller pressing of sugar cane bagasse.

22.3.2 Screw press dewatering

In a screw press, the material is introduced in a perforated chamber where an endless screw forces the material along the length of the chamber towards the discharge (Fig. 22.2). The pressure force of the screw drives the water out through the perforations of the holding chamber. For this type of dewatering process, the waste feed must have a certain particle size large enough not to clog the perforations of the holding system and to flow through without excessive resistance.

When feeding through becomes more problematic due to the waste product's characteristics (particle size, viscosity, etc.), a twin screw press may be more appropriate (Fig. 22.3). The two press screws are designed to compress the product as they rotate in opposite directions, which prevents the waste material from rotating with the screws and clogging up the system. Twin screw dewatering has been successfully developed and proven efficient for citrus waste and for oil extraction from agricultural products (Isobe *et al*., 1997).

Screw press dewatering of citrus pulp is practised in the industry to yield a higher dry matter pulp and a liquid fraction high in soluble solids. The liquid fraction is further processed to produce citrus molasses, whereas the

Fig. 22.2 A screw press manufactured by Press Technology & Mfg. Inc. (Press Technology & Mfg. Inc., 2006).

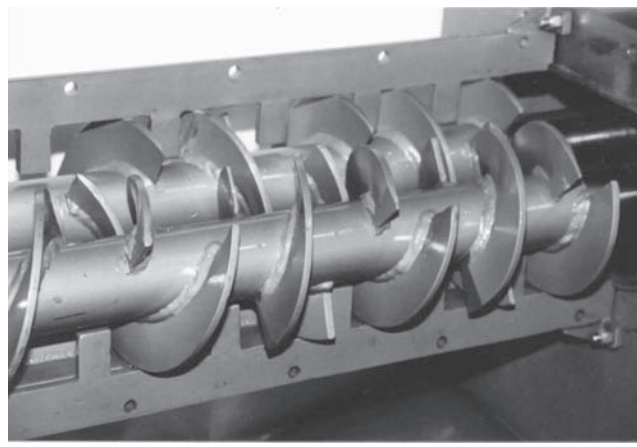

Fig. 22.3 A twin screw press manufactured by Vincent Corporation, USA (Vincent Corporation, 2006).

citrus pulp can be used as animal feed for ruminants, thus fulfilling the requirement of high fiber content leading to high digestibility (Crawshaw, 2001).

22.3.3 Rotary and centrifugal presses

A centrifugal dewatering system consists of a basket or a solid bowl and a conveyor, both of which can rotate at high speed. As the bowl rotates, the

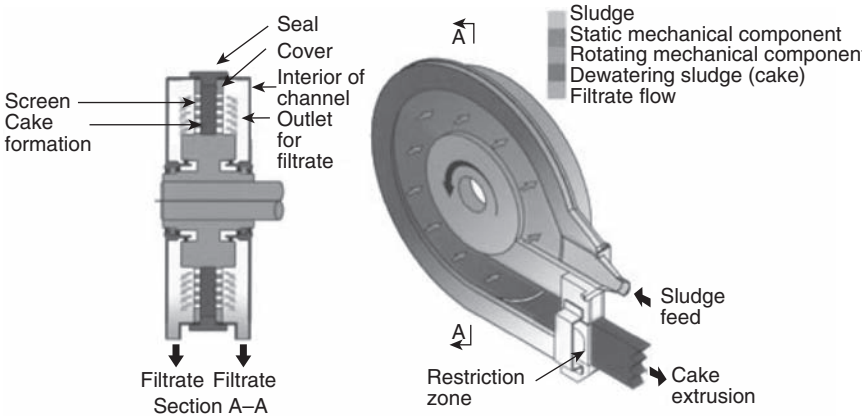

Fig. 22.4 Schematic of the Rotary Press manufactured by Fournier Industries Inc. (Fournier Industries Inc., 2006).

heavier solids gravitate to the bowl wall where they accumulate. The separation of solids from the liquid depends on the G-force, time and permeability of the waste mass (Leung, 1998).

The Rotary Press manufactured by Fournier Industries Inc., Quebec, Canada (Fournier Industries Inc., 2003) offers the industry an interesting piece of dewatering equipment (Fig. 22.4). The waste material is fed into a rectangular channel, and rotated between two parallel revolving stainless steel, chrome-plated screens. The filtrate passes through the screens as the particulate sludge advances within the channel. The sludge continues to dewater as it travels through the channel, eventually forming a cake near the outlet side of the press. The frictional force of the slow moving screens, coupled with the outlet restriction, results in the extrusion of low moisture material.

22.3.4 Membrane filter press

A membrane filter press comprises a stack of filter plates held tightly closed by pressure (Fig. 22.5). The filter plates have a filtration drainage surface that supports a filter media, in most cases a polypropylene filter cloth held in place by a more rigid polypropylene structure. The mixed solid–liquid waste is pumped into the chambers under pressure. The filtered liquid passes through the filter cloth, against the drainage surface of the plates, and is directed towards discharge collectors. The pressure gradient between the cake and the filter material provides the driving force for the flow. Solids are retained on the filter cloth forming a filter cake. The filter plates are separated and the filter cake is discharged. At this stage a vacuum step

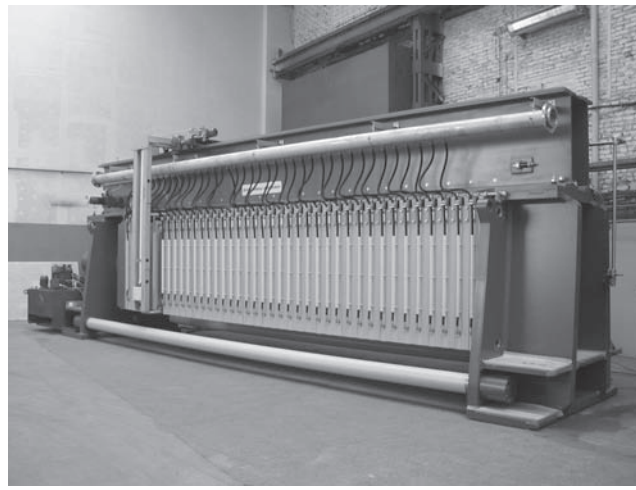

Fig. 22.5 An Andritz Netzsch Filter Press (Andritz Netzsch Filtration, 2006).

may be introduced to further reduce the moisture content. In a study by El-Shafey *et al.* (2004), brewer's spent grain was dewatered to a low moisture level of 20–30% when combining membrane filter pressing (500 kPa) with vacuum drying.

22.3.5 Electro-osmotic dewatering

Electro-osmosis is caused by the electrical double layer that exists at the interface of suspended particles subjected to an applied voltage across a solid–liquid mixture. In waste slurries, the solid particles possess a slight electric charge known as the zeta potential. Hence, when exposed to an electric field, the charged particles and the liquid fraction are entrained to move in opposite directions: one towards the anode, the other towards the cathode (Orsat *et al.*, 1996). On the one hand, electrophoresis is the movement of charged particles within solution under the influence of an electrical field, and on the other hand with electro-osmosis, the electric field causes the movement of the electrically neutral solution (Weber and Stahl, 2001). The position of the electrodes is selected in order to promote the gravity flow of water (Chen and Mujumdar, 2002). The product's properties and mainly its zeta potential will dictate the position of the negative and positive electrodes in order to favour dewatering by gravity flow. The zeta potential of a material is dependent on its composition and the ion concentration of the surrounding fluid. With an increase in the ion concentration of the surrounding fluid there is an improvement of the coagulation of the

suspended particle; this results in a decrease in zeta potential and thus a reduced electro-osmotic flow.

As the waste is dewatered by electro-osmosis, a layer of the waste near one of the electrodes has a greater water removal, causing an increase in the local electrical resistance which hinders the dewatering process (Yoshida and Yasuda, 1992). To overcome this drawback, an increase in mechanical pressure can limit the negative effect of the formation of an unsaturated layer. This is supported by studies on the electro-osmotic dewatering of food waste, where best results were obtained when combining highest pressure and highest electric field, since the electro-osmotic flow is proportional to the current density and electric field strength affects the movement of dissolved ions within the solid suspension (Al-Asheh *et al*., 2004; Gazbar *et al*., 1994; Orsat *et al*., 1996). A schematic presentation of combined pressure and electro-osmotic dewatering apparatus is presented in Fig. 22.6.

An increase in salinity from 5000 to 20 000 ppm can increase the dewatered cake solid content by 3–7% due to the increased conductivity of the slurry allowing an increase in the electric current. This benefit subsequently cancels out with the ensuing decrease in the zeta potential (Chen *et al*., 2003).

Electro-osmotic dewatering can be conducted in direct (DC) or alternating current (AC) electric fields with varying results. In general, the use of an alternating or intermittent electric field helps to reduce the electrical contact resistance that occurs at the dewatering front with an increased dewatering yield (Yoshida *et al*., 1999; Iwata, 2000; Yoshida, 2000).

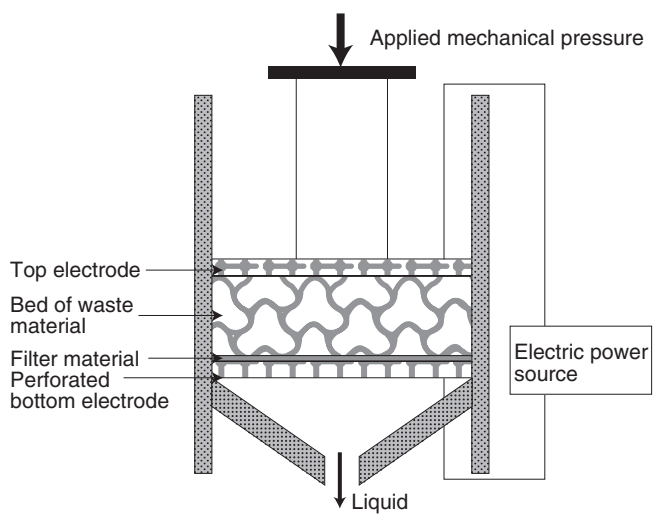

Fig. 22.6 Schematic of a combined pressure and electro-osmotic dewatering cell.

In most applications of electro-osmotic dewatering, a vertical electric field is usually applied along with mechanical pressure; however, Zhou *et al*. (2001) proposed a modification with a horizontal electric field which yielded comparable results to a vertical field in terms of dewatering efficiency while offering an alternative design for equipment construction. Furthermore, the application of a horizontal electric field may facilitate the discharge of the gases emitted by oxidation (oxygen) at the anode and by reduction (hydrogen) at the cathode (Jin *et al*., 2003).

Ho and Chen (2001) studied a rotating anode arrangement for dewatering fine particle suspensions. The system operated at variable speed. The rotating anode mixed the waste material which prevented the formation of a dry layer and thus increased the electric current and the dewatering efficiency.

The electro-osmotic dewatering process may be operated at constant voltage or constant current (Orsat *et al*., 1996; Banerjee and Law 1998). In constant voltage mode, the flow rate of expressed liquid increases with voltage applied. In such cases, heating of the sludge bed occurs due to joule heating which causes vaporization of part of the water (Banerjee and Law, 1998). In constant current mode, the electrical resistance of the bed increases with time and current settings in a quadratic relation.

Overall, electro-osmotic dewatering can remove a significant amount of water from a waste suspension at a fraction of the energy consumption that would have been required to vaporize it.

22.3.6 Dissolved air flotation separation

Dissolved air flotation (DAF) is a liquid–solid separation process for liquid suspended colloidal mixtures. DAF is principally used for the clarification of wastewaters that contain suspended solids. DAF is widely used for the recovery of valuable solids from food processing wastewaters, especially from the meat processing industry (Le Roux and Lanting, 2000). DAF involves the dissolution of air in the waste mixture at a high pressure to achieve saturation. By bringing the pressure of the mixture back to atmospheric, the air – in the form of very small bubbles – rises to the product's surface carrying with it the colloidal particles which can be recovered. To improve the DAF process, depending on the type of waste material, the addition of a chemical flocculant or coagulant (such as a polyelectrolyte) may be required as a pretreatment step for the waste slurry (Ng *et al*., 1988; Genovese and Gonzalez 1994). In general a DAF system consists of a flocculator tank, a flotation tank and a pressure vessel (Fig. 22.7). Its operation can be continuous or intermittent and a DAF system can be designed and constructed to meet the requirements of a variety of wastewaters in terms of the characteristics of its suspended solids and the plant volume requirement for separation (Viitasaari *et al*., 1995).

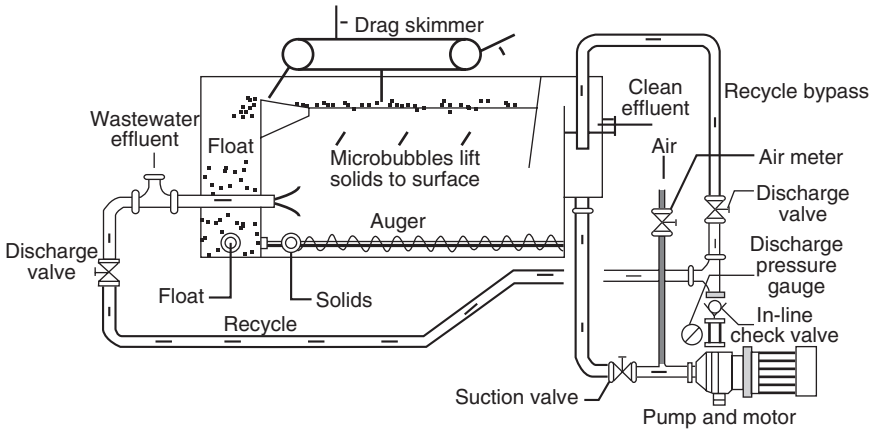

Fig. 22.7 Schematic of a DAF system (Pan America Environmental, Inc., 2006).

22.4 Combining dewatering methods

22.4.1 Electro-osmotic belt filter

Mechanical dewatering removes mostly free water from waste material, while electro-osmotic dewatering aims at pushing more tightly bound water out of the solid matrix. In the case of combined electro-osmosis with a belt filter, the waste material must pass between stainless steel or carbon fiber-coated woven belts that serve as the electrode. In certain cases, to prove effective, the waste material may require pre-conditioning with an electrolyte to achieve appropriate conductivity for an efficient combined electro-osmotic belt press dewatering (Hwang and Min, 2003). The total amount of water removed by combined pressure and electro-osmotic dewatering is a measure of both effects with high significance (Vijh, 1999).

22.4.2 Ultrasonic vibrations

Ultrasonic energy may be used to improve the efficiency and capacity of traditional separation/dewatering methods. The ultrasonic vibrations can help the agglomeration of particles to facilitate their collection in the separation process. Furthermore high-intensity ultrasonic energy causes alternative contraction and expansion of a solid–liquid mixture which facilitates the migration of moisture through the porous channels, acting as a sponge (Gallego-Juarez *et al*., 1999; Riera-Franco de Sarabia *et al*., 2000). In some cases, high-intensity ultrasounds can produce cavitation which efficiently moves moisture away. Typically the sound transducers operate in the 10–40 kHz range, and they can be coupled with belt filter press, rotary or centrifuge dewatering (Swamy *et al*., 1983). Additional dewatering efficiency

can also be achieved by adding vibrations to existing press equipments especially when dewatering viscous or thixotropic waste products. A vibratory action improves the capillary channels in dewatering cakes.

22.4.3 Electro-acoustic dewatering

Some applications have been experimentally developed for combining electrical and acoustic fields to enhance dewatering. The results have demonstrated that the acoustic field has little to bring in terms of improving dewatering in comparison to the significant improvement in the rate of dewatering brought about by electro-osmosis, which is a fraction of the cost of acoustic equipment (Smythe and Wakeman, 2000; Wakeman and Smythe, 2000). In some applications where clogging or fouling of a filter material is common, the application of optimal sound waves (at the distance prescribed by the wavelength), may keep the filtration surface clear of debris accumulation (Tuori, 1992).

22.4.4 Vapour pressure dewatering

Vapour pressure dewatering involves mechanical pressure dewatering coupled with a contact drying process (involving a thermal treatment). The waste material is compressed by mechanical means while vaporization of the moisture occurs within the draining capillaries of the waste mass with an indirect application of heat through a heated plate. The indirect application of heat to the dewatering material causes a pressure build-up with the vaporization of the liquid. The temperature gradient in the filter cake causes vaporization and condensation effects within the capillaries, thus improving the dewatering process (Korger and Stahl, 1993). With pressure differences of 100–300 kPa, Peuker and Stahl (2001) obtained a residual moisture content of 16% for the steam pressure filtration of a saturated waste cake. In this study, the waste cake was exposed directly to a steam atmosphere in a closed environment. The steam pushes the liquid out of the pores while the cake is being mechanically compressed (Peuker and Stahl, 2001).

22.5 An environmental and economic choice

In conventional drying, the latent heat supplied to vaporize the water content is a considerable energy sink. Moisture reduction by dewatering and/or drying may be an expensive option for waste handling since the concentrated material may show very little improvement in its monetary value as a dried product. The processors may also have on their hands a nutrient-rich filtrate liquid component that also becomes an environmental issue and an additional expense for its disposal as an effluent in municipal

collectors. In some cases, the filtrate has commercial value. In the UK, the expressed juice from brewer's spent grain dewatering is being marketed as a liquid protein feed for pigs (Crawshaw, 2001).

Penzim Produce, a wholesale and retail fruit and vegetable distributor located in New York City, was generating approximately 2370 tonnes of landfill waste per year and spending over $16 000 per month to dispose of the waste. Before each refuse collection, the perishable produce emitted foul odors, especially during summer months. In collaboration with New York Wa$teMatch (New York City's materials exchange and solid waste reduction program) and Earth Conserve Management Consultants, the fresh produce company has installed dewatering machinery to reduce the volume of organic material from 8–10 cubic yards (6.1–7.6 m^3) a day to 1.5–2 cubic yards (1.1–1.5 m^3) a day. Through the use of dewatering machinery, Penzim expects to reduce its waste stream by 480 tonnes per year and save about $17 000 a year in waste removal and disposal costs (New York Wa$te Match, 2006).

A simple screw press may represent a small investment to reduce the expensive cost of hauling large quantities of water from the processing plant to the farm or landfill. Dewatering methods are gaining attention in answer to rising environmental concerns and the rising cost of transport (fuel).

22.6 Conclusions

Dewatering represents an alternative to thermal drying that is more energy efficient. Combination of dewatering forces (pressure, acoustic, electric, chemical, etc.) offers potential for increased yields and improved quality of both liquid and solid fractions, for further processing or application development. Dewatering of biomass is advisable both technically and economically, and should strongly be considered for the handling of agri-food waste. Today, and in future years, the concept of 'combined field separation' should be emphasized where two or more fields/properties are exploited in a single piece of equipment for optimized energy usage and synergy of separation effects (Muralidhara, 1992).

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23

Fermentation, biogas and biohydrogen production from solid food processing

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23.1 Introduction: fermentation, biogas and biohydrogen production from food waste

Food processing waste has significant potential to pollute land, air, and water because of its high chemical oxygen demand (COD) and sheer volume. The COD concentration can be 90 000 mg/L or more, which is more than 100 times greater than common domestic sewage. It may also have a moderately high salt or acidity content and it might be contaminated with pathogens. Generally, food processing waste does not contain significant amounts of toxic chemicals.

The fact that food waste is generally organic and non-toxic means it lends itself to biological treatment. Biological wastewater treatment is primarily used to remove dissolved and colloidal organic substances in a wastewater stream. Organic substances in water decay due to the presence of microorganisms that are naturally occurring. Several biological processes have been utilized (Mawson, 1994; Gonzalez Siso, 1996). In aerobic treatment, organic materials are converted into oxidized end products, which are mostly carbon dioxide and new bacterial mass (Schugerl, 1997). The anaerobic wastewater treatment process converts organic materials primarily into methane, a fuel that can yield a net energy gain from process operations and carbon dioxide (McCarty and Smith, 1986).

During anaerobic fermentation, hydrogen is produced. In a normally functioning anaerobic digester, it is rapidly consumed by hydrogen-utilizing methanogens as they reduce carbon dioxide to methane (Gujer

and Zehnder, 1983; Huang *et al*., 2000). For that reason, hydrogen gas had been considered only as a process control index and an indicator of organic shock loading because it is rarely detected unless methanogenesis is disturbed by external environments, despite significant amounts of hydrogen being produced in reality (Archer *et al*., 1986; Huang *et al*., 2000). Hydrogen is considered a promising alternative clean energy source, which produces no greenhouse gases and is more economical than methane at less than stoichiometric yields, if it is captured during anaerobic fermentation. To extract hydrogen effectively from an anaerobic reactor requires special procedures to block out the co-metabolic chains (Adams and Stiefel, 1998), this will be discussed further in the chapter.

Most of the research and development on hydrogen production from organic materials has focused on the use of photosynthetic and fermentative bacteria. The latter is preferred, because it does not rely on the availability of a solar conversion process with a large surface area and transparency of the media (Zaborsky, 1998). An advantage of the anaerobic fermentative route is the fact that hydrogen can be produced directly from organic wastewater as raw material. This has considerable potential as an environmentally friendly process that does not consume fossil fuels (Billings, 1991; Nandi and Sengupta, 1998).

23.2 Key reasons to consider using anaerobic processes

Anaerobic processes are regarded as the most efficient of biological technologies (Speece, 1996). In contrast to aerobic biological treatment, anaerobic fermentation processes do not require air input and generate considerably smaller amounts of sludge. Anaerobic treatment normally produces 10 times less refractory biomass than aerobic treatment. Under anaerobic conditions, the majority of food processing waste COD is converted to biogas (methane and carbon dioxide) as an end product. This equivalent energy is not available for biomass synthesis, and thereby considerably lessens wastewater biomass disposal requirements and the financial burden associated with disposal. Biogas produced from anaerobic treatment has been promoted as a part of the solution to energy problems. Bio-methane has a calorific value of 9000 kcal/m^3 and can be burned on-site for heat production, to generate electricity or to do both. Little energy (3–5%) is lost as heat in the biological process (Saham, 1984; Speece, 1996; Droste, 1997).

Lettinga and Hulshoff Pol (1991a) have listed criteria to select proper treatment for any given wastewater as follows:

1 They should lead to prevention of the production of additional wastewater(s), or at least a sharp reduction.

2 They should not require any dilution of the pollutants with clean water.
3 They should provide a high efficiency with respect to environmental pollution control.
4 They should lead to maximum recovery and reuse of polluting substances (e.g. to integrated systems) particularly from food processing wastewaters.
5 They should be low cost with respect to construction, required infrastructure (including energy requirement), operation and maintenance.
6 They should be applicable on a small as well as large scale.
7 They should lead to a high self-sufficiency in all respects.
8 They should be acceptable for the local population.
9 The method should provide sufficient treatment efficiency for removal of various categories of pollutants, i.e. biodegradable organic matter, suspended solids, ammonia, organic-N compounds, phosphates and pathogens.
10 The system should be stable for interruptions in power supply, peak loads, feed interruptions and/or for avoiding toxic pollutants.
11 The process should be flexible with respect to future extensions and possibilities to improve the efficiency.
12 The system should be simple to operate, maintain and control, so that good performance does not depend on the continuous presence of highly skilled operators and engineers.
13 Land requirements should be low, especially when little land is available and/or the price of the land is high.
14 The number of process steps should be as low as possible.
15 The lifetime of the system should be long.
16 The system should not suffer from serious sludge disposal problems.
17 The application of the system should not be accompanied by malodor or other nuisance problems.
18 The system should offer good possibilities for recovery of useful byproducts, such as for irrigation or fertilization.
19 There should be sufficient experience with the system to manage it easily.

Based on these criteria, anaerobic treatment technology is regarded as a highly effective method for the treatment of most wastewaters.

The development of new bioreactors is one reason that anaerobic treatment technologies will probably gain in popularity. Organic loading rates over 20 kg COD/m^3/day (g COD/L day) are possible for some types of food processing wastes in a modern high-rate anaerobic digester (Table 23.1). High loading rates result in relatively low hydraulic retention times, which reduces capital costs because the vessels holding the waste are smaller.

Table 23.1 Comparison of various anaerobic treatment processes for cheese waste

Reactor	Waste	Temp. (°C)	HRT (d)	Influent strength (g COD/L)	OLR (g COD/ L d)	Treatment efficiency (%)	Reference
UFFLR	Sour whey	35	5	79	14	95	Wildenauer and Winter (1985)
DSFFR	Whey	35	5	13	2.6	88	De Haast *et al.* (1985)
FBR	Whey	35	0.4	7	7.7	90	Boening and Larsen (1982)
FBR	Whey	35	0.1–0.4	0.8–1.0	6–40	63–87	Denac and Dunn (1988)
AAFEB	Powder	28–31	0.4–1.1	10	8.9–27	77–93	Switzenbaum and Danskin (1982)
	Whey	35	0.6–0.7	5–15	8.2–22	61–92	Switzenbaum and Danskin (1982)
AnRBC	Whey	35	5	64	10.2	76	Lo and Liao (1986)
	Whey	35	6–11	61–70	6.3–10	76	Lo and Liao (1986)
SDFA	Whey	4.3		69.8	16.1	99	Barford *et al*. (1986)
UASB	Deproteinized Whey	35	1.5	11	7.1	94	Schroder and De Haast (1989)
UASB	Whey	33	5	5–28.7	0.9–6	97–99	Yan *et al*. (1989)
DUHR	Whey	35	7	68	10	97	Malaspina *et al*. (1995)
UASB	Whey	35	2.3–11.6	5–77	1–28.5	95–99	Kalyuzhnyi *et al*. (1997)
UASB	Whey	22–30	5.4–6.8	47–55	7–9.5	90–94	Kalyuzhnyi *et al*. (1997)
UASB	Whey	20–29	3.3–12.8	16–50	1–6.7	90–95	Kalyuzhnyi *et al*. (1997)
UASB	Whey	34	5	25–30		90–98	Yan *et al*. (1993)
CSTR and DUHR	Whey				10	98	Malaspina *et al*. (1996)
CSTR and AT	Whey	33–36	2	2–6	5	90–95	Ince (1998)
MPAR	Whey	34	2.3–2.4	20–37	9–14.7	92–98	Guiot *et al*. (1995)

Abbreviations are: HRT, hydraulic retention time; OLR, organic loading rate; COD, chemical oxygen demand; UFFLR, upflow fixed-film loop reactor; DSFFR, downflow stationary fixed-bed reactor; FBR, fluidized-bed reactor; AAFEB, anaerobic attached-film expanded-bed reactor; AnRBC, anaerobic rotating biological contact reactor; SDFA, semicontinuous digester with flocculent addition; UASB, upflow anaerobic sludge-blanket; DUHR, downflow–upflow hybrid reactor. CSTR, continuously stirred tank reactor; AT, anaerobic filter; MPAR, multiplate anaerobic reactor.

23.3 Biochemical and microbiological principles of the anaerobic process: hydrolysis, acidogenesis, methanogenesis

The anaerobic process is accomplished through biological conversion of organics to methane and carbon dioxide in an oxygen-free environment. The overall conversion process is often described as a three-stage process which may occur simultaneously in an anaerobic digester. These stages are: (1) hydrolysis of insoluble biodegradable organic matter; (2) production of acid from smaller soluble organic molecules; (3) methane generation. The three-stage scheme involving various microbial species can be described as follows: (1) hydrolysis and liquefaction; (2) acidogenesis; (3) methane fermentation.

23.3.1 Hydrolysis and liquefaction

Hydrolysis and liquefaction are the breakdown of large, complex and insoluble organics into small molecules that can be transported into microbial cells and metabolized. Hydrolysis of the complex molecules is catalyzed by extracellular enzymes such as cellulase, protease and lipase. Essentially, organic waste stabilization does not occur during hydrolysis; the organic matter is simply converted into a soluble form that can be utilized by the bacteria (McCarty and Smith, 1986; Parkin and Owen, 1986).

23.3.2 Acidogenesis

The acidogenesis stage is a complex phase involving acid-forming fermentation, hydrogen production and an acetogenic (acetic acid-forming) step. Once complex organics are hydrolyzed, acidogenic (acid-forming) bacteria convert sugars, amino acids and fatty acids to smaller organic acids, hydrogen and carbon dioxide. The products formed vary with the types of bacteria as well as with environmental conditions. The community of bacteria responsible for acid production may include facultative anaerobic bacteria, strict anaerobic bacteria or both (e.g. *Bacteroides, Bifidobacterium, Clostridium, Lactobacillus, Streptococcus*). Hydrogen is produced by the acidogenic bacteria including hydrogen-producing acetogenic bacteria. Acetogenic bacteria such as *Syntrobacter wolini* and *Syntrophomonas wolfei* convert fatty acids (e.g. propionic acid and butyric acid) and alcohol into acetate, hydrogen and carbon dioxide, which are used in methanogenesis. These microorganisms are related and can tolerate a wide range of environmental conditions (Novaes, 1986; Parkin and Owen, 1986).

Hydrogen has been shown to play a key role in regulating organic acids production and consumption. Under relatively high H_2 pressure, acetic acid

formation is reduced and the substrate is converted into propionic acid, butyric acid and ethanol, rather than methane. Thus, to maintain efficient anaerobic treatment of sludge to produce methane, the hydrogen levels must be maintained at least below 10^{-4} atom level. The syntrophic association between acetogenic bacteria and CO_2-reducing methanogenic bacteria can ensure maintenance of low hydrogen concentration (Harper and Pohland, 1986). Because of its key role, hydrogen is considered a performance indicator of anaerobic methane fermentation. Acetogenic bacteria grow much faster than methanogenic bacteria. The former group has a growth rate (μ_{max}) of approximately 1/h, whereas the latter is around 0.04/h (Stronach *et al.*, 1986).

23.3.3 Methanogenesis

The formation of methane, which is the ultimate product of anaerobic treatment, occurs by two major routes. Formic acid, acetic acid, methanol and hydrogen can be used as energy sources by the various methanogens. The primary route is the fermentation of the major product of the acid-forming phase, acetic acid, to methane and carbon dioxide. Bacteria that utilize acetic acid are acetoclastic bacteria (acetate splitting bacteria). The overall reaction is

$$CH_3COOH \rightarrow CH_4 + CO_2$$

The acetoclastic group comprises two main genera: *Methanosarcina* and *Methanothrix*. During the thermophilic digestion of lignocellulose waste, *Methanosarcina* is the dominant acetoclastic bacterium encountered in the bioreactor. About two-thirds of methane gas is derived from acetate conversion by acetoclastic methanogens.

Some methanogens use hydrogen to reduce carbon dioxide to methane (hydrogenophilic methanogens) according to the following overall reaction (Novaes, 1986; Morgan *et al.*, 1991):

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

There is a synergistic relationship between the hydrogen producers and the hydrogen scavengers. Subtle changes in hydrogen conditions can change the end products of the acid-forming phase. Alcohol degradation is also inhibited by high hydrogen levels (Gujer and Zehnder, 1983).

The methane-forming bacteria are more fastidious in their environmental requirements than acid-forming bacteria. Their metabolic rates are lower than those of acid-forming bacteria and methane production is generally the rate-limiting step in anaerobic digestion (Speece, 1996).

23.4 Environmental and operational variables of anaerobic treatment

23.4.1 Environmental factors

Temperature

Temperature is a key variable in biological treatment. Anaerobic digesters are generally operated in one of two temperature ranges: mesophilic (30–40 °C) or thermophilic (50–65 °C). Most anaerobic reactors are operated in the mesophilic ranges. Methane has been produced at temperatures of 10 °C or lower, but for reasonable rates of methane production, temperatures should be maintained above 20 °C. The rate of methane production approximately doubles for each 10 °C temperature change in the mesophilic range (Stronach *et al*., 1986). In municipal wastewater plants, anaerobic treatment is usually carried out near the mesophilic range (25 °C to 40 °C) with an optimum at approximately 35 °C (Parkin and Owen, 1986).

There are some advantages of using thermophilic digestion including higher rates of degradation and therefore smaller digester size with less capital cost, faster solid–liquid separation and better control of bacterial and viral pathogens (Mackie and Bryant, 1995). In thermophilic temperature ranges, reaction rates proceed faster than under mesophilic conditions, so that the loading potentials of anaerobic bioreactors are significantly higher. However, the fact that thermophilic wastewater treatment is hardly ever applied can probably be attributed to the conflicting and sometimes disappointing results. In comparison to mesophilic operational systems, thermophilic reactors seem to be less stable. Another disadvantage is the energy required to heat the influent to reactor temperature (Parkin and Owen, 1986; van Lier *et al*., 1996).

Psychrophilic digestion operates at temperature of 15–20 °C. There have been studies of anaerobic processes at psychrophilic temperatures aimed at decreasing or eliminating expenses required for anaerobic digester heating systems. Safley and Westermann (1990) suggest that reasonable methane yields are possible for anaerobic digestion at low temperatures if organic loading rates are appropriately reduced. In addition, Dague *et al*. (1998) report that an anaerobic sequencing batch reactor (ASBR) performs well while treating low-strength wastewater at 5–20 °C.

In practice, a stable and uniform temperature is imperative for consistent and efficient reactor operation that results in the best treatment. Temperature fluctuation has a net adverse effect on digester performance and contributes to instability of anaerobic treatment (Droste, 1997).

pH and alkalinity

The pH is perhaps the most important anaerobic process control parameter. A favorable pH range for methanogenic bacteria is between 6 and 8 with an optimum pH for the group as a whole near 7.0. Many studies report

that the pH required in anaerobic systems for good performance and stability is in the range of 6.5–7.5, although stable operation has been observed outside this range. Clark and Speece (1971) reported effects of adverse pH levels of 3.8–9.4. They found that steady methane production occurs at pH levels as low as 4. However, the rates were lower than for the same reactor operating at more optimal pH. No inhibition of methane production was observed between pH 6 and 8, and temporary pH shock did not have long-lasting effects.

The anaerobic process may eventually fail if the pH gets as low as around 6.0 and remains there for some time. Acidogenic bacteria produce organic acid, which tends to lower the pH of the anaerobic reactor. Under normal conditions, this pH reduction is buffered by the bicarbonate produced by methanogens. Under adverse environmental conditions, the buffering capacity of the system can be upset, eventually stopping the production of methane. An increase in volatile acids thus serves as an early indicator of system upset. Therefore, excess alkalinity or the ability to control pH must be present to guard against the accumulation of excess volatile acids. Anaerobic processes can operate over a wide range of volatile acid concentrations if proper control is maintained. A constant pH lends stability to the process. Automatic pH control is considered more economical than adding pH chemicals in a random manner because fewer chemicals are consumed. Common materials to increase the alkalinity are lime, soda ash, ammonia, ammonium bicarbonate, sodium hydroxide or sodium bicarbonate. Generally, lime, sodium hydroxide and ammonia are the least expensive of these chemicals (Parkin and Owen, 1986; Anderson and Yang, 1992).

Nutrient requirements

The low growth yields of anaerobes from a given amount of substrate result in lower nutrient requirements compared with aerobes. The nutrients required in highest concentration are nitrogen (N) and phosphorous (P). A common empirical formula of bacterial composition is $C_5H_7O_2N$. Using that formula, nitrogen comprises approximately 12% of bacterial cells. Nitrogen is used in the synthesis of protein, enzymes, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Phosphorous is required to synthesize energy-storage compounds, RNA and DNA (Parkin and Owen, 1986). For a typical activated sludge process, the COD:N:P requirement is approximately 100:5:1 on a mass basis. The theoretical minimum COD:N:P ratio of an anaerobic system is 350:7:1 for a highly loaded system, whereas for a lightly loaded system it is 1000:7:1 due to the reduced net synthesis of biomass. In addition to the nitrogen and phosphorous required for anaerobic microbial systems, some sulfide precursor may be needed. Anaerobic systems have significantly higher sulfur content in the biomass than aerobic cells. Therefore, the empirical cell formulation of anaerobic cells can be considered as $C_5H_7O_2NP_{0.06}S_{0.1}$ (Speece, 1996).

According to Zehnder *et al.* (1980), a substrate sulfur content of approximately 0.001–1.0 mg/L is required for optimal growth and methane production.

There are a number of trace elements required for successful anaerobic digestion: nickel and cobalt have been shown to promote methanogenesis. For typical wastes, these elements will normally be present in excess amounts (Rittmann and McCarty, 2001).

Toxicity

Toxicants, components in the wastewater causing adverse effects on bacterial metabolism, are responsible for the occasional failure of anaerobic digesters. Inhibition of methanogenesis is generally indicated by reduced methane production and increased concentration of volatile acids. Most toxic materials are stimulatory at low concentrations, but become inhibitory as the concentrations increase. From a control standpoint, toxic materials need to somehow be reduced in concentration to below a toxic threshold (McCarty, 1964). The following are some toxicants that are known to cause problems in anaerobic digesters.

Ammonia-nitrogen. Ammonia-nitrogen-containing waste or its precursors are of concern because of the potential inhibitory effects of ammonia on the anaerobic digestion microbial consortia (Angelidaki *et al.*, 1993; Poggi-Varaldo *et al.*, 1997). Ammonia is usually formed in anaerobic processes as a result of mineralization of organic nitrogen in wastes rich in protein or urea. The excess ammonia-nitrogen in the fermentation medium could cause an inhibitory effect in three different ways. First, free ammonia, which is more toxic for anaerobic microflora than the ammonium ion, is formed during the fermentation process. Second, amination of α-ketoglutaric acid by ammonia-nitrogen coupled with rapid disappearance of α-ketoglutaric acid from the metabolic pool of the tricarboxylic acid cycle could cause difficulties in the metabolism of organic compounds. Finally, buildup of ammonia-nitrogen may result in undetected accumulation of volatile fatty acids (VFAs) because ammonia will keep the pH above 7 (Krylova *et al.*, 1997; Sterling *et al.*, 2001).

Ammonia-nitrogen is generally inhibitory to methanogens at levels of 1500–3000 mg/L. However, ammonia inhibition can be tolerated in concentrations as high as 7000 mg/L with no significant decrease in methane production if a long acclimation time is allowed. Toxicity caused by continuous addition of ammonia decreases as solids retention time (SRT) is increased (Parkin and Owen, 1986).

Sulfide. Sulfide toxicity is a common problem with wastewaters containing high concentrations of sulfate. Sulfate is used primarily as an electron acceptor in anaerobic wastewater treatment and is converted to sulfide.

Sulfide in complex with heavy metals – such as iron, zinc or copper – is not toxic. It is the soluble form – primarily un-ionized hydrogen sulfide – that is most inhibitory. Concentrations of soluble sulfide from 50 to 100 mg/L are tolerated with little or no acclimation. Concentrations up to 200 mg/L are tolerated after some acclimation. Concentrations above 200 mg/L are quite toxic (McCarty, 1964). Theoretically, 600 mg/L of sulfate will produce 200 mg/L of sulfide. Hydrogen sulfide (H_2S), one of the sulfide species formed, is a relatively insoluble gas and is partially stripped from solution through normal gas production. At a normal pH during anaerobic treatment, almost all soluble sulfide is H_2S or HS^- (Rittmann and McCarty, 2001). H_2S is formed by bacterial sulfate reduction and the decomposition of sulfur-containing organic substrates. Acid-forming bacteria are less sensitive to H_2S than methanogens. Within the latter group, hydrogen oxidizing bacteria are considered to be more sensitive than acetoclastic methanogens.

H_2S is also a toxic and odorous gas even at low concentrations. It poses a health hazard and esthetic problems for workers and those who live around anaerobic systems. The H_2S in anaerobically produced gases not only causes an odor nuisance, but is also corrosive and quite detrimental to the operation of combustion engines used in energy recovery. Furthermore, H_2S is oxidized to sulfur dioxide during combustion, creating air pollution (Arogo *et al*., 2000; Rittmann and McCarty, 2001).

Sulfide production in anaerobic systems has some benefits. Sulfide serves as an essential nutrient for biological growth, and a certain amount (50–100 mg/L) is actually desirable because it helps remove heavy metals from solution in the digester. It also helps maintain a low oxidation–reduction potential, which is required for successful treatment operation (Speece, 1996).

Cation toxicity. Some organic wastes have relatively higher concentrations of normal alkali and alkaline earth salts and this can inhibit the anaerobic process. If one attempts to control very high volatile acid concentrations through the addition of sodium hydroxide or other sodium-containing bases, high salt concentration could readily affect the activity of microorganisms and interfere with their metabolism (Rittmann and McCarty, 2001). McCarty (1964) reports that sodium concentrations in the range of 100–200 mg/L are beneficial for the growth of mesophilic anaerobic microorganisms. For anaerobic granular biomass at mesophilic temperatures, sodium concentrations of 5, 10 and 14 g/L caused 10, 50 and 100% inhibition of methanogens, respectively, at neutral pH (Rinzema *et al*., 1988).

Acclimation is a factor that could affect the characteristics of sodium inhibition. Acclimation appears in anaerobic digesters treating wastewaters from mussel or sea-food processing units that contain high concentrations of sodium. Adaptation of methanogens to high concentrations of sodium

Table 23.2 Cation concentrations reported to be inhibitory to anaerobic microorganisms

Cation	Moderately inhibitory (mg/L)	Strongly inhibitory (mg/L)
Sodium	3500–5500	8000
Potassium	2500–4500	12000
Calcium	2500–4500	8000
Magnesium	1000–1500	3000

over prolonged times could increase the sodium tolerance of these microbes (Soto *et al*., 1993; Feijoo *et al*., 1995).

Another phenomenon associated with sodium toxicity is the antagonistic effect. Here, if a cation such as sodium is present in an inhibitory concentration, this inhibition might be relieved if another cation such as potassium is added. With the stimulatory concentrations of the various cations present, they help reduce the extent of inhibition caused by any of the other cations present at a moderately inhibitory concentration (Feijoo *et al*., 1995; Speece, 1996). Table 23.2 shows a summary of concentrations of various common cations that may cause inhibition (Parkin and Owen, 1986).

Feedback inhibition. Anaerobic treatment systems may also be inhibited by several intermediates produced during the process. High concentrations of these intermediates such as VFAs are toxic by virtue of feedback inhibition. In order to avoid some of these problems, Ghosh and Klas (1978) suggested that two-phase anaerobic digestion be used to spatially separate acidogenic bacteria from methanogenic bacteria. They report that some advantages of phase separation are enhanced stability and increased resistance to toxicants.

23.4.2 Operational variables

Hydraulic retention time (HRT)

The HRT is a measure of the rate of liquid flow in to and out of a reactor. Under steady state conditions, the HRT is defined as follows:

$$\text{HRT} = \text{total volume of liquid in the system/volume of liquid changed per day}$$

In a completely mixed system that employs continuous mixing, all the contents of the system have the same residence or retention time. In such a system, the detention time is governed by the replication time of the slowest growing organism of the microbial community. Below this value, the system fails from washout of the slowest growing organism that is necessary to the process. On the other hand, in systems such as the anaerobic sequencing

batch reactor (ASBR), upflow anaerobic sludge blanket reactor (UASB) and induced blanket reactor (IBR), solids retention time (SRT) is decoupled through internal settling and biomass retention from HRT. The HRT can be varied independently of the SRT (Parkin and Owen, 1986; Droste, 1997).

Solids retention time (SRT)

The SRT has been recognized as a key parameter in the design and operation of anaerobic treatment processes. The SRT is the average time that a solid particle, particularly a biological particle, stays in the reactor. Under steady state conditions, the SRT is defined as follows:

SRT = mass of total biomass in the reactor/biomass wastage from the reactor per day

For successful operation of any system, a minimum SRT must be maintained to allow the working microorganisms to regenerate themselves in the system or the system eventually fails from washout of bacteria. If the slowest growing organisms have a unique role that no other species can perform, the washout causes a loss of function and a disruption or failure of the process.

The rate of regeneration or growth of a microorganism depends on various environmental factors. The factor that influences growth rate most is the temperature at which organisms are maintained. The growth rate and minimum SRT can be related to temperature using the Arrhenius equation (Dague *et al*., 1970). From laboratory studies of many wastes, the minimum SRT for methane formers is 3–5 days at 35 °C. All biological systems require a safety factor of 3–20 times the minimum SRT for successful operation (Lawrence and McCarty, 1969).

Droste (1997) described four methods of maintaining and increasing the amount of biological solids in anaerobic systems.

1 Separation of solids from the anaerobic digester effluent and recycling of these solids to the reactor.
2 Provision of fixed surfaces on which bacteria grow and are retained in the system.
3 Development of a dense sludge blanket to hold solids in the anaerobic system.
4 Operation of the anaerobic digester with long hydraulic detention times.

Organic loading rate

The organic loading rate represents the amount of organics that must be handled by the anaerobic system, measured in mass of organic influent to the system per unit volume per time. This parameter is used as an index of

the stress imposed on the microbial population and affects the amount of total gas, methane production, COD stabilization and alkalinity.

High loading rate is one of the most important advantages of the anaerobic process compared with the aerobic process because there is no oxygen transfer limitation and no biomass thickening limitation with proper biomass immobilization (Lettinga and Hulshoff-Pol, 1991b; Speece, 1996). Maximum organic loading rate for an anaerobic reactor depends on a number of parameters, such as reactor design, wastewater characteristics, the ability of the biomass to settle and activity, etc.

In practical design, a rate of about 10 g/COD/L/day has been commonly used for high-strength wastewater in high-rate digesters. Fang and Chui (1993) showed a maximum organic loading of 100 g COD/L/day with soluble chemical oxygen demand (SCOD) removal of 90–98% in a UASB fed sucrose and milk powder.

Speece (1996) reported that the factors that control organic loading rates are:

1 Concentration of viable biosolids which can be retained in the anaerobic reactor.
2 Mass transfer between the incoming wastewater and the retained biomass.
3 Biomass proximity for metabolism of hydrogen intermediates.
4 Temperature and pH within the anaerobic reactor.
5 Level of toxicity in the wastewater.
6 Reactor configuration and presence of staging.

23.4.3 Biogas potential

Biochemical methane potential (BMP) and anaerobic toxicity assay (ATA)

The methane potential of wastewater is related to COD and the treatment efficiency. The maximum theoretical yield of methane for carbohydrates is 0.35 L CH_4/g COD removed at standard temperature and pressure (Droste, 1997). The maximum methane potential of wastewater may not be realized in a treatment process for reasons such as the refractory nature of some of the organics.

The BMP test is a procedure developed to determine the methane yield of an organic material during anaerobic decomposition by mixed microbial flora in a defined medium. This test, analogous to the biological oxygen demand (BOD) test, provides a simple means of monitoring the relative biodegradability of substrates using a serum bottle. The protocol for this assay was designed to ensure that the degradation of the compound is not limited by nutrients, inoculum, substrate toxicity, pH, oxygen toxicity or substrate overloading (Owen *et al.*, 1979; Speece, 1996). The procedure is readily modified to become a toxicity assay. The ATA uses the same

procedure as for BMP except that an acetate–propionate spike is added to a serum bottle to provide a readily degradable substrate. Methane production from various sample concentrations is compared with gas production from a control to assess the toxicity of the sample (Owen *et al.*, 1979; Owens and Chynoweth, 1993).

23.5 High-rate anaerobic bioconversion system

Higher treatment rates reduce capital costs and make it easier to build large systems because the vessels needed to contain the waste are smaller. Successful anaerobic treatment requires a microbial balance between the fast-growing acidogens and the slow-growing methanogens. Because of the slow growth rate of methanogens, efficient biomass retention is required for successful high-rate anaerobic system performance (Stronach *et al.*, 1986; Wohlt *et al.*, 1990). To achieve rapid anaerobic treatment of liquid wastewater, researchers recognized that maintenance of a high population of biomass in the reactor is necessary in order to compensate for the various problems associated with anaerobic processes (Dague *et al.*, 1970; Ndon and Dague, 1997).

The retention of active biomass has been achieved in advanced reactors such as the UASB, which has been used widely. More recently, the IBR, the ASBR, various anaerobic contact reactors, anaerobic bio-filters and fluidized bed reactors have become available. These reactors are often referred to as 'high-rate' anaerobic reactors. These methods are considered to be innovative process technologies (Switzenbaum, 1995). These processes all have a high concentration of biomass, which provides long SRTs while maintaining short HRTs. Many of these systems are also suited for treating diluted wastewater and have been used commercially for treatment of municipal and industrial wastewaters with high loading rates and stability (Lo and Liao, 1985; Lettinga and Hulshoff-Pol, 1991b). Anaerobic processes are sufficient for pre-treatment of wastewater only and some form of post-treatment is required.

23.6 Requirements for high-rate anaerobic bioconversion systems

In order to successfully create a high-rate anaerobic reactor and provide anaerobic treatment processes that can handle high organic loading rates and hydraulic loading rates, the following conditions should be met.

23.6.1 High retention of biomass and biomass–substrate contact

A high concentration of biomass is required for a high loading potential for anaerobic processes. The process loading, based on the food to microorganism

(F/M) ratio, should be low in order to achieve efficient biomass formation and solids separation. A low F/M ratio at high loading rates can be achieved when the biomass concentration is high. The sludge in high-rate anaerobic systems often forms granules that help in retaining biomass and may also serve as an internal clarifier.

High-rate anaerobic processes often require relatively rapid internal settling to keep high levels of biomass in the reactor (Lettinga and Hulshoff-Pol, 1991b; Sung and Dague, 1995). Sufficient contact between the substrate and the biomass in UASB reactors and IBRs is maintained by a hydraulic upflow pattern and natural mixing by gas production (Lo *et al*., 1994). When a hydraulic upflow pattern is absent, sufficient biomass–substrate contact can be maintained by using intermittent, gentle mixing.

23.6.2 Simple design

To become competitive, an efficient high-rate reactor system should be simple. For example, introducing waste into the bottom of a UASB reactor or IBR at pressure greater than the head pressure is all that is needed to operate the reactor, providing there is an effective method to separate solids, liquids and gas at the top. The ASBR does not require an upflow pattern and a gas–solids separation system is unnecessary, however it does require equipment and controls for filling, mixing, settling and decanting (Fernandes *et al*., 1993).

23.6.3 Acclimated and adapted biomass

High organic loading rates are obtained after the biomass is sufficiently acclimated and adapted to wastewater. In most cases, seed biomass for a newly built anaerobic process system needs an acclimation period to the wastewater before high organic loading is applied (Speece, 1996).

23.7 Single-stage high-rate anaerobic digesters

Three single-stage bioreactors that can be considered to be high rate are described in this section. All of these bioreactors retain biomass.

23.7.1 Upflow anaerobic sludge blanket (UASB) reactor

The UASB reactor (Fig. 23.1) was initially developed for widespread use in the Netherlands, and is currently the most popular high-rate anaerobic system in the world, used, in particular, to treat different types of wastewaters (Lettinga and Hulshoff-Pol, 1991b; Lettinga, 1995). Unlike some systems, such as fluidized bed and biofilter systems, the UASB reactor does not require attachment material. Wastewater enters into a UASB reactor at

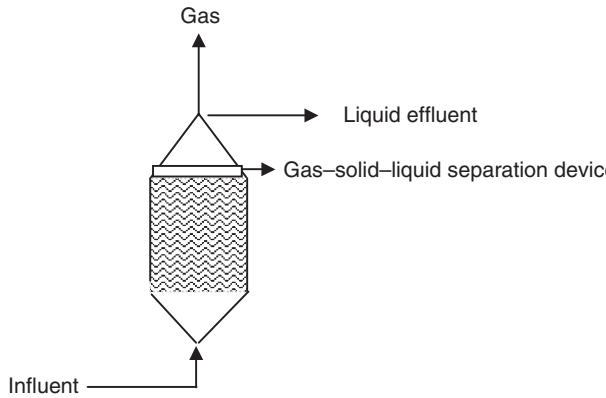

Fig. 23.1 UASB reactor schematic.

the bottom and exits at the top, and the biomass is developed as a flocculent mass in an upward flowing water stream. The UASB reactor can be successfully applied for the anaerobic treatment of wastewater if well-settling biomass with high methanogenic activity is developed. Mixing in a UASB reactor is not necessary. Sufficient contact between the biomass and the substrate occurs because of the upflow velocity of the wastewater in the sludge blanket, as well as biogas production. Good distribution of the substrate is obtained by having more inlet points at the bottom of the reactor. A gas–solids separation system is used in this type of reactor to collect the biogas and to separate the biomass from the effluent. Biomass falls back in the reactor because of a decreased upflow velocity after biogas is separated in the settling section. A gas–solid separation system is even required for the treatment of much diluted wastewater (Hulshoff Pol and Lettinga, 1986; Lettinga *et al.*, 1991).

A distinctive advantage of the UASB reactor is the very high loading rate achieved by the systems (Speece, 1996). UASB reactors can be used for high-strength wastewaters with a volatile suspended solids (VSS) : COD ratio of less than one and with COD concentrations between 500 and 20 000 mg/L (Mulligan, 2002). The UASB reactor is not therefore, an effective treatment process for wastewater containing high total suspended solids (TSS) concentration and organics in the wastewater must posses a proportionately high degree of solubility. The TSS should ideally be limited to around 500 mg/L (Verstraete *et al.*, 1996). Some advantages and disadvantages of UASB digesters are shown in Table 23.3 (Weiland and Rozzi, 1991).

23.7.2 Induced blanket reactor (IBR)

The IBR is an in-vessel high-rate (<6 day HRT) anaerobic digester developed at Utah State University (Fig. 23.2). An IBR tank has a height :

Table 23.3 Characteristics of the UASB reactor

Advantages	Disadvantages
High organic loading capacity	Granulation process difficult to control
Short HRTs	Granulation depends on wastewater properties
High COD removal efficiency	Granule floatation
No need for support media	Restriction on nearly solids-free wastewater
Simple reactor construction	Sensitive response to organic shock loads
Low energy demand	

Fig. 23.2 IBR anaerobic digester.

diameter ratio of 2.5:3.5, which can vary depending on waste type. The tanks are usually ~10 m tall to facilitate settling. There is a septum located near the top of the tank. Treated waste must pass through a relatively small orifice in the septum. An anti-plugging device fills the orifice and can be made to push solids back below the septum or can be reversed to pull solids through the hole, if necessary (Hansen and Hansen, 2005). IBRs are also

equipped to break up foam or a floating layer that may otherwise form at the top. Waste enters the bottom of the tank and is treated as it moves vertically through the tank and is discharged at the top. The upward velocity is much less than that recommended for UASB reactors. Livestock waste has been successfully treated with short HRTs in an IBR that was about 10 m high by 4 m in diameter (Hansen and Hansen, 2002).

The IBR captures solids from the influent where they are kept in the reactor vessel so that SRT greatly exceeds HRT. These solids also become attachment media for bacteria. If the solids concentration becomes too great, a portion is removed to avoid plugging. The IBR thus forms a sludge blanket within the digester vessel and can help maintain the size of the blanket so as to avoid plugging. As previously stated, high-rate anaerobic digesters must have some means of retaining the slow-growing anaerobic bacteria. The blanket that is induced within the IBR retains bacteria. Media can be placed in the reactor vessel so that the bacteria attach to it, such as sand in a fluidized bed bioreactor. However, the attachment media tend to plug when trying to treat waste containing a high concentration of fibrous solids such as is found in animal manure.

An integral part of the IBR system is a modular approach. IBR tanks are usually not larger than 100 000–120 000 L. Very large agricultural production or processing facilities can be accommodated. A 1000-animal unit (1000 pounds (454 kg) of live weight per animal unit) farm would require three tanks. However, there are advantages in that an IBR system can easily be built to fit small or large facilities and can be easily expanded. If part of the system fails, it does not stop the entire process.

23.7.3 Anaerobic sequencing batch reactor (ASBR)

The ASBR process is able to handle soluble influent streams and also those with higher TSS (Dague *et al.*, 1992; Schmit and Dague, 1993). The ASBR is a non-steady state or pseudo-steady state, anaerobic treatment system. By definition of non-steady state, substrate conversion rate and biomass production rate of the system vary during the cycle. An intermittent feed and decant regime results in alternating high/low substrate (feast/famine) conditions in the reactor. One of the most important operation characteristics of the ASBR is a high substrate concentration at the end of the feed cycle and a low substrate concentration at the end of the react cycle. The reactor cycle includes four steps: feed, react, settle and decant (Arora, 1985; Sung and Dague *et al.*, 1995) (Fig. 23.3).

During feeding, substrate is added to the reactor. Normally, the volume of waste being added to the reactor during feeding is the same as the volume being decanted as effluent. At the end of feeding, the reactor is mixed to distribute the waste throughout the liquid volume. The second step in the cycle is to react. Proper mixing during this step is found to be important in the conversion of organic substrate to biogas. The time

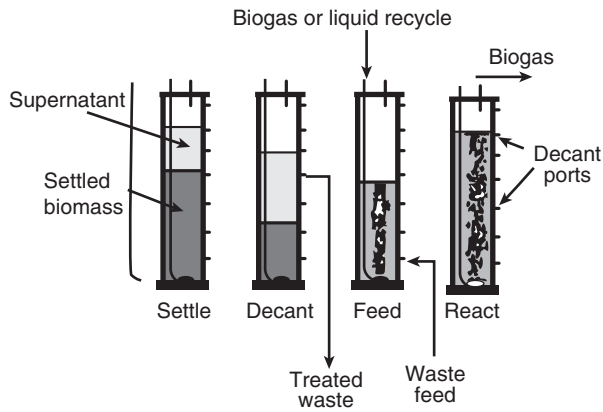

Fig. 23.3 ASBR cycle.

required for this step depends on several parameters such as substrate composition, substrate strength, required effluent quality, biomass concentration and reactor temperature (Sung and Dague, 1992; Hur *et al*., 1998). In the third step, mixing is shut off to allow biomass solid separation while the reactor itself acts as the clarifier. The time required for clarification varies, depending on biomass concentration and its ability to settle, and ranges from a few minutes to one hour. The settling time needs to be short to wash out the poorly settling biomass, but not so short that granular biomass is washed out of the reactor (Angenent and Dague, 1995). The last step is the decanting of the effluent out of the reactor. The decant step takes place after sufficient solids separation has occurred. The time required for this step is governed by the total volume to be decanted during each cycle and the decanting rate. The total volume is dependent on the HRT and the volume of the reactor. Since the ASBR is a closed system, a reduced pressure results when effluent is withdrawn unless a provision is made for biogas backflow. To overcome this, a separate gas bag is installed to maintain constant pressure. While decanting, the gas bag decreases in volume, refilling again during the feed step. Once the decant step is completed, the reactor is ready to be fed another batch of substrate (Sung and Dague, 1995).

23.7.4 Other bioreactors that retain biomass

There are other types of bioreactors that retain biomass including the anaerobic contact process, the filter or expanded bed process and rotating biological contactors. There is not sufficient room to discuss all of these processes here. Descriptions of most of them can be found in Tchobanoglous and Burton (1991).

23.8 Continuously stirred tank reactor (CSTR)

The CSTR is also commonly called a completely mixed reactor. It is described here because it is so commonly used around the world. In the CSTR, the liquid or slurry stream is continuously introduced, and liquid contents are continuously removed from the reactor.

Anaerobic treatment using a CSTR normally consists of a well-mixed reactor without solids recycling because all solids are in suspension and exit with the effluent. The SRT is equal to the HRT in this type of reactor. If operated properly, biomass that grows within the reactor continuously replaces the biomass removed from the reactor in the effluent stream (Droste, 1997). The basic characteristic of the ideal CSTR is that the concentration of substrate and biomass are the same everywhere throughout the reactor. In addition, the concentrations leaving in the effluent are the same as those in the reactor. This uniformity of concentration makes analysis of a CSTR comparatively simple. The CSTR is commonly used for aerobic or anaerobic treatment of highly concentrated organic mixtures, such as waste primary and biological sludge, as well as high-strength industrial wastes (Rittmann and McCarty, 2001). Since the biomass in the CSTR configuration is not retained by settling or attachment, the percentage of COD removal tends to be limited. The reactor must provide a minimum HRT of about 10 days at 35 °C depending on the waste; 10–30 days is a reasonable HRT range. Nevertheless, these vary with wastewater composition and degree of agitation (Stronach *et al.*, 1986; Mulligan, 2002).

A disadvantage of the CSTR system is that it is susceptible to malfunction upon shock loading or subsequent introduction of a variety of toxic substances. Malfunction manifests itself in terms of reduced gas production, reduced degradation of organic materials and simultaneous increase in acidity. Improved digestion of animal waste by simple gravitational settling of the contents of a conventional mixed digester prior to effluent removal has been demonstrated. However, the time required for this additional treatment step is the major drawback, and widespread adoption of the technique seems unlikely (Stronach *et al.*, 1986).

23.9 Separation of anaerobic processes in reactor systems

Anaerobic bioreactors can be classified into two-phase anaerobic digestion (TPAD) and two-stage anaerobic digestion (TSAD). The TPAD system refers to the development of unique biomasses in separate reactors. The first phase is referred to as ‘acid fermentation’ and is designed to produce primarily VFAs, while the second phase is referred to as ‘methane fermentation’ because in this phase the VFAs are converted to mainly methane and carbon dioxide. Due to short SRT in the first phase, acidogens are predominant, while both acidogens and methanogens are found in the

longer SRT of the second phase. The TSAD system refers to two consecutive reactors in which a common microbial consortium is recycled between the second-stage reactor and the first-stage reactor. The same microbes are thus exposed to different environmental conditions, because the first reactor tends toward acidification of the waste and the second is more methane formation. Thus, the same microbes are exposed to diverse substrate and metabolic intermediate concentrations in a TSAD system. Staging can be accomplished in both suspended growth and attached growth systems (Stronach *et al*., 1986; Azbar and Speece, 2001).

23.9.1 TPAD system

The TPAD system was adapted to the two-stage nature of anaerobic metabolism, and the concept has been evaluated in detail by various researchers. The system is also referred to as 'phase separation' (Fox and Pohland, 1994).

Pohland and Ghosh (1971) first proposed the physical separation of acid formers and methane formers in two separate reactors where optimum environmental conditions for each group of bacterial communities would be provided to enhance the overall process performance. In order to accomplish phase separation, several techniques have been employed such as membrane separation, kinetic control and pH control. Currently, a combination of kinetic and pH control has proven to be most successful for application of the TPAD system (Ince, 1998). Low pH and short SRT limits the growth of methanogens in the acid-forming reactor. If a low pH in the proper range is maintained in the acid-forming reactor, then any of the process modifications can be used and acid-forming bacteria will dominate with negligible presence of a methanogenic population (Zoetemeyer *et al*., 1982; Kasapgil *et al*., 1995; Hwang and Hansen, 1998). Any type of reactor may be designed for the second reactor.

Zhang and Noike (1991) used both a single-stage reactor and TPAD processes to compare the characteristics of substrate degradation and reported that acetate-utilizing methanogens in the second phase of the TPAD system were established at 2–10 times higher concentrations than were present in the single-stage reactor. Some investigators (Ince and Ince, 2000) studied the changes in microbial populations in TPAD systems and found that they have several advantages over single-stage reactors, such as the facilitation of the selection and enrichment of different bacteria in each reactor, increased process stability and enhanced buffering of the methanogenic phase pH by the prior acidogenic phase. The major advantages and disadvantages of the TPAD system are described in Table 23.4 (Fox and Pohland, 1994; Ince, 1998).

23.9.2 Comparison of TSAD and TPAD systems

Although few studies have been reported that compare the TSAD system to the TPAD system, subtle differences in system configuration such as

Table 23.4 Major advantages and disadvantages of the TPAD system

Advantages
Isolate and optimize the conditions for potential rate-limiting steps:
• Hydrolysis encouraged during first phase
• Methanogenesis encouraged during second phase
Improve reaction kinetics and stability:
• pH control in each phase
• improved reactor stability to organic and hydraulic overloads
• select for faster-growing microbes
Potential for detoxification in acidogenic phase
Disadvantages
Disruption of syntrophic relationships
Configuration more difficult to implement and operate
Lack of applicability to a variety of wastes
Uncertainty of linkage between substrate type and reactor configuration
Requirement of additional reactor and the cost

gas-phase management and combinations have been demonstrated to improve process performance. Wiegant *et al.* (1994) investigated the performance efficiency of UASB reactors using single and TSAD systems and found 10–13% better performance in the TSAD system due to biogas removal evolved in the first stage. Dugba and Zhang (1999) studied a temperature-phased TSAD system using ASBRs for treatment of dairy wastewater and they reported that thermophilic–mesophilic staging is recommended over mesophilic–mesophilic staging for best solids removals, biogas production and coliform bacteria destruction. One drawback was the increased heating requirement.

23.10 Biohydrogen production by anaerobic fermentation

23.10.1 Properties of hydrogen as a fuel resource

Hydrogen gas is the only alternative clean fuel source that produces no greenhouse gases, with water as the only combustion by-product. Combustion of hydrogen in automobiles is 50% more efficient than gasoline, since hydrogen can be combusted very lean, and gasoline must rely on stoichiometric mixtures and catalytic control of the exhaust gases for emission control (Swain *et al.*, 1983; Mizuno *et al.*, 2000). It may be possible to store compactly as a metallic hydride (Billings, 1991). It is the lightest fuel possible, with a molecular weight of only 2.02. Even as a liquid, hydrogen is only about one-tenth the weight of gasoline. The latent heat of vaporization of hydrogen is 28% higher than gasoline and 92% higher than diesel fuel (Bechtold, 1997).

23.10.2 Value of biohydrogen as a fuel resource

Despite its clean and green nature, most hydrogen is currently produced primarily from non-renewable sources, such as natural gas, oil and coal. Fig. 23.4 shows the ratio of sources used in worldwide production of hydrogen (US DOE Website, 2004a). Hydrogen production is 500 billion nm^3/year and electrolysis, the only non-fossil fuel source, comprises only 4% of total hydrogen production at 20 billion nm^3/year. Unfortunately, hydrogen production via electrolysis and thermal decomposition of water is more costly than methods that utilize fossil fuels. One commonly used method is steam reforming of natural gas (US DOE Website, 2004b). Natural gas is a finite resource, so new sources of hydrogen need to be found.

Biological production of hydrogen, using microorganisms, is an exciting new area of technology that offers the potential production of usable hydrogen from a variety of renewable resources. Table 23.5 shows a comparison of the energy costs of different hydrogen generation processes including biological as compared with alcohol and gasoline processes (Das and Veziroglu, 2001). This comparison indicates that hydrogen production from coal is the cheapest process. Fermentative hydrogen production, which has a conversion efficiency of 10%, is economically less attractive when compared with conventional fuels or hydrogen from coal. However, costs

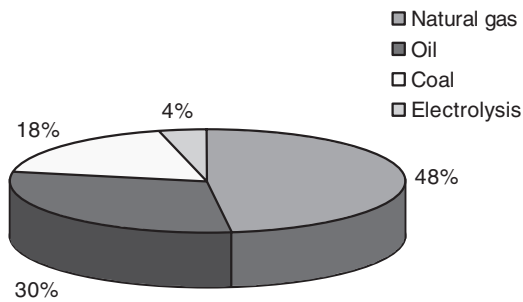

Fig. 23.4 Sources used in the worldwide production of hydrogen.

Table 23.5 Unit cost of energy obtained by different processes

Type of energy	Unit cost of energy content of fuel (US$/MJ)
Hydrogen from fermentation	~42
Hydrogen from coal	4.20
Hydrogen from electrolysis	10.50
Hydrogen from thermal decomposition of natural gas	13.70
Ethanol	~33
Gasoline	6.30

can be lowered if the conversion efficiency is increased. For example, if the fermentative hydrogen conversion ratio from sucrose is 75%, hydrogen yields correspond to 6.0 mol/mol sucrose. Assuming an overall fuel cell efficiency of 90%, Gibbs free energy of hydrogen as 56.7 kcal/mol and the lower heating values (LHVs) of hydrogen and sucrose as 58.3 and 1234 kcal/mol, respectively, the following adjusted energy costs can be calculated (Kumar and Das, 2000; Das and Veziroglu, 2001):

$$\begin{aligned}\text{Energy recovery from substrate} &= (\text{LHV of } H_2 \times H_2 \text{ yield})/\text{LHV of sucrose} \\ &= (58.3 \times 6.0)/1234 \\ &= 28.3\%\end{aligned}$$

$$\begin{aligned}\text{Final conversion efficiency} &= \text{Gibbs free energy for } H_2 \times H_2 \text{ yield} \times \text{efficiency of fuel cell/LHV of sucrose} \\ &= (56.7 \times 6.0 \times 0.90)/1234 \\ &= 24.8\%\end{aligned}$$

Based on this approximate energy cost calculation, fermentative anaerobic hydrogen production looks attractive. The anaerobic fermentation producing hydrogen is actually more cost-effective than indicated, and has considerable potential as an environmentally friendly process, since the process utilizes waste materials of low or negative value.

23.10.3 Microbiology of anaerobic biohydrogen production

Anaerobic hydrogen production can be divided into two main categories: one uses photosynthetic bacteria cultured under anaerobic or semi-anaerobic conditions in light and the other uses anaerobic bacteria that produce hydrogen via fermentation metabolism in dark conditions (Benemann, 1996). Hydrogen production by the dark fermentation process is much simpler than the photo-biological process, and the fermentation process generates hydrogen from a large number of carbohydrates frequently obtained as refuse or waste products (Nandi and Sengupta, 1998).

When only considering theoretical hydrogen yields, the photo-biological process using photosynthetic bacteria and algae has good economic potential. However, the process requires large surface areas for photobioreactors to achieve the most efficient solar conversion, and its fermented broth adds to the existing water pollution problems (Zaborsky, 1998). One of the merits of anaerobic fermentative hydrogen production is a higher hydrogen synthesis rate compared with many other biological processes, as shown in Fig. 23.5 (Levin *et al*., 2004). The anaerobic fermentation system may have a more practical application by producing hydrogen on-site with a reduced reactor size.

Hydrogen production by anaerobic fermentation has been studied for a large group of pure fermentative bacteria; *Escherichia coli, Enterobacter*

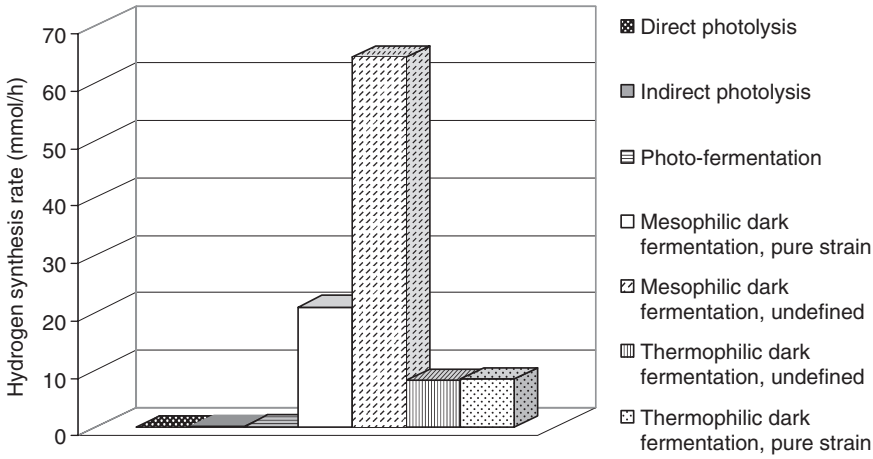

Fig. 23.5 Comparison of the rates of hydrogen synthesis in different biological production processes.

aerogenes, Clostridium butyricum, Clostridium acetobutyricum and *Clostridium perfringens* have been found to produce hydrogen under anaerobic conditions. *Escherichia coli* and *Enterobacter aerogenes* are facultative anaerobes, utilizing both glucose and lactose as carbon sources, and producing hydrogen as one of the fermentation by-products (Zajic *et al*., 1978; Minton and Clarke, 1989). The most effective hydrogen production (2 mol H_2/mol glucose) was observed upon fermentation of glucose in the presence of *Clostridium butyricum* (Zeikus, 1980; Heyndrickx *et al*., 1987; Taguchi *et al*., 1992). *Clostridium* species are Gram-positive, spore-forming, rod-shaped bacteria. When stressed, these bacteria produce spores that tolerate extreme conditions that vegetative bacteria cannot (Reimann *et al*., 1996). Their endospores are very resistant to heat or harmful chemicals, including acids, and cannot be destroyed easily (Reimann *et al*., 1996).

23.10.4 Treatments to enrich hydrogen-producing bacteria from mixed anaerobes

During anaerobic digestion, methanogenic or sulfate-reducing bacteria consume hydrogen produced by acidogenic bacteria, contributing negatively to bio-hydrogen production. Effectively extracting hydrogen from an anaerobic bioreactor depends on special procedures that block out the co-degradable chains (Adams and Stiefel, 1998). Heat treatment of sludge used as an inoculum is one feasible method of increasing hydrogen production by inhibiting the activity of non-spore-forming hydrogenotrophic bacteria. Lay (2000) and Okamoto *et al*. (2000) used wet heat treatment of anaerobic digester sludge, whereas van Ginkel *et al*. (2001) used dry

heat treatment of compost and soils to inactivate hydrogen-consuming microorganisms and to select hydrogen-producing bacteria. Cheong and Hansen (2006) achieved maximum specific hydrogen production of up to 380 mL H_2/g cell mass after 96 h using inocula that had been enriched at pH 3.

23.10.5 Environmental factors influencing anaerobic biohydrogen production

The selection of the correct pH level is crucial to hydrogen production, due to the effects of pH on the hydrogenase activity or specific metabolic pathways (Dabrock *et al*., 1992). At pH values lower than 6.3 or higher than 7.8, the methanogenic rate may decrease or stop. Using a low pH environment to inhibit methane production in the acidogenic phase and to obtain dominant microbes for hydrogen production from sludge is considered feasible. Van Ginkel *et al*. (2001) reported the highest rate of hydrogen production occurred at an initial pH of 5.5, with a sucrose substrate concentration of 7.5 g COD/L, in batch experiments using composts as the inoculum. Lee *et al*. (2002) investigated the effects of initial pH in the batch culture using sucrose from mixed micro-flora. Their results show that maximum specific hydrogen production yields were achieved at an initial pH of 9.0, whereas small amounts of hydrogen production were observed at pH values of 5.0 and 5.5. These experiments investigated the effects of initial pH on hydrogen production without a well-maintained buffering capacity for preventing a sharp decrease of pH. Some investigators reported effects of pH on the continuous production of hydrogen by mixed culture, the optimal values being 5.5, 4.0–4.5 and 4.7–5.7 for fermentation of glucose (Fang and Liu, 2002), sucrose (Ren *et al*., 1995), and starch (Lay, 2000), respectively.

HRT is also a key factor for enhanced hydrogen production. Some investigators have observed the effect of HRT for hydrogen production along with the effect of pH on anaerobic continuous systems. When fed with glucose or sugary synthetic wastewaters, the reported maximum hydrogen production was 1.0–2.1 mol H_2/mol hexose at a short SRT and low pH (Ueno *et al*., 1996; Lin and Chang, 1999; Sung *et al*., 2002).

23.10.6 Development of anaerobic biohydrogen production processes

Most continuous studies for hydrogen production have investigated hydrogen production using a completely mixed system. Although various organic loading rates have been investigated, conversion efficiencies have not been equal to the theoretical value of 0.467 L H_2/g COD for acetic acid fermentation (Majizat *et al*., 1997; Lin and Chang, 1999; Fang and Liu, 2002). Some have tried to try increase hydrogen yields in a CSTR system by applying a vacuum to the head space of the reactor (Kataoka *et al*., 1997),

by sparging with nitrogen gas (Mizuno *et al*., 2000) or by vigorously stirring to allow the dissolved hydrogen to escape into the gas phase (Lamed *et al*., 1988). Another process aimed at increasing hydrogen production was investigated using various immobilized microorganisms (*Bacillus licheniformis, Clostridium butyricum*, a mixed microbial culture) on brick dust, calcium alginate beads or polyacrylamide gel (Suzuki and Karube, 1983; Kumar and Sharma, 1995).

Two-phase or two-stage systems have potential as processes to achieve greater extraction of hydrogen from organic wastewaters. One example is a two-phase process using a combined culture system of anaerobic fermentative bacteria and photosynthetic bacteria to produce the most hydrogen from organic carbon sources. In this manner, metabolites, such as VFAs produced during anaerobic hydrogen fermentation, can be further degraded with photosynthetic bacteria, and more hydrogen is produced during this process (Kataoka *et al*., 1997). However, using the photosynthetic bacteria may cause additional waste recycling; thus, increasing the treatment needs of host facilities (Henley *et al*., 2003). High biomass-retaining reactors also have potential for economical production of hydrogen from anaerobic fermentation (Cheong and Hansen, 2006).

23.11 Producing other chemicals and useful products from food waste

Although bioenergy, such as methane and hydrogen, may reduce the cost of food wastewater treatment, it cannot satisfy entirely the energy demands of our society. Therefore, the production of high-value chemicals from the organic material in wastewater might be more feasible than bioenergy production. Table 23.6 shows that 19 different useful products have been fermented from whey products with various types of organisms (Yang and Silva, 1994). Organisms listed in the table could most probably be used with some other carbonaceous substrates. Wastes from the food processing industry often become inexpensive raw materials for integrated fermentation processes. High-carbohydrate wastewaters that are unsuitable for feeding to animals or humans are particularly appropriate for conversion to valuable products in pure-culture or co-culture processes. It is the cost-efficiency of the bioconversion process that ultimately determines whether a specific food waste stream is suitable for production of a specific product. Examples of chemicals that are obtained via fermentation of organic materials in food waste streams are shown in Table 23.7. Lactic acid fermentation and ethanol fermentation have been proposed as the main frame of a bioconversion system for the development of biobased chemicals from food and agricultural wastes (Ohara, 2003). The bioconversion technologies for ethanol fermentation are mainly crop-based, utilizing substrates such as sugar cane juice and corn starch. Since the cost of raw materials can be as high as 40%

Table 23.6 Some products from whey fermentations

Product	Organism	Medium
SCP	*Kluyveromyces fragilis, Rhodopseudomonas sphaeroides/Bacillus megaterium*	Sweet whey permeate
	K. marxianus	Acid whey permeate
	Candida pseudotropicalis	Whey plus yeast extract
	Various yeasts	Whey plus yeast extract
Alcohol	*K. fragilis*	Concentrated cottage cheese whey permeate
	K. marxianus	Acid whey permeate
Bakers yeast	*Saccharomyces thermophilus* followed by *S. cerevisiae*	Sweet whey permeate plus corn steep liquor
Lactic acid	*Lactobacillus delbrueckii* ssp. *lactis*	Acid whey permeate plus yeast extract
	Homolactic acid bacteria	Unsupplemented acid whey
	L. helveticus	Supplemented whey permeate
Acetic acid	*Streptococcus lactis* ssp. *lactis* and *Clostridium formicoaceticum*	Whey permeate plus yeast extract
Propionate	*Propionibacterium acidipropionici*	Whey permeate
Polysaccharide	*Propionibacterium* sp.	Supplemented sweet whey
Oil	*Apiotrichum curvatum*	Whey permeate
	Various fungi	Deproteinized cheese whey
	Candida curvata	Whey permeate
Enzymes	*Aspergillus niger*	Lactose
β-Galactosidase	*Candida pseudotropicalis*	Whey plus yeast extract
Acetone-butanol	*Clostridium acetobutylicum*	Whey permeate plus yeast extract
Lysine	Mutant *Escherichia coli*	Whey
Vitamin B12	*Propionibacterium* sp.	Acid whey
	P. shermanii	Sweet whey plus yeast extract
Citric acid	*A. niger*	Whey permeate
L-Ascorbic acid	Mutant *Candida norvegensis*	Sweet whey permeate
Glycerol	*K. fragilis*	Whey permeate
	K. marxianus	Supplemented whey permeate
Anthocyanins	*Ajuga reptans*	Supplemented whey permeate
Insecticides	*Bacillus thuringiensis*	Unsupplemented sweet whey
Xanthan gum	*Xanthomonas campestris*	Hydrolyzed whey permeate plus yeast extract
	Adapted strain of *X. campestris*	Supplemented whey medium

SCP, single-cell protein.

Table 23.7 Chemicals production from food industry waste streams by different microorganisms

Chemical	Waste stream	Function	Organism	References
Lactic acid	Food	Bioplastic	*Lactobacillus delbrueckii*	Kim *et al.* (2003)
Polyhydroxyalkanoates	Food	Bioplastic	*Ralstonia eutropha*	Du and Yu (2002)
Poly-3-hydroxybutyrate	Whey	Bioplastic	Recombinant *E. coli*	Park *et al.* (2002)
Lipase	Corn steep liquor	Enzyme	*Galactomyces geotrichum*	Burkert *et al.* (2005)
Protease	Whey	Enzyme	*Mucor* spp.	Tubesha and Al-Delaimy (2003)
Xylitol	Sugar cane bagasse	Sweetner	*Geotrichum Guilliermondii*	Carvalho *et al.* (2002)
Biomass protein	Starch processing	Feed	*Rhizopus* sp.	Jin *et al.* (1999)
Monascus pigment	Gluten-free effluent	Dye	*Monascus purpureus*	Dominguez-Espinosa and Webb (2003)
Acetic acid	Milk permeate	De-icing salt	*Clostridium thermolacticum* plus *Moorella*	Collet *et al.* (2003)
Citric acid	Orange wastes	Preservative	*Aspergillus niger*	Aravantinos-Zafris *et al.* (1994)
Chitin	Shrimp wastes	Functional additive	*Lactobacillus plantarum*	Rao *et al.* (2000)
Nisin, pediocin	Mussel-processing	Antibacterials	*Lactococcus lactis* *Pediococcus acidilactici*	Guerra and Pastrana (2002)
Ethanol	By-products from olive oil extraction	Fuel	*Kluyveromyces marxianus*	Ballesteros *et al.* (2001)

of the ethanol cost, recent efforts have concentrated on utilizing lignocellulose for producing a biomass-derived fuel (Zaldivar *et al.*, 2001). Lactic acid produced through lactic acid fermentation is polymerized to form poly acetate, which is used as a plastic. Lactic acid and ethanol are esterified to produce ethyl acetate, which is used as a biodegradable solvent (Ohara, 2003). Polyhydroxyalkanoates (PHAs) have also attracted extensive research interest for their potential use as a biodegradable alternative to the petroleum-based synthetic plastics such as polypropylene (PP) and polyethylene (PE). Much effort has been put into the production of PHAs by microbial fermentation from organic matters in agricultural and industrial wastes because producing the biodegradable thermoplastics from pollutants can provide multiple benefits to the environment and contribute to sustainable development (Park *et al.*, 2002; Aldor and Keasling, 2003).

Bioconversion for chemical production has been enhanced by process modification, such as culture immobilization or coupling two separate bioreactors (Carvalho *et al.*, 2002). For example, anaerobic hydrogen fermentation and aerobic conversion of volatile fatty acids by *Ralsonia eutropha* were combined to enhance the efficiency of PHA production from food wastes (Du and Yu, 2002). Another fermentation strategy for increasing waste bioconversion yield was the use of co-cultures of pure microbial organisms in a single process. Within these step-wise fermentation strategies, one species performs most of the complex nutrient hydrolysis, and in turn provides its metabolic by-products to the second species, which forms the desired products. This study achieved high acetic yields during bioconversion of milk permeate by combining cells of *Clostridium thermolacticum, Moorella thermoautotrophica* and *Methanothermobacter thermoautotrophicus* in a microbial consortium. Addition of the hydrogentrophic methanogen decreased the hydrogen partial pressure, which increased the acetic acid production (Talabardon *et al.*, 2000; Collet *et al.*, 2003). In bioconversion of agricultural and food wastes to profitable chemicals, separation and purification of the chemical products from the bulk liquid still represents the higher percentage of the manufacturing cost. Therefore, the economic feasibility of reusing wastes will strongly depend on the fermentation processing efficiency in the downstream.

23.12 Future trends

Anaerobic digestion of agricultural and food wastes to methane is a mature process that is being used within full-scale facilities worldwide (Table 23.8). Although methane is a relatively low-value product, methanogenic anaerobic digestion still represents the most economically viable technology. Hydrogen production via anaerobic fermentation has the greatest potential as a pre-process step that can be followed by a suitable secondary process step, such as bioconversion of VFAs to other products, such as PHAs,

Table 23.8 Comparison of three biological processing strategies for food wastes treatment

Processing strategy	Culture type	Degree of products separation	Level of maturity	Value added
Anaerobic digestion	Mixed	Easy – gas	Mature, operational on a commercial scale	Low to medium
Hydrogen fermentation	Mixed	Easy – gas	Laboratory phase	Medium
Chemical production	Pure or limited mixture	Difficult – soluble products	Scale-up phase	Medium to high

lactic acid and ethanol. Methanogenic anaerobic digestion and hydrogen fermentation use mixed bacterial communities that are selected according to their function. This is well-suited to the non-sterile, complex environment of wastewater treatment. Also, the products from these biological processes can be easily separated as gases and used for electricity generation or other beneficial uses (Table 23.8). Anaerobic digestion has been used for many years to treat wastewaters but, so far, not to produce heat or electricity from hydrogen. With the advancement of fuel-cell technologies, this hydrogen gas can generate electricity in a clean and efficient manner. The anaerobic hydrogen fermentation would use similar hardware to that used currently in methane fermentation anaerobic digesters and the technology has been proven for many years in laboratory and pilot-scale treating using various agricultural and food wastewaters. Of even greater potential is combining hydrogen fermentation with methane fermentation in on-site wastewater treatment systems. However, in our current, cost-effective energy economy, such large-scale implementation may not be economically feasible compared with conversion to more valuable chemical products. Fortunately, specialized high-value biochemical products might soon be produced from food wastewaters as a more economical alternative. The effects of bioreactor design (e.g. IBR, UASB), configuration and operating conditions (e.g. biomass retention, high-rate hydraulic retention) need to be more widely studied and optimized before the processes can be scaled up. More recently, a number of molecular biology techniques have been developed for the qualitative and quantitative analysis of microbial communities from biological processes treating food and agricultural wastes. Monitoring the phylogenic diversity of mixed microbial species may support enhancement of the bioconversion to hydrogen and useful chemicals, and improve understanding of the metabolic characteristics of key microorganisms.

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Index