Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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# Food processing technology

## Principles and practice

Third edition

P. J. Fellows

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**P. J. Fellows**

**CRC Press**
**Boca Raton Boston New York Washington, DC**

WOODHEAD PUBLISHING LIMITED
Oxford Cambridge New Delhi

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Published by Woodhead Publishing Limited, Abington Hall, Granta Park,
Great Abington, Cambridge CB21 6AH, UK
www.woodheadpublishing.com

Woodhead Publishing India Private Limited, G-2, Vardaan House, 7/28 Ansari Road, Daryaganj,
New Delhi – 110002, India

Published in North America by CRC Press LLC, 6000 Broken Sound Parkway, NW,
Suite 300, Boca Raton, FL 33487, USA

First edition 1988, Ellis Horwood Ltd
Second edition 2000, Woodhead Publishing Limited and CRC Press LLC
Reprinted 2002, 2003, 2005, 2007, 2008
Third edition 2009, Woodhead Publishing Limited and CRC Press LLC
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British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data
A catalog record for this book is available from the Library of Congress.

Woodhead Publishing Limited ISBN 978-1-84569-216-2 (book)
Woodhead Publishing Limited ISBN 978-1-84569-634-4 (e-book)
CRC Press ISBN 978-1-4398-0821-4
CRC Press order number: N10075

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Typeset by Godiva Publishing Services Limited, Coventry, West Midlands, UK
Printed by TJ International Limited, Padstow, Cornwall, UK

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# For Wen and Paddy

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# Acknowledgements

I am indebted to the large number of people who have given freely of their time and experience, provided me with information, checked the text and given support during this revision of *Food Processing Technology*. My particular thanks to Dr Mike Lewis of the Department of Food Biosciences, Reading University, for his support and technical editing skills; to Dr Mike Dillon, Director, Grimsby Institute of Food and Fisheries, for his assistance with the section on quality assurance; and to Sarah Whitworth of Woodhead Publishing for her ideas, suggestions and administrative support. My thanks also to the many companies that responded positively to my requests for information about their equipment and products; and finally, but not least, my special thanks to Wen for her constructive thoughts, encouragement and forbearance at my long hours away from family life over many months.
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# Introduction

## A brief history of food processing

Archaeological and ethnographic evidence indicates that the first food processing had its origins in hunter–gatherer societies that used heating over open fires or in boiling water to make meat, roots and vegetables more palatable. However, because of their lifestyle they did not need to preserve foods to any significant extent. The gradual change to agricultural societies necessitated storage and preservation of foods and by 3000–1500 BC, the Egyptians had developed processing techniques, including sun drying to preserve fish and poultry, fermentation to produce alcohol, and cereal grinding and ovens to bake leavened bread. These were slowly adopted by pastoral societies throughout the Middle East and then further afield, to preserve foods against times of famine, to improve their eating quality and to give a more varied diet. By 1500 BC, all of the main food plants that are used today, except sugar beet, had been cultivated somewhere in the world.

During the ensuing thousand years similar food technologies may have developed independently in many places, with local variations due to differences in climate, crops or food preferences. Early processes developed in China include tofu (soybean curd) and roasted dried millet and dried beef as military rations. In Japan, rice wine (saki) was developed, salt made from dried seaweed was used to preserve foods, and soya was processed to soy sauce and miso (soy paste) to flavour foods. In Europe, the first water-powered flour mills and the first commercial bakeries were developed by the Romans, and in India, the manufacture of sugar from cane had developed in the Indus Valley, both by 100 BC (Trager 1995).

In the first millennium AD, the comparative isolation of different civilisations began to change, and first travellers and then traders began to exchange ideas and foods across the world. For example in AD 400, the Vandals introduced butter to southern Europe, which began to replace olive oil. By AD 600, Jewish merchants had established the spice trade with the Orient. Technological developments during this period included the use of maltose from germinating grains as a sweetener, the development of kumiss (fermented mare's milk) and ales made from fermented millet in China. By AD 700, the first written

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law, which established regulations for the production of dairy products and preservation of foods, was encoded in China.

By the turn of the second millennium, a rapid expansion of trade and exchange of foods and technologies took place with European explorers and military expeditions: for example in 1148, knights returning from the second crusade brought sugar to Britain from the Middle East; Marco Polo brought noodles from China; in the 1500s, the Portuguese brought cloves from the East Indies for use in preserves and sauces, and to disguise spoiled meat. Spanish conquistadors discovered sun-dried llama, duck and rabbit, which were eaten uncooked in Peru; and they returned with foods that had never been seen before in Europe, including avocado, papaya, tomato, cacao, vanilla, kidney beans and later (1539) potatoes. At the same time, the Portuguese introduced chilli peppers and cayenne from Latin America to India, where they were used to prepare spiced dishes.

As societies developed, specialisation took place and trades evolved (e.g. millers, cheese-makers, bakers, brewers and distillers). Small variations in raw materials or processing methods gave rise to thousands of distinctive local varieties of cheeses, beers, wines and breads. These were the forerunners of present day food industries, and some foods have been in continuous production for nearly 800 years. During this period, mechanical processing equipment using water, wind and animal power was developed to reduce the time and labour involved in processing; for example, the Domesday Book (1086) in England lists nearly 6000 water-powered flour mills, one for every 400 inhabitants.

In countries with a temperate climate, processing techniques were developed to preserve food through winter months, including salting and smoking of meats and fish, fermentation to produce vinegar which was also used to preserve meat and vegetables, and boiling fruits or vegetables to reduce the moisture content and produce jams or chutneys. Ice from mountains had been used to refrigerate fruits and vegetables by the Romans, and later (1626) in England, Francis Bacon published his ideas on freezing chickens by stuffing them with snow. The growth of towns and cities gave impetus to the development of preservation technologies and the extended storage life allowed foods to be transported from rural areas to meet the needs of urban populations. During the 1600–1700s, the slave trade helped change food supplies, eating habits, agriculture and commerce. Ships returning from delivering slaves to Brazil took maize, cassava, sweet potato, peanuts and beans to Africa, where they remain staple foods. Cocoa from West Africa was brought to Europe and in 1725 the first chocolate company began operation in Britain. At this time, in Massachusetts, USA, more than 60 distilleries produced rum from molasses that was supplied by slave traders. The rum provided the capital needed to buy African slaves, who were then sold to West Indian sugar planters. A similar circular trade existed in salted cod fish and slaves between Britain, America, Africa the Caribbean and Latin America (Kurlansky 1997, 2002).

The scale of operation by food processing businesses accelerated during the Industrial Revolution in the eighteenth century, but there was an almost total absence of scientific understanding. The processes were still based on craft skills and experience, handed down within families that held the same trades for generations. By the late 1700s, the first scientific discoveries were being made: chlorine was used to purify water and citric acid was used to flavour and preserve foods. At this time, the first 'new' food process was developed in France after Napoleon Boneparte offered a prize of 12 000 francs to invent a means of preserving food for long periods, for military and naval forces. Nicholas Appert, a Parisian brewer and pickler, opened the first vacuum bottling factory (cannery) in 1804, boiling meat and vegetables and sealing the jars with corks and tar, and he won the prize in 1809.

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In the nineteenth century, the pace of scientific understanding increased: Russian chemist, Gottlieb Iorchoff, demonstrated that starch breaks down to glucose and a Dutch chemist, Johann Mulder, created the word 'protein'. Technological advances in canning and refrigeration accelerated at an unprecedented rate. In 1810, the first patent for a tin-plated steel container was issued in Britain, and in 1849 a can-making machine was developed in the USA that enabled two unskilled workers to make 1500 cans per day, compared with 120 cans per day that could be made previously by two skilled tinsmiths. In 1861 a canner in Baltimore reduced the average processing time from 6 hours to 30 minutes by raising the temperature of boiling water to 121 ºC with calcium chloride; and in 1874, a pressure-cooking retort using live steam was invented, leading to rapid expansion of the industry. In 1858 the first mechanical refrigerator using liquid ammonia was invented in France and in 1873, the first successful refrigeration compressor was developed in Sweden. The pasteurisation process, named after French chemist and microbiologist Louis Pasteur, was developed in 1862. Towards the end of the nineteenth century, scientific understanding had started the change away from small-scale craft-based industry, and by the start of the twentieth century, the food industry as we now know it was becoming established. In 1929, the merger of Lever Brothers and the Margarine Union formed the world's first multi-national food company.

Technological advances gathered speed in all areas of food technology as the twentieth century progressed. For example, 'instant' coffee was invented in 1901, the first patent for hydrogenating fats and oils was issued in 1903, transparent 'cellophane' wrapping was patented in France in 1908, the same year that the flavour enhancer, monosodium glutamate was isolated from seaweed. In 1923 dextrose was produced from maize, and widely used in bakery products, beverages and confectionery.

The widespread introduction of electricity revolutionised the food industry and prompted the manufacture of new specialist food processing machinery. For example in 1918, the Hobart Company developed the first electric dough mixer, electric food cutters and potato peelers. Most food processing at this time supplied staples (e.g. dried foods, sugar, cooking oil) and foods that were further prepared in the home or in catering establishments (e.g. canned meat and vegetables). The impetus for development of some of these foods came from military requirements during World War I. Later a 'luxury' market included canned tropical fruits, confectionery and ice cream. After World War II, development of a wide range of ready-to-eat meals, snackfoods and convenience foods began. Again these developments had been partly stimulated by the need to preserve foods for military rations. From the 1950s, food science and technology were taught at university level, and the scientific underpinning from this and work at food research institutions has created new technologies, products and packaging that have resulted in several thousand new foods being developed for sale each year.

## The food industry today

The aims of the food industry today, as in the past, are fourfold:

1 To extend the period during which a food remains wholesome (the shelf-life) by preservation techniques which inhibit microbiological or biochemical changes and thus allow time for distribution, sales and home storage.
2 To increase variety in the diet by providing a range of attractive flavours, colours, aromas and texture in food.

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3 To provide the nutrients required for health.
4 To generate income for the manufacturing company and its shareholders.

Each of these aims exists to a greater or lesser extent in all food processing, but a given product may emphasise some more than others. For example, the aim of freezing is to preserve organoleptic and nutritional qualities as close as possible to the fresh product, but with a shelf-life of several months instead of a few days or weeks. In contrast, sugar confectionery and snackfoods are intended to provide variety in the diet, and a large number of shapes, flavours, colours and textures are produced from basic raw materials. All food processing involves a combination of procedures to achieve the intended changes to the raw materials. Each of these unit operations has a specific, identifiable and predictable effect on a food and the combination and sequence of operations determine the nature of the final product.

In industrialised countries the market for processed foods is changing, and consumers no longer require a shelf-life of several months at ambient temperature for the majority of their foods. Changes in family lifestyle, and increased ownership of refrigerators, freezers and microwave ovens are reflected in demand for foods that are convenient to prepare, are suitable for frozen or chilled storage, or have a moderate shelf-life at ambient temperatures. There is also an increasing demand by consumers for foods that more closely resemble the original raw materials and have a 'healthy' or 'natural' image, and have fewer synthetic additives, or have undergone fewer changes during processing. Manufacturers have responded to these pressures by reducing or eliminating synthetic additives from products and substituting them with natural or 'nature-equivalent' alternatives; and by introducing new ranges of low-fat, sugar-free or low-salt products in nearly all sub-sectors. Functional foods, especially foods that contain probiotic micro-organisms and cholesterol-reducing ingredients, have shown a dramatic increase in demand, and products containing organic ingredients are also now widely available. Consumer pressure has also stimulated improvements to processing methods to reduce damage caused to organoleptic and nutritional properties, and led to the development of novel 'minimal' processes.

Trends that started during the 1960–1970s, and have accelerated during the last 30 years, have caused food processors to change their operations in four key respects: first is increasing investment in capital intensive, automated equipment to reduce labour and energy costs, and improve product quality; secondly, investment in computer control of processing operations, warehousing and distribution logistics to meet more stringent requirements for traceability, food safety and quality assurance; thirdly, high levels of competition and slower growth in food markets in industrialised countries have prompted mergers or take-overs of competitors; and fourthly, there has been a shift in power and control of food markets from manufacturers to large retail companies. Changes to the food industry during the last quarter of the twentieth century are reviewed by Anon (2001).

The changes in technology have been influenced by substantial increases in the costs of both energy and labour, and by public pressure and legislation to reduce negative environmental effects of processing. Food processing equipment now has increasingly sophisticated levels of microprocessor control to reduce processing costs, enable rapid change-over between shorter production runs, to improve product quality and to provide improved records for management decisions. The automation of entire processes, from reception of materials, through processing and packaging to warehousing is a reality. This has allowed producers to generate increased revenue and market share from products that

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have higher quality and value. Food processing has now become a global industry, dominated by a relatively few multinational conglomerates. A 2005 report (Anon 2005) noted that 30 companies now account for a third of the world's processed food and five companies control 75% of the international grain trade. Two companies dominate sales of half the world's bananas and three trade 85% of the world's tea.

A substantial increase in the power and influence of large retailing companies, especially in the USA and Europe during the past 20 years, has caused food companies to form international strategic alliances that enable them to develop pan-regional economies of scale. These companies are now focusing on development of new markets and are either buying up or forming alliances with local competitors in South East Asia, India, Eastern Europe and Latin America. Global sourcing of raw materials has been a feature of some industries from their inception, but this has now expanded to many more sectors to reduce costs and ensure continuity of supply. These developments have in turn prompted increased consumer awareness of both ethical purchasing issues, such as employment and working conditions in suppliers' factories, and the environmental impact of international transportation of foods by air. There has also been a resurgence of consumer interest in locally distinctive foods and 'fair-traded' foods in some European countries.

Rapid development of global production and distribution (global value chains, GVCs) has been made possible by developments in information and communications technologies. Growth in the buying power of major retail companies, particularly in Europe and the USA, enabled them to drive down prices paid to food processors. As a result, transnational processing corporations (TNCs) have adopted a series of strategies to increase their competitiveness, including mergers and acquisitions with food manufacturers in other countries. TNCs have established tightly integrated global-scale systems in widely separated locations and have reduced the need for highly skilled, highly paid workforces. This makes it possible for companies to move their operations to new countries, often in the developing world, where unskilled and lower-paid workers can be employed. These developments enable food production to be coordinated between distant sites, and suppliers can now be called upon to transfer goods across the world at short notice. These developments have been assisted by international agreements to remove tariff and non-tariff barriers, privatisation and deregulation of national economies to create 'free' markets in trade and foreign investment. The Uruguay Round of the General Agreement on Tariffs and Trade (GATT) held from 1986 to 1994 expanded the principle of 'free' trade in key areas, including agriculture, where countries must reduce subsidies paid to producers and also reduce tariffs on imported goods (Hilary 1999). Agreements related to investment under the World Trade Organization (WTO) extended the scope of GATT negotiations to include services and intellectual property (The General Agreement on Trade in Services), foreign direct investment and copyright, trademarks, patents and industrial designs. This was facilitated by changes introduced by the International Monetary Fund and World Bank that opened up investment opportunities in many developing countries and helped the creation of GVCs. Saul (2005) describes the rise of free trade from the mid-nineteenth century, with globalisation reaching its high point in the mid-1990s with the establishment of the WTO. However, he believes that globalisation had begun to falter within five years, with the Asian financial crisis of 1997–98 underlining its inherent instability, and it is presently characterised by stasis. Whether this is the case or not, the effects of globalisation are likely to remain a significant influence on large-scale food industries for many years.

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## About this book

This book aims to introduce students of food science and technology or biotechnology to the wide range of processing techniques that are used in food processing. It shows how knowledge of the properties of foods is used to design processing equipment and to control processing conditions on an industrial scale, to achieve the aims of making attractive, saleable, safe and nutritious products and extending the shelf-life of foods. It is a comprehensive, yet basic text, offering an overview of most unit operations (Fig. I.1). It provides details of the processing equipment, operating conditions and the effects of processing on micro-organisms that contaminate foods, the biochemical properties of foods and their sensory and nutritional qualities. It collates and synthesises information from a wide range of sources, combining food processing theory and calculations, and results of scientific studies, with descriptions of commercial practice. Where appropriate, references are given to related topics of food microbiology, nutrition, fundamentals of food engineering, mathematical modelling of food processing operations, biochemical and physical properties of foods, food analyses, and business operations, including quality assurance, marketing and production management.

The book is divided into five parts in which unit operations are grouped according to the nature of the heat transfer that takes place:

- Part I describes some important basic concepts;
- Part II describes unit operations that take place at ambient temperature or involve minimum heating of foods;
- Part III includes operations that heat foods to preserve them or to alter their eating quality;
- Part IV describes operations that remove heat from foods to extend their shelf-life with minimal changes in nutritional quality or sensory characteristics; and
- Part V describes post-processing operations, including packaging and distribution logistics.

In each chapter, the theoretical basis of the unit operation is first described. Formulae required for calculation of processing parameters and sample problems are given where appropriate, and sources of more detailed information are indicated. Details of the equipment used for commercial food production, and developments in technology that relate to cost savings, environmental improvement or improvement in product quality are described. Finally the effects of each unit operation on sensory characteristics and nutritional properties of selected foods, and the effects on micro-organisms are described. The book describes each topic in a way that is accessible without an advanced mathematical background, while providing references to more detailed or more advanced texts. The book is therefore suitable for students studying nutrition, hospitality management/catering or agriculture, as an additional perspective on their subject areas.

This third edition has been substantially rewritten, updated and extended to include the many developments in food technology that have taken place since the second edition was published in 2000. Nearly all unit operations have undergone significant developments, and these are reflected in the large amount of additional material in each chapter. In particular, advances in microprocessor control of equipment, 'minimal' processing technologies, genetic modification of foods, functional foods, developments in 'active' or 'intelligent' packaging, and storage and distribution logistics are described. Additionally, sections on the composition of foods and food-borne micro-organisms are included for the first time.

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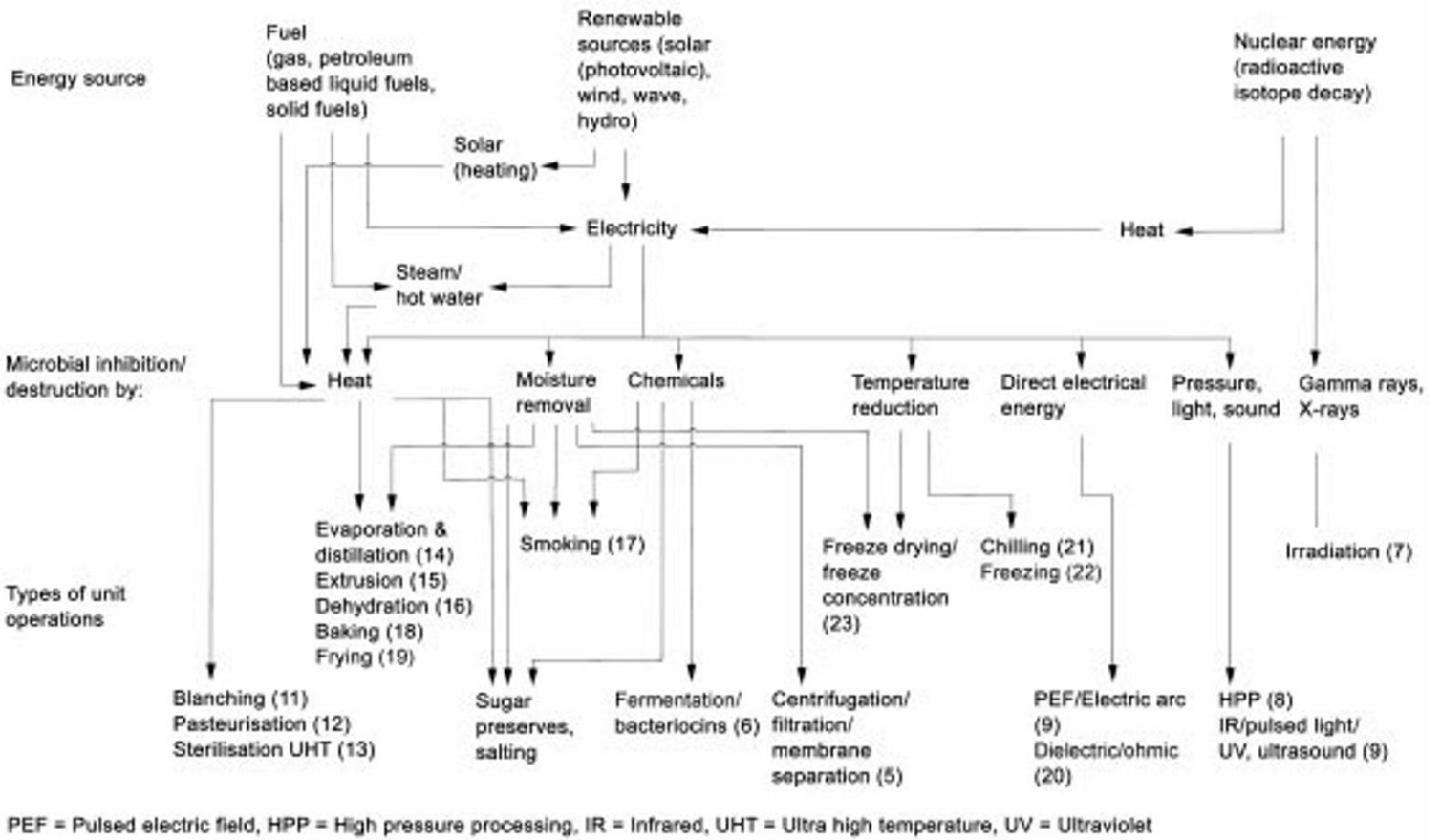


**Fig. I.1** Types of processing and their preservative effects (numbers in parentheses indicate chapter numbers).

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# 6

# Food biotechnology

**Abstract:** This chapter describes recent developments in food biotechnology, including genetic modification of foods and micro-organisms, nutritional genomics and the development of functional foods. Fermentation improves the palatability and acceptability of raw materials by producing flavour and aroma components and modifying food texture. This chapter describes the theory of microbial growth in food fermentations and the equipment used to produce fermented foods in submerged cultures and solid substrate fermentations. It outlines the production of microbial enzymes and selected commercially important fermented foods, including alcoholic drinks, bakery and dairy products, and mycoprotein; and concludes with a summary of developments in the production of bacteriocins and antimicrobial ingredients.

**Key words:** genetic modification, functional foods, fermentation, microbial enzymes, bacteriocins.

The Convention on Biological Diversity (Anon 2000a) defines biotechnology as 'any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use'. It therefore includes all forms of plant and animal breeding and development, traditional food fermentations and waste treatment. The Institute of Food Science and Technology has defined food biotechnology as 'the application of biological techniques to food crops, animals and micro-organisms to improve the quality, quantity, safety, ease of processing and production economics of food' (Anon 2004a). This chapter describes developments in food biotechnology including genetically modified foods, probiotics, prebiotics, microbial enzymes and selected food fermentations.

## 6.1 Genetic modification

Genetic modification is the alteration of the genetic makeup of an organism that can be passed on to its descendants. Strictly, this includes traditional methods of selective breeding of crops or animals for specific attributes by normal reproduction, breeding closely related species or isolating mutants, which have been practised by farmers and breeders for thousands of years. However, the term is now more often used to describe 'genetic engineering' in which techniques in molecular biotechnology are used to

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manipulate genes, outside the normal reproductive process of a plant, animal or micro-organism, to produce new physiological or physical characteristics in a food. Internationally, the FAO and WHO have defined genetically modified organisms (GMOs) as 'organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally' (Anon 2007). EU legislation defines GM food as 'food containing, consisting of, or produced from, a genetically modified organism' (Anon 2008a).

There are a number of methods for making GM foods, each based on alteration of one or more genes. The production of an individual protein by an organism is specified by a gene (although a single gene can encode for more than one protein) and this can be modified by changing the DNA of the gene. There are a number of methods to do this:

- Chromosomal substitution (all, or part, of a chromosome from a donor organism replaces that of the recipient).
- Gene addition (a functional gene or part of a gene is inserted into an organism, e.g. by recombination).
- Rendering a gene non-functional (e.g. by mutation or the removal of all or part of the gene).
- Induced mutation (the modification of genetic material by mutagenic compounds or irradiation).
- Transfection (the introduction of genetic material into an organism often in the form of a plasmid).

Of these, recombination is important for food crops and the stages involved in producing a recombinant GMO are as follows:

1 Identification and isolation of the piece of DNA containing the targeted gene.
2 Precisely cutting out the nucleotide sequence of the gene using bacterial enzymes. The sequence is identified by a 'promoter' (a DNA sequence that is recognised by RNA polymerase and initiates transcription), and a termination sequence stops transcription of the new gene, producing several copies of the isolated gene using the polymerase chain reaction (PCR) method to replicate nucleotide sequences and help isolate the required DNA.
3 Reintroducing (splicing) the sequence into a different DNA segment in the cell. Where genetic material is transferred between different species the new GMO is known as a 'transgenic' organism. Splicing can be done by exploiting natural pathogens, such as *Agrobacteriaum tumefaciens* or *A. rhizogenes*, that infect cells by injecting genetic material; or by direct methods such as microinjection (also known as the 'gene gun' or 'biolistic transformation') where small particles of gold or tungsten, coated with DNA material, are accelerated to four times the velocity of sound using compressed helium, to penetrate cells without causing significant damage (Wilm 2005). Plant viruses can also be used to insert genetic material into cells, but the technique is limited by the range of hosts that the virus is able to infect.

Transposons (also called 'jumping genes' or 'mobile genetic elements') are useful to alter DNA inside an organism. They are sequences of DNA that can move to different positions within the genome of a cell to cause mutations and change the amount of DNA in the genome (a process called 'transposition'). Transposons can be grouped based on their mechanism of transposition into those that move by being transcribed to RNA and then back to DNA by reverse transcriptase (known as 'retrotransposons'), and those that move directly from one position to another using a transposase to 'cut and paste' them within the genome. Another genetic modification method is to interfere with the

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expession of a particular gene and 'silence' it using particular fragments of double-stranded RNA (dsRNA) (RNA interference (RNAi) is a mechanism that exists in both plants and animals and has a role in defence against viruses). RNAi and transposon silencing mechanisms are used to create GMOs for new food and animal feed crops.

Whichever method is used, GM plants are regenerated from a single new embryo cell into multicellular organisms. Plants also have a trait known as 'totipotency' in which cells from an adult plant are able to regenerate into new adults, enabling a range of cell types to be used for manipulation. A marker gene, often for antibiotic resistance, is included so that successfully transformed cells can be selected by their ability to grow on media containing antibiotics, whereas those that have not been transformed die. Mammals can also now be cloned from adult cells. Details of GM mechanisms and methods are described in more detail by Heller (2003).

### 6.1.1 GM food crops

The first commercially grown GM food crop in 1994 was a tomato (the 'FlavrSavr') that carried a gene to reduce the level of polygalacturonase and hence reduce softening and increase the shelf-life. A variant of this was used to produce tomato paste that was first sold in 1996 (Tester 2000). Commercially produced GM foods now include crops that are made resistant to herbicides or insects, new protein ingredients, or production of enzymes by GM micro-organisms (section 6.1.3). Examples of GM food crops include the following:

- Soybeans that tolerate glyphosate herbicides, maize and cotton that are herbicide-tolerant and have insect protection traits (the latter due to a gene for endotoxin production from *Bacillus thuringiensis* – Bt insecticidal protein) and maize with enhanced levels of lysine to improve the quality of protein for animal feeds.
- 'Golden rice', developed to synthesise the precursors of $\beta$-carotene in the edible parts of the grain for areas where there is a dietary shortage of vitamin A (not currently (2008) available for human consumption) and transgenic rice containing the milk proteins, lactoferrin and lysozyme, to improve oral rehydration therapy for diarrhoea.
- Insect-protected and herbicide-tolerant rapeseed (canola), oilseeds and oil-bearing plants that have altered fatty acid and lipid compositions (under development), in particular to produce oils that have a predominant fraction of conjugated linoleic acid (Dunford 2001) (also section 6.3).
- Virus resistant sweet potato.
- Plants that are better able to tolerate water and nitrogen limitation, or survive high-salinity, acidic soils or high or low ambient temperatures.

By 2005, more than 80 GM crops had been grown commercially or in field trials in over 40 countries on six continents. The most important GM crops are soybeans, maize, rapeseed and cotton, which have been widely adopted in the USA and in several developing countries (Table 6.1). Brazil in particular has increased cultivation of GM soybeans from 5 million hectares in 2004 to 9.4 million hectares in 2005. In 2004, the share of crops in global trade that contained GM material was estimated to be 90% for soybeans, 80% for maize, 44% for cottonseed and 73% for rapeseed (Brookes *et al.* 2005). The importance of these four crops is seen by their uses in a wide range of food ingredients (Table 6.2).

The development of transgenic farm animals has lagged behind crop development because of higher costs and lower reproductive rates (Anon 2005a). There is also

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**Table 6.1** Global area of transgenic crops

| Country | Global area of transgenic crops | | | |
|---|---|---|---|---|
| | 2001 | | 2004 | |
| | Area (million ha) | % of total | Area (million ha) | % of total |
| Argentina | 11.8 | 23 | 16.2 | 20 |
| Brazil | – | – | 5.0 | 6 |
| Canada | 3.2 | 7 | 5.4 | 6 |
| China | 1.5 | 1 | 3.7 | 5 |
| Paraguay | – | – | 1.2 | 2 |
| South Africa | 0.2 | 0.5 | 0.5 | 1 |
| USA | 35.7 | 68 | 47.6 | 59 |
| Total | 52.6 | 99.5 | 79.6 | 99 |

From Embarek (2005)

widespread lack of public support for genetic modification of animals to improve productivity, with almost three-quarters of global consumers opposed to this (Hoban 2004). By 2006, GM fish were available and in some countries a recombinant version of the bovine growth hormone, somatotropin, was injected into dairy cattle to increase milk production, but there were no foods derived from GM livestock or poultry (Anon 2005a). Possible future applications of GMOs include the production of drugs in food (e.g. fruits that produce antibodies against infectious diseases for human vaccines) (Tester 2000), gluten-free wheat for people suffering from coeliac disease, GM fruit and nut trees that mature more quickly and produce crops years earlier than at present, and plants that produce new types of plastics that have unique properties (Anon 1999).

**Table 6.2** Uses of ingredients from the four main GM crops

| Crop | Ingredient | Uses |
|---|---|---|
| Soybeans | Beans | Soya sauce, soya milk, tofu, miso and tempeh |
| | Bran | Breakfast cereals |
| | Full-fat, enzyme-active soyflour | Bread improver |
| | Defatted flour | Texturised vegetable protein meat analogue or extender |
| | Soy concentrates/isolates | Infant formula, meal replacement preparations used in weight loss programmes, supplements for athletes' training diets, non-dairy creamer, meat extender |
| | Hydrolysed vegetable proteins (from acid- or enzymic-hydrolysis of soyflour) | Flavourings with savoury meat-like flavour used in a wide range of processed foods – soups, stock cubes, gravy powders, snack foods and condiments. Flavour enhancers and hypo-allergenic infant foods |
| | Soya oil (pure oil or hydrogenated) | Direct consumption or used in a wide range of foods and commercial frying oils |
| | Fatty acids and glyceride esters | Emulsifiers used in a wide range of products |
| | Lecithin (refined, fractionated, hydrolysed or enzymically modified) | Emulsifier used in bakery products, chocolate and dairy products |
| | Tocopherols | Antioxidant or vitamin E precursor |
| | Sterols, stanols and phytosterols | Use in functional foods (section 6.3) |
| | Isoflavones | Use in functional foods |

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**Table 6.2** Continued

| Crop | Ingredient | Uses |
|---|---|---|
| Maize | Grains and flours | Direct consumption, flakes, flours for bakery products, breakfast cereals, extruded snackfoods, tortillas, crumb coatings, carriers. Pre-gelatinised for infant foods and bakery products. Feedstock for fermentation to alcohol |
| | Bran | Breakfast cereals and poultry feeds. Isoflavones may be obtained from bran |
| | Oil (pure, refined, hydrogenated, enzymically modified) | Direct consumption or used in a wide range of foods and commercial frying oils |
| | Maize germ oil | Health foods |
| | Starch and derivatives (maltodextrins, chemically and enzymically modified, glucose syrups, dextrose, high fructose corn syrup | Very wide applications as thickeners, sweeteners, encapsulants, carriers or diluents for other ingredients |
| | Fermentation or enzymic conversion from starch to: | |
| | Sugar alcohols (sorbitol, xylitol, etc.) | Used in sugar-free foods and low-calorie drinks |
| | Ethanol | Flavourings and colourants<br>Carrier for flavourings, blended alcoholic beverages |
| | Ascorbic acid and sodium ascorbate | Antioxidant, widely used in bakery products |
| | Lactic acid/lactates, citric acid/citrates, acetic acid, gluconic acid/gluconates, glucono-delta-lactone | Very wide range of applications as acidulants, e.g. for the preservation of meat and fish products and in ice cream, sorbets, liqueurs, baking powder, yeast substitutes, preparation of egg products |
| | Caramels from glucose syrups | Flavourings and colourants |
| | Sterols and stanols | Use in functional foods |
| | Monosodium glutamate | Flavour enhancer |
| | Xanthan gum | Many uses in a variety of food applications for thickening, suspending and gelling in low pH dressings, ice cream, processed cheese dips and spreads and frozen desserts |
| Rapeseed | Oil | Salad oils, shortening, margarine, coffee whiteners, bakery products, frying oils, anti-dust agent |
| | Esterified oil (diacylglycerol) | Novel and functional foods (see section 6.3) |
| | Sterols and vitamin E | Margarine |
| | Monoglycerides | Emulsifiers in bakery products |
| | Ammonium phosphatide | Emulsifier in chocolate |
| Cottonseed | Oil and hydrogenated oil | Cooking oils, salad oils/dressings, frying oils, mayonnaise, shortening, margarines |
| | Cellulose and chemically modified cellulose gums from linters | Emulsifiers, stabilisers, viscosity modifiers and thickeners in a wide range of products including meat products, sauces, salad dressings, bakery products, low-calorie products, ice cream, yoghurt and high-fibre foods |

Adapted from Brookes *et al.* (2005)

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### 6.1.2 Public perceptions of GM foods

Hoban (2004) reviewed the results of surveys of public attitudes towards GM foods conducted from the mid-1990s to 2002. The largest survey of 35 000 respondents in 35 countries, conducted in 2000, found that support for GM foods varied widely in different parts of the world. Support was most evident in the 13 countries surveyed in North and South America, 2 countries surveyed in Africa, 6 of the 8 countries surveyed in Asia and the Pacific, but only 3 of the 8 countries surveyed in Europe. In each case more respondents agreed that 'the benefits of biotechnology outweigh the risks' than those who disagreed. The countries in which GM foods were least supported were Japan and Korea in SE Asia, and France, Germany, Greece, Italy and the UK in Europe, where more respondents disagreed than agreed. In reviewing previous surveys, the author noted that in the USA although there is strong support for GM foods, awareness has fluctuated since the mid-1990s. Few consumers believed that GM foods were in wide use in the food supply, with only 14% believing that 'more than half of US foods contain GM ingredients' (Anon 2001), whereas it is estimated that 60% of all US processed foods contain a GM ingredient (Wagner 2006).

In Europe, consumer support for GM foods in all countries except Spain and Austria declined throughout the mid–late 1990s. Attitudes turned slightly more positive in 2000, except for Italy, France and the Netherlands, which experienced further declines. A later 2002 survey (Anon 2003a) found that in seven countries surveyed, support for 'scientifically altered' (i.e. GM) fruits and vegetables was strongest in the USA and Canada, but some Western Europeans and Japanese were overwhelmingly opposed (89% in France, 81% in Germany and 76% in Japan). This perception may have arisen from a series of food crises in Europe in the 1990s, which increased consumer apprehension over food safety, and weakened consumer trust in government oversight of the food industry and the ability of scientists to guarantee food quality. Although there may be benefits to producers from GM crops, these consumers perceived no evident benefit to them. These aspects are explored in more detail by Santaniello and Evenson (2004a).

There remains substantial controversy between supporters and opponents of GM foods, focused mainly on environmental and health concerns, labelling and consumer choice. Supporters of GM foods claim environmental benefits from reduced pesticide and herbicide use. For example:

- GM cotton has reduced synthetic pesticide use in the USA, Australia and India (Anon 2005b), increased cotton yields by 50% as a result of reduced losses to insects, and insect protected crops can reduce exposure of farmers to synthetic insecticides. Insect protected GM maize can also have lower mycotoxin levels due to reduced insect damage to the crop (Wu 2006). GM crops can be developed for cultivation in soils or climates where they previously could not grow, thus releasing more land for crop production and increasing crop yields per unit area of land to meet the needs of population growth, and thus contribute to sustainable food security. Reduced ploughing or conservation tillage associated with GM crops reduces fuel use and increases carbon sequestration in soils, thus contributing to reduced greenhouse gas emissions from agriculture.

Supporters also point to economic benefits:

- Higher yields increase farm outputs and lower food prices, which benefit farmers and consumers respectively; insect-protected GM crops have greater economic benefits in developing countries because agriculture is often a large part of the economy and

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reduced crop losses due to insect damage and improved yields benefit large numbers of people. Higher yields of feed crops also meet demand from increased consumption of animal protein.

In relation to safety concerns, GM supporters note that:

- There have been no major health hazards identified since GM foods were introduced, and more than 100 studies have been published indicating their safety (Anon 2004b). There are no significant differences in nutritional value between GM crops and corresponding conventional crops, and no residues of recombinant DNA or novel proteins have been found in organs or tissues of animals fed on GM feeds. They conclude that manufacturers of GM foods are responsible for the safety of their products and are required to demonstrate this before their use is approved. It is in the manufacturers' interests to ensure that safety testing is adequately done.

The main concerns raised by opponents of GM foods are that they threaten unintended, undesirable or unforeseeable consequences to the environment, and create a risk to consumer health and safety. Environmental concerns have arisen due to the following:

- The possibility that unwanted traits could be introduced along with the desired ones, or the risk of cross-contamination of GM genes to traditional crops or wild plants. For example, the uncontrolled spread of GM insecticidal proteins into wild plants could give a competitive advantage to those plants or disrupt the role of insects in natural ecosystems. The use of herbicides substantially reduces weed densities, which can have significant impact on wildlife that consume the weed seeds and hence reduce biodiversity (Tester 2000). For example, the results of a UK farm-scale trial showed that some broadleaf weeds were less numerous in fields containing GM rapeseed and produced one-third fewer seeds for farmland birds to eat at the end of the season, compared with those in a conventional crop. Two years later, there were still fewer seeds, even though the weedkiller had not been applied again, and there were also fewer bees and butterflies in the GM crops (Burke 2005).

Consumer rights groups also emphasise the long-term health risks that GM foods could pose, or that the risks have not yet been adequately investigated. These include the following:

- Exposure of populations to large amounts of novel proteins could cause unpredictable problems with allergenicity that are difficult to detect because symptoms can take several years to develop. Examples include a Bt-maize, which was reported to have caused allergic responses, some life-threatening, in over 200 people and was subsequently banned (Anon 2003b), and the unintentional transfer in 1993 of a gene for an allergenic trait from the Brazil nut into GM soybeans. The intention was to increase the methionine content to improve the protein quality of soybeans for animal feed, but the methionine-rich protein was also the source of Brazil nut allergy and further development was discontinued (Nordlee *et al.* 1996).
- In the USA, the Food and Drug Administration (FDA) reported no evidence for adverse health effects of GM bovine somatotropin. However, there have been reports of the hormone having a negative impact on the ability of cows to conceive, and that cows often developed severe infections requiring treatment with antibiotics (Smith 2003). As a result, some dairies have stopped using the hormone and it is banned in Europe because of health concerns.

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Opponents also claim inadequate oversight by regulatory bodies of the safety of GM foods and that their safety relies on the veracity of manufacturers who do the testing. Many believe that the existing regulatory models fail to protect consumers and are subject to influence by the industry. There is concern that the patenting of GM technologies and private funding of developments enables corporations to gain excessive power in the marketplace (Smith 2003). There are also issues over the requirement of farmers, particularly in developing countries, to pay licensing fees for use of modified seeds and preventing them from saving seeds for future planting, especially if crops are developed that contain the so-called 'Terminator Gene', which allows seeds to germinate only once.

Developments in genomics have created a new agricultural biotechnology named marker-assisted selection (MAS) that does not have the potential disadvantages of GM technologies. MAS is a sophisticated method that can accelerate crop and animal breeding by identifying genes that are associated with traits such as yield, pest resistance or increased nutritional or sensory values (known as 'quantitative trait loci' or QTL). When particular genes are identified, related wild varieties of the crop are scanned for the presence of those genes. Instead of using gene splicing technologies to create a GM crop, the wild varieties are cross-bred with the food crop to create the desired traits. This can reduce the time needed to develop new varieties by 50% or more. Crops so far developed include an aphid-resistant lettuce, rice that remains firm after processing, pearl millet that is drought-tolerant and resistant to mildew (Rifkin 1999, 2006) and new varieties of hops, soya and potato. Further details of marker-assisted selection in animals are given by Van der Werf (2006) and an overview of MAS developments in cereal and forage crops, livestock, fruit trees and farmed fish is given by Anon (2003c).

### 6.1.3 Genetically modified micro-organisms

The use of micro-organisms in food processing is described in sections 6.3.1 and 6.4.3 and 'technical' enzymes produced by micro-organisms are described in section 6.5. This section describes developments in the use of GM micro-organisms (GMMs) and their products, particularly extracellular enzymes. The first GMM food product, bacterial chymosin (rennet) for cheese manufacture, was approved for use in the USA in 1990. Since then there has been widespread and increasing use of enzymes produced by GM bacteria, moulds and yeasts, but this has not given rise to consumer concerns in the same way the GM plants and animals have (section 6.1.2). The enzymes that are commercially produced by GMMs, especially *Bacillus* sp., *Aspergillus* sp. and *Trichoderma* sp. are shown in Appendix B.3.

Enzymes from GMMs and non-GM sources are also incorporated into animal feeds to enhance digestion and complement either the gut microflora of the animal or natural enzymes in the feedstuff. In particular added enzymes are used to hydrolyse antinutritional factors and increase the availability of nutrients in the feed. Examples include the following:

- Phytase produced from GM *Aspergillus niger* or *A. oryzae*, which breaks down phytate to increase the availability of phosphate in the feed. This reduces the amount of added phosphate by up to 30%, so reducing levels of phosphate in manure and contributing to reduced contamination of groundwater.
- Partial reductions in indigestible polysaccharides and oligosaccharides in feeds, including reduced $\beta$-glucan in barley feeds by $\beta$-glucanase from *Aspergillus* sp. or *Penecillium* sp., reduced arabinoxylan in wheat feeds by xylanase from GM *Asper-*

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*gillus* sp., *Bacillus* sp. or *Trichoderma* sp., and reduced oligosaccharides in vegetable (e.g. soybean) feeds using a GM $\alpha$-galactosidase from *Aspergillus* sp. In each case the enzymes increase the efficiency of feed utilisation by the animal (Brookes *et al.* 2005).

Examples of food applications of GMMs include GM rennet (chymosin) derived from genetically modified *Escherichia coli*, *Kluyveromyces lactis* and *Aspergillus niger* for cheese production, lactic acid bacteria that are resistant to viruses for the production of safer dairy and meat products (section 6.4.3), GM *Bacillus subtilis* to hydrolyse starch for the production of glucose, citric acid and other products, and microbial production of vitamins $B_1$ and $B_2$, aromas, amino acids and flavour enhancers. There are also developments to produce oils and fats that have high concentrations of conjugated linoleic acid (CLA) using micro-organisms that produce linoleic isomerase, thereby converting linoleic acid to CLA (Dunford 2001). CLA has been shown to suppress tumour development and is being studied for its role in cancer prevention (Lee *et al.* 2005).

### 6.1.4 Legislation and safety testing

A summary of the development of EU legislation is described by Lee and Carson (2004) and the OECD (Anon 2008b). Santaniello and Evenson (2004b) give further details of EU, US and international legislation. Anon (2006) summarises the regulatory status of countries worldwide. There are significant differences in approach between regulators in the USA and EU that are summarised below. In the USA, a legislative policy of 'substantial equivalence' permits GMOs to qualify as traditional or GRAS foods if their nutritional composition is essentially the same (i.e. GM foods that have similar proteins, and toxins are deemed to be no different from conventional food, without further investigation of the effects of any other differences) (Anon 2002). A comprehensive review of the US position on biotechnology was published by the Institute of Food Technologists in 2000 (Anon 2000b–e). Since 2004, EU regulations require all GMO products to be labelled as GM, whether or not they contain GM material. This includes any product derived directly from a GM plant, animal or micro-organism (e.g. maize starch, which includes traces of GM material) or indirectly (e.g. glucose syrup made from maize starch, in which all GM material is removed during processing) (Appendix B.3). Foods that contain less than 0.9% GM material that arises from accidental contamination during cultivation, harvesting and processing need not be labelled.

The EU regulatory system requires authorisation for placing a GM food on the market, in accordance with the precautionary principle that requires a comprehensive pre-market risk assessment and traceability of products at each stage of their production and distribution. Rules for traceability and labelling apply to all GM-derived products except those derived from microbial genetic engineering, To prevent GM crops from contaminating traditional varieties, EU legislation also requires a quarantine zone around GM fields where traditional varieties of the same crop cannot be grown (Anon 2008a).

The Cartagena Protocol is an international agreement on the movement of GMOs from one country to another that seeks to protect biological diversity from the potential risks posed by GMOs. It established procedures that require specific notification to, and agreement of, the importing country before export of a GMO may go ahead. An importing country can declare via the Biosafety Clearing House (an information exchange mechanism) that it wishes to base a decision on risk assessment before agreeing to accept an imported GMO for food, feed or processing.

The differences in approach between regulatory agencies in the EU and the USA have resulted in strong disagreements over the labelling and traceability requirements for GM

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foods. As a result, the USA claims that restrictions on sales of GM products in the EU violate free trade agreements. This is described in detail by Toke (2004).

One result of the EU legislation and the negative consumer attitudes to GM foods in Europe has been an increase in 'GM avoidance' policies by major EU retailers. This in turn has led to increased demand by food processors for 'Identity Preserved' (GM-free) ingredients, which has increased costs in the supply chain up to but not including the retail sector (Brookes *et al.* 2005). A manufacturer wishing to label products as 'GM-free' must institute systems and records to ensure that only ingredients from non-GM sources are used. Similar considerations also apply to livestock or poultry meat products where feed ingredients contain soya or maize, and to imported finished products from countries that do not segregate GM and non-GM crops. Identity Preserved ingredients with non-GM certification (e.g. soybean lecithin, $\beta$-carotene, curcumin, lutein and caramelised sugar) are now becoming available and it is possible that xanthan gum derived from GM-free sugar will be available to replace that from maize derivates because of doubts about the non-GM status of maize.

In 2006, the legal status of foods that contain GM fermentation products was that such products are excluded from regulation if foods are produced with, rather than from, the GMM. If the GMM is present in the final product, whether alive or dead, the food falls within the scope of the regulations. The distinction is based on whether there is genetic material in the food (i.e. if cellular material from GMMs is incorporated) or whether modifications to the product can be demonstrated. The legislation controlling use of enzymes in food processing, whether from GMM or non-GMM sources, categorises them into 'additives' (substances that have a technological function in the food) and 'processing aids' (substances that are added during processing for technical reasons, but do not have a technological function in the final product). At present, lysozyme and invertase are the only two enzymes that are considered as additives. All other enzymes are processing aids that do not have to be declared on the label under GM or food labelling regulations. The position is under review (in 2008) and a proposal to reclassify most enzymes as additives is under discussion. If approved, this would have important implications for processors that operate GM avoidance policies and obtain enzymes from GMM sources.

*Safety testing*

GM foods are subject to safety assessment for possible changes to allergenic potential or nutritional profile. If the originating material is known to be allergenic (Brazil nuts, peanuts, kiwifruit, eggs, crustaceans, etc.) the testing regime should verify whether allergens are transferred into the host organism. Other factors that are taken into account include the stability to processing and digestion, the final amount of allergen in food and the identity of other non-allergenic proteins. Allergenity is tested *in vitro* using RAST (radioallergosorbent) and ELISA (enzyme-linked immunosorbent assay) and if this is negative, *in vivo* skin prick tests are used. (The RAST is a blood test for 'true allergies' that are immediate IgE reactions such as hives, swelling, rapid heartbeat, whereas the ELISA test is for IgG reactions that are delayed reaction allergies in which symptoms can appear 2–48 h after exposure to the allergen. IgE and IgG are acronyms for the two different kinds of immunoglobulin antibodies produced by the immune system in allergic reactions to food.) Further details are given by Allen (1990).

In 2003, the Codex Alimentarius Commission approved principles for the risk analysis of GM foods and guidelines for the safety assessment of GM plants and micro-organisms (Anon 2003d,e, 2008a). These procedures form the basis of assessments of GM foods and ingredients by national regulatory authorities.

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Analytical methods including gas chromatography mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) and capillary electrophoresis are unable to detect these materials and immunological methods (e.g. ELISA) are used. The DNA is extracted and the promoter sequence and gene are amplified using the polymerase chain reaction (PCR). The products of the PCR can then be made visible using gel electrophoresis. The modified genetic material is often present in very small amounts in foods and the transgenetic protein may be located in non-edible parts of the plant (e.g. Bt toxin is present in leaves but not in maize kernels). Also other compounds, such as polysaccharides, can inhibit the PCR and interfere in the detection, leading to false negative results.

## 6.2 Nutritional genomics

Nutritional genomics (or 'nutrigenomics') is the application to human nutrition, especially the relationship between nutrition and health, of:

- *genomics* (the study of an organism's genome and use of the genes);
- *proteomics* (the study of structures and functions of proteins and their interactions with the genome and the environment); and
- *metabolomics* (the study of unique chemical fingerprints that specific cellular processes leave behind).

Research focuses on the prevention of disease by understanding nutrient-related interactions at the gene, protein and metabolic levels and their effects on tissues and organs. With progress in genetics research, including the Human Genome Project, genetic variation is known to affect food tolerances and may also influence the dietary requirements of individuals. Nutritionally related biochemical disorders have been linked to genetic origin and the influence of diet on health may therefore depend in part on the genetic makeup of an individual (Stover 2006).

When food is digested, some dietary chemicals are not metabolised and become ligands (molecules that bind to proteins that are involved in turning on certain genes). These dietary chemicals change the expression of genes and even the genome itself. The theory is that a diet that is out of balance will cause gene expressions that may cause chronic illness. This has been observed in the Alaskan Inuit, whose metabolism of high-fat food was suited to high levels of activity in the cold climate and who now show high levels of obesity, diabetes and cardiovascular disease as a result of changes in their lifestyle; those members of the Maasai in East Africa who have abandoned their traditional meat, blood and milk diet for maize and beans, and have since developed new health problems; and differences in lactose tolerance between people of northern European and south-east Asian or African ancestry (Grierson 2003).

Future developments in nutrigenomics may enable identification of people who are genetically predisposed to diet-related diseases, and the development of foods or personalised precisely tailored 'intelligent diets' for disease prevention and health promotion (Hasler 2000, 2002). This is also known as targeted or 'prescription' nutrition, or 'eat right for your genotype'. A few companies have started offering a genetics testing service to produce a preventive health profile and nutritional supplements (Grierson 2003). However, the interactions between diet and genes are complex and poorly understood. There may be genetic subpopulations that would incur different benefits or risks from generalised fortification policies based on genomic criteria. Research is

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ongoing to understand the molecular mechanisms that underlie gene–nutrient interactions and their modification by genetic variation before dietary recommendations and nutritional interventions can be made to optimise individual health. Another development in genomics, MAS, is described in section 6.1.2.

## 6.3 Functional foods

The term 'functional foods' (also 'nutraceuticals') was first used in Japan and is used to refer to foods that provide benefits other than the nutrients required for normal health. It can be said to be a concept rather than particular types of products (Roberfroid 2000). It is not defined in law but a number of definitions have been proposed, including 'any substance that is a food or food ingredient that provides medical or health benefit, including the prevention and treatment of disease' (Defelice 1995). Interest in functional foods has grown since the mid-1990s due to a number of factors, including new evidence of the links between diet and health that has resulted in an increasing interest by many consumers in 'healthy eating', compounded by increasing healthcare costs and an ageing population in many industrialised countries. Consumer interest has prompted the commercial development of new foods that are designed to address a range of specific medical conditions or health concerns, including cardiovascular disease, cancer, osteoporosis, neural tube defects, immune deficiencies, intellectual performance, gut health/bowel disorders, ageing and obesity. These are summarised by Henry and Heppell (1998). Research is also focused on functional attributes that are being discovered in many traditional foods, and new food products that are being developed to contain beneficial components.

Functional foods can be grouped into the following four categories:

1 Products in which an ingredient that is normally present is increased or reduced (e.g. breakfast cereals with added vitamins or bran, drinks fortified with antioxidant vitamins or dairy products that have reduced fat).
2 Products in which an ingredient that is not normally present is introduced (e.g. fibre added to fruit juice, folic acid or plant oestrogens added to bread and margarines, and snack bars enriched with stanols to reduce cholesterol absorption).
3 Dairy products, fermented using probiotic bacteria that are selected for their functional benefits to aid digestion and protect against infections (section 6.3.1). Some products have added oligosaccharides to support the growth of these bacteria.
4 Products that are specially formulated to meet a particular nutritional requirement (e.g. sports drinks that give a balanced replacement of fluids lost during exercise or provide additional energy, or cereals that are formulated to slowly release carbohydrates and supply energy over a prolonged period).

Other functional foods may contain herbal extracts that claim to help address a range of problems from premenstrual syndrome to lack of energy. Caffeine stimulation drinks can also be described as functional foods.

A summary of the presently known (2008) functional components of foods is given in Appendix A.5. The addition of functional ingredients to confectionery products is described by Pickford and Jardine (2000), and to spreadable fats by de Deckere and Verschuren (2000). Functional dairy products are described by Mattila-Sandholm and Saarela (2003) and Saarela (2007), and the legislative and health aspects of functional foods are described by Gibson and Williams (2000). Lindsay (2000) describes the use of GM technology to increase the levels of functional components in plant foods.

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Some functional ingredients, such as plant extracts, are microencapsulated (section 6.6 and Chapter 24, section 24.3) to mask their taste, and microencapsulation can also be used to improve the nutritional value of products for children (e.g. microencapsulated minerals or vitamins in chewing gum or sweets) or to produce processed foods for targeted nutrition (section 6.2).

### 6.3.1 Health claims and regulation

New functional foods are being developed at an increasing rate and they are usually accompanied by health claims for marketing purposes. In most countries they are treated as other foods and manufacturers are not allowed to claim that foods can prevent, treat or cure disease. So whereas it is acceptable to state that a food 'provides calcium which is important for strong bones', or 'helps lower blood cholesterol when consumed as part of a low-fat diet', it is illegal to claim that the food 'provides calcium, which helps prevent osteoporosis' or 'helps prevent heart disease' (Anon 2004c). A number of reports, including Heller *et al.* (1999), Jacobson and Silverglade (1999) and Katan and De Roos (2004), reviewed the regulatory and marketing aspects of functional foods in Japan, the USA and the EU. They concluded that some foods were being marketed without sufficient evidence of their health benefits, or that some manufacturers use symbols such as a heart on the packaging that implied health benefits that were not proven, and that regulations should be strengthened. Health claims should take into account the required level of intake within normal dietary patterns and how often/how long it is necessary to consume a product to make any functional impact.

The Japanese Government was the first to develop a specific regulatory approval process for functional foods, named Foods for Special Health Use (FOSHU), and approved foods bear a seal of approval from the Japanese Ministry of Health and Welfare. Over 100 products have been licensed as FOSHU foods in Japan (Hasler 2005). Other countries, including Canada, have specific laws covering the labelling of functional foods. In the USA, claims are regulated by the FDA under a complex set of rules that are described in detail by Schmidl and Labuza (2000). In 1997 in the UK, the Joint Health Claims Initiative (JHCI), a panel of nutritional and health professionals, drew up a Code of Practice on Health Claims on Foods. This sets out the general principles for making a claim, describes precise criteria for substantiating claims, monitors the operation of the code and considers the scientific validity of any particular claim. Food producers may optionally use the JHCI to determine whether their claims are likely to be legally sustainable. In Europe in 2005, the PASSCLAIM (Process for the Assessment of Scientific Support for Claims on Foods) project developed criteria for the scientific substantiation of food claims (Anon 2005c,d). Arvanitoyannis and van Houwelingen-Koukaliaroglou (2005) have reviewed health claims and legislation. Berry Ottaway (2000) describes the EU legislation that relates to different functional ingredients and Schmidl and Labuza (2000) describe US legislation and functional health claims. The issues of guidance to manufacturers and the substantiation of claims are ongoing, and updated information is available at Anon (2008c).

In the following sections, the types of functional foods known as probiotics and prebiotics are examined in more detail.

### 6.3.2 Probiotic foods

Daily consumption of bioyoghurts and other fermented dairy products that contain probiotic bacteria has been shown to promote gut health. Probiotic bacteria include

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*Lactobacillus acidophilus*, *L. casei* Shirota, *L. johnsonii*, *L. rhamnosus* GG, *L. lactis, L. plantarum, Streptococcus thermophilus, Bifidobacterium lactis, B. adolescentis, B. longum, B. breve, B. bifidus* and *B. infantis* (Krishnakumar and Gordon 2001). Their beneficial effects are described by Sanders (1999) and Mattila-Sandholm and Saarela (2000) and include increased immunity to a range of intestinal pathogens, including *E. coli* O157, *Salmonella* spp. and *Shigella* spp. (see Chapter 1, section 1.2.4). The mechanisms by which they do this are described by Isolauri and Salminen (2000). Dairy products that contain *L. acidophilus* can also reduce the incidence of vaginal infections by reducing gut colonisation by *Candida albicans* (Surawicz and McFarland 1996). Other health benefits may include reduction in cancer risk, particularly colon cancer, because lactic acid bacteria can alter the activity of faecal enzymes that may play a role in the development of this disease. They may also have hypocholesterolemic (cholesterol-lowering) and anticarcinogenic actions (Mital and Garg 1995). However, there is not yet a full understanding of the roles that intestinal bacteria play in health and the influence that diet has on their composition.

Their effectiveness depends on the ability of bacteria to survive storage as frozen or freeze dried cultures, processing, and passage through stomach acids to colonise the large bowel and change the composition of its flora. Champagne and Gardner (2005) have reviewed the factors that should be considered to enable probiotic micro-organisms to survive processing and storage. Microencapsulation of probiotics improves their survival rate during processing and digestion and is described in detail by Siuta-Cruce and Goulet (2001) (see also section 6.6). Microencapsulation using calcium-alginate gel capsules, kappa-carrageenan, gelatin or starch (Kailasapathy 2002), and the use of cryoprotectants such as trehalose or gum acacia (Ross *et al.* 2005), have improved the viability of cells and microencapsulated cultures are commercially available. Fermentation technology for the production of probiotic bacteria is described in section 6.4.

### 6.3.3 Prebiotic foods

Prebiotics are foods that contain ingredients that are not digested but stimulate the growth of probiotic bacteria in the colon. They are complex fermentable carbohydrates including fructo-, gluco-, xylo- and galacto-oligosaccharides, inulin, chitin, dietary fibres, other non-absorbable sugars and sugar alcohols (Roberfroid and Slavin 2000, Playne and Crittenden 1996). Of these, oligosaccharides (Chapter 1, section 1.1.1) have received the most attention, and numerous health benefits have been attributed to them (Gibson *et al.* 2000). They are found naturally in many fruits and vegetables (including banana, garlic, onions, artichokes), milk and honey, but most commercially available prebiotics are manufactured using enzymic reactions. These may involve building the oligosaccharide from sugars using transglycosylation, or by hydrolysis of large polysaccharides. The advantage of prebiotics is that their benefit does not rely on the viability of probiotic bacteria in a given product and they can be added to any food that normally contains carbohydrates. To date they have mostly been added to fermented dairy products, but cereal products, infant formulae, confectionery or savoury pastes and spreads are each potential applications (Rastall *et al.* 2000).

Synbiotics consist of a live probiotic micro-organism and a prebiotic oligosaccharide. These products have advantages in that the prebiotic aids the establishment in the colon of the particular probiotic bacterium, which has known benefits. There is therefore great flexibility in the combination of probiotic micro-organisms and different oligosaccharides to achieve the desired health benefits (Shah 2001, Rastall *et al.* 2000).

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## 6.4 Fermentation technology

Fermented foods are among the oldest processed foods and have formed a part of the diet in almost all countries for millennia. Today they form major sectors of the food processing industry, including fermented cereal products, alcoholic drinks, fermented dairy products and soy products among many others. Mycoprotein has also been developed as a meat-substitute. A summary of commercially important food fermentations is given in section 6.4.3.

During food fermentations, the controlled action of selected micro-organisms is used to alter the texture of foods, preserve foods by production of acids or alcohol, or to produce subtle flavours and aromas that increase the quality and value of raw materials. The preservative effect is supplemented by other unit operations (e.g. pasteurisation, chilling or modified atmosphere packaging (Chapters 12, 21 and 25, section 25.3). These combined effects are examples of hurdle technology, described in Chapter 1 (section 1.3.1). The main advantages of fermentation as a method of food processing are:

- the use of mild conditions of pH and temperature which maintain the nutritional properties and sensory characteristics of the food;
- enrichment of foods with vitamins, proteins, essential amino acids or fatty acids, or detoxification by removal of antinutritional factors (e.g. phytate, trypsin inhibitor) or toxins (e.g. aflatoxins) (Steinkraus 2002);
- the production of foods that have a diversity of flavours, aromas or textures that cannot be achieved by other methods;
- low energy consumption due to the mild operating conditions;
- relatively simple technologies that have low capital and operating costs (section 6.4.2).

### 6.4.1 Theory

Food fermentations can be grouped into those in which the main products are lactic acid, alcohol and carbon dioxide, and acetic acid. Details of the metabolic pathways that are used to produce these products are readily available (for example Jay *et al.* 2005). Many fermentations involve complex mixtures of micro-organisms or sequences of microbial populations that develop as changes take place in the pH, redox potential or substrate availability. The factors that control the growth of micro-organisms are described in Chapter 1 (section 1.2.3) and selected examples are given in section 6.4.3.

*Batch culture*

Cell growth during the logarithmic (or exponential) phase is at a constant rate (Chapter 1, Fig. 1.15), which is shown by:

$$\ln c_b = \ln c_0 + \mu t \qquad 6.1$$

where $c_0$ = original cell concentration, $c_b$ = cell concentration after time $t$, (biomass produced), $\mu$ ($h^{-1}$) = specific growth rate and $t$ (h) = time of fermentation. Graphically, the natural logarithm (ln) of cell concentration versus time produces a straight line, the slope of which is the specific growth rate. The highest growth rate ($\mu_{max}$) occurs in the logarithmic phase.

The rate of cell growth eventually declines owing to exhaustion of nutrients and/or accumulation of metabolic products in the growth medium. If different initial substrate concentrations are plotted against cell concentration in the stationary phase, it is found that an increase in substrate concentration results in a proportional increase in cell yield

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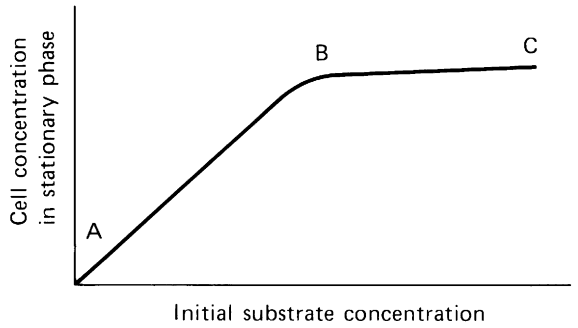


**Fig. 6.1** Effect of initial substrate concentration on cell concentration at the end of the logarithmic phase of growth (after Stanbury *et al.* 1995).

(AB in Fig. 6.1). This indicates substrate limitation of cell growth, which is described by:

$$c_b = Y(S_0 - S_r) \quad \boxed{6.2}$$

where $c_b$ = concentration of biomass, $Y$ (dimensionless group) = yield factor, $S_0$ (mg $l^{-1}$) = original substrate concentration, $S_r$ (mg $l^{-1}$) = residual substrate concentration. The portion of the curve BC in Fig. 6.1 shows inhibition of cell growth by products of metabolism.

The reduction in growth rate is related to the residual substrate concentration by Monod's equation:

$$\mu = \frac{\mu_{max} S_r}{(K_s + S_r)} \quad \boxed{6.3}$$

where $\mu_{max}$ ($h^{-1}$) = maximum specific growth rate, $K_s$ (mg $l^{-1}$) = substrate utilisation constant. $K_s$ is a measure of the affinity of a micro-organism for a particular substrate (a high affinity produces a low value of $K_s$).

Growth parameters that enable the production of biomass or a specific metabolic product (e.g. ethanol, amino acids or citric acid) can be determined. The yield coefficient ($Y$) is calculated from the amount of a limiting nutrient, usually the carbohydrate source, that is converted into the microbial product. For biomass, it is found using:

$$c = Y_{c/s}(S - S_r) \quad \boxed{6.4}$$

where $c$ = biomass concentration (g $l^{-1}$), $Y_{c/s}$ = yield coefficient (g biomass/g substrate utilised), $S$ = initial substrate concentration (g $l^{-1}$), and $S_r$ = residual substrate concentration (g $l^{-1}$).

The yield coefficient describes the amount of biomass produced per gram of substrate utilised: the higher the yield coefficient, the greater the amount of substrate that is converted into biomass. For the production of metabolic products, the yield coefficient ($Y_p$) describes the amount of metabolic product that is produced in relation to the amount of substrate used ($Y_{p/s}$). Yield coefficients are important because the cost of the growth medium, particularly the carbon source, can be a significant proportion of the total production cost.

The specific rate of product formation for primary products varies with the specific growth rate of cells. The rate of production of secondary products (those produced from primary products such as aromatic compounds and fatty acids), which are produced in the stationary growth phase, does not vary in this way and may remain constant or change in more complex ways.

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**Sample problem 6.1**
An inoculum containing $3.0 \times 10^4$ cells $ml^{-1}$ of a fast-growing yeast is grown on $50\,g\,l^{-1}$ glucose in a batch culture for 20 h. Cell concentrations are measured at 4 h intervals and the results are plotted (Fig. 1.15, in Chapter 1). The harvested broth contained $0.024\,g\,cells^{-1}$ and $30\,g\,l^{-1}$ of glucose. Calculate the maximum specific growth rate and the yield coefficient of the culture.

*Solution to sample problem 6.1*
From Fig. 1.15, final cell concentration $= 2 \times 10^8$ cells $ml^{-1}$ after logarithmic growth for 8.5 h.

From Equation 6.1 for the logarithmic phase,

$$\ln 2 \times 10^8 = \ln 3 \times 10^4 + \mu_{max} 8.5$$

Therefore,

$$\mu_{max} = \frac{\ln (2 \times 10^8) - \ln (3 \times 10^4)}{8.5}$$

$$= 0.95\ h^{-1}$$

From Equation 6.4,

$$0.024 = Y_{c/s}(50 - 30)$$

$$Y_{c/s} = 0.024 \times 20$$

$$= 0.48 \text{ or } 48\%$$

The average productivity of a culture (the amount of biomass produced in unit time is found using:

$$P_b = \frac{(c_{max} - c_0)}{t_1 - t_2} \qquad 6.5$$

where $P_b$ ($g\,l^{-1}\,h^{-1}$) = average productivity, $c_{max}$ = maximum cell concentration during the fermentation, $c_0$ = initial cell concentration, $t_1$ (h) = duration of growth at the maximum specific growth rate, $t_2$ (h) = duration of the fermentation when cells are not growing at the maximum specific growth rate and including the time spent in culture preparation and harvesting.

*Continuous culture*
Cultures in which cell growth is limited by the availability of substrate in batch operation have a higher productivity if the substrate is added continuously to the fermenter and biomass or products are continuously removed at the same rate. Under these conditions the cells remain in the logarithmic phase of growth. The rate at which substrate is added under such 'steady state' conditions is found using:

$$D = \frac{F}{V} \qquad 6.6$$

where $D$ ($h^{-1}$) = dilution rate, $F$ ($l\,h^{-1}$) = substrate flowrate and $V$ (l) = volume of the fermenter.

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The steady state cell concentration and residual substrate concentration respectively are found using:

$$\bar{c} = Y(S_0 - \bar{S}) \tag{6.7}$$

$$S = \frac{K_s D}{\mu_{max} - D} \tag{6.8}$$

where $\bar{c}$ = steady state cell concentration, $Y$ = yield factor, $S$ = steady state residual substrate concentration, $K_s$ (mg l$^{-1}$) = substrate utilisation constant.

The maximum dilution rate that can be used in a given culture is controlled by $\mu_{max}$ and is influenced by the substrate utilisation constant and yield factor (Fig. 6.2). The overall productivity of a continuous culture ($P_c$) is found using:

$$P_c = D\bar{c}\left(1 - \frac{t_3}{t_4}\right) \tag{6.9}$$

where $t_3$ (h) = time before steady state conditions are established and $t_4$ (h) = duration of steady state conditions. Further details of the above equations are given by Blanch and Clark (1995) and in microbiological texts.

**Sample problem 6.2**

Brewers' yeast is grown in a fermenter with an operating volume of 12 m$^3$. A 2% inoculum, which contains 5% yeast cells, is mixed with the substrate and the yeast is harvested after 20 h. Calculate the mass of yeast harvested from the fermenter. (Assume that the yeast has a doubling time of 3.2 h and the density of the broth is 1010 kg m$^{-3}$.)

*Solution to sample problem 6.2*

$$\text{Mass of broth} = 12 \times 1010$$
$$= 1.212 \times 10^4 \text{ kg}$$

$$\text{Yeast concentration} = \frac{\text{concentration in the inoculum}}{\text{dilution of inoculum}}$$
$$= \frac{5/100}{100/2}$$
$$= 0.001 \text{ kg kg}^{-1}$$

The doubling time is 3.2 h.
Therefore in 20 h there are 20/3.2 = 6.25 doubling times.
As 1 kg of yeast grows to 2 kg in 3.2 h, 1 kg grows to $1 \times 2^{6.25} = 76$ kg in 20 h.
Therefore,

$$\text{mass of product} = \text{yeast concentration} \times \text{growth} \times \text{mass of broth}$$
$$= 0.001 \times 76 \times (1.212 \times 10^4)$$
$$= 921 \text{ kg}$$

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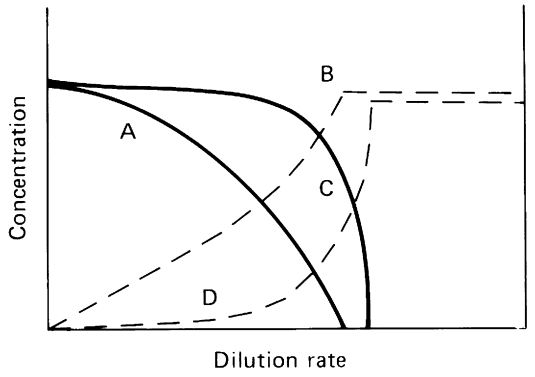


**Fig. 6.2** Effect of dilution rate in continuous culture on steady state cell concentration (——) and residual substrate concentration (— — —) for limiting substrate compared with initial substrate concentration: curves A and B, micro-organisms with a low *K* value; curves C and D, micro-organisms with a high *K* value.

### 6.4.2 Equipment

*Submerged cultures*

Liquid substrates are fermented in stainless steel tanks that are fitted with cleaning-in-place (CIP) and sterilising-in-place (SIP) facilities. Figure 6.3 shows a batch fermenter, and continuous fermenters have similar controls but with additional arrangements to

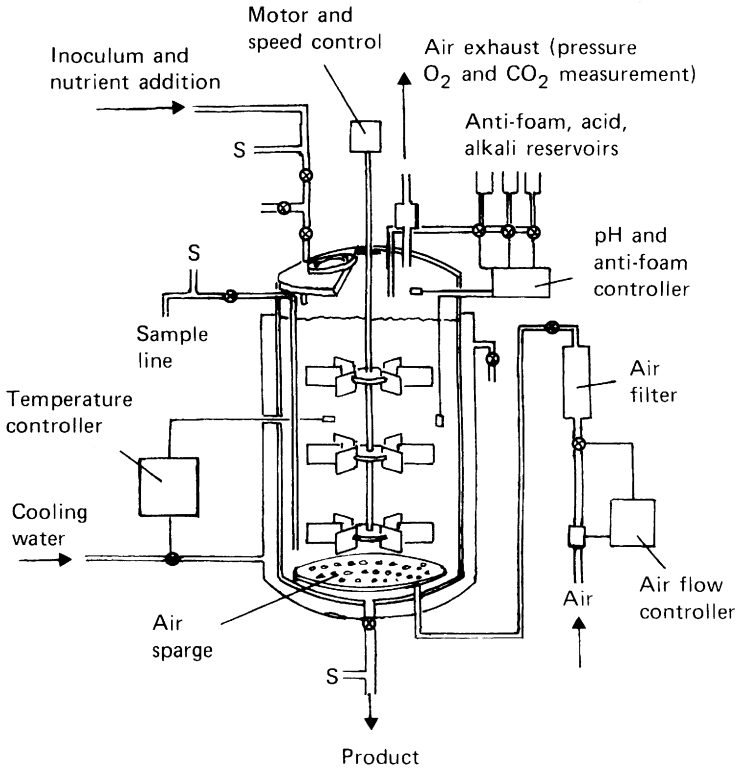


**Fig. 6.3** Batch fermenter showing controls and instrumentation: S, steam sterilising points.

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continually remove the product. In operation, the inoculum is produced from a freeze-dried stock culture and introduced into a fermentation medium in a sterilised fermentation vessel. The medium contains a carbon source (e.g. glucose syrup) with added nutrients depending on the requirements of the micro-organism for growth and/or metabolite or enzyme production. In aerobic fermentations, air is pumped into the fermenter to initiate a period of growth under constant conditions of temperature, dissolved oxygen (DO) and pH, which are maintained by automatic control mechanisms (types of control mechanisms and systems are described in Chapter 27, section 27.2). In batch operation, the fermenter is emptied at the end of the fermentation, judged by accumulated biomass and/or enzymes or metabolites, or by depletion of nutrients. In continuous fermentations, a feed of nutrients is maintained with the simultaneous removal of fermenter broth so that an excess of nutrients remains in the supernatant. In aerobic fermentations the supply of air (and if necessary supplementary oxygen) is automatically regulated in response to continuous monitoring of the DO concentration in the broth. The concentration of biomass, enzymes or metabolites is controlled by the flow of medium and by monitoring factors such as the carbon dioxide evolution rate using on-line analysis. In anaerobic fermentations, a low DO concentration is maintained using nitrogen or carbon dioxide.

In both batch and continuous fermentations the main factors that require control are temperature, pH, dissolved oxygen, degree of agitation and foaming. Methods of control are described by a number of equipment suppliers including Anon (2008d,e).

*Temperature control*

There are three ways to control the optimum temperature for cell growth and/or metabolite production: (1) the growth medium is continuously recirculated through a heat exchanger, linked to a thermostatic controller; (2) the fermentation vessel is fitted with a thermostatically controlled water-jacket and water is recirculated through the jacket and a heat exchanger; or (3) the fermenter is fitted with an electric heating blanket, linked to a thermostat.

*pH measurement and control*

A digital pH probe is linked to a solid state controller that measures and controls pH to an accuracy of $\pm 0.01$ pH units. The microprocessor controls the calibration of the pH probe using standardised buffers and regulates pumps to add acid or alkali. The limits of the working range are established by adjustable high and low set-point controls, and pH values that exceed the set-points in either direction cause the controller to activate the pumps. Adjustable electronic timers determine the length of time that pumps operate to provide a delay between corrective additions, thus minimising overshooting. The set-point limits have an associated alarm to alert the operator to depleted reagent supplies or to signal the need for adjustment or intervention.

*Aeration/dissolved oxygen control*

A digital DO probe continuously monitors the DO concentration in the medium. It is calibrated by the software in the controller with temperature compensation, and a display indicates DO concentration in a range of 0–100%. The required DO concentration range is maintained using upper and lower set-points, and an alarm indicates when the set-point range is exceeded. The controller is linked to gas flowmeters and the agitator speed monitor. It controls oil-free air pumps or regulators on pressurised gas tanks to add oxygen, air, carbon dioxide or nitrogen to the vessel, and/or to control the speed of

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agitation, to maintain the DO concentration according to the needs of process. In-line filters maintain the sterility of incoming and outgoing process air or gas.

*Agitation*

A controller equipped with digital speed measurement monitors the degree of agitation and adjusts a variable speed motor on the mixer shaft, typically operating between 50 and 1250 rpm.

*Foaming*

Agitation and aeration may result in excessive foaming, depending on the growth medium and the types of metabolites produced by the fermentation. Foaming is controlled to prevent foam being forced out of the probe ports, thereby risking contamination, and to prevent it inhibiting oxygen transfer. Teflon-coated stainless steel foam-sensing probes are mounted within the fermentation vessel. The sensitivity of the probes is adjustable to eliminate signals caused by occasional splashing. Brown *et al.* (2001) describe an improved method using continuous on-line conductance measurement of foam. When foam contacts the probes they activate a controller that drives a pump to introduce chemical antifoam reagent into the vessel. Antifoam chemicals used in food fermentations include silicone, mineral oils, polyols, fatty acids, alcohols, hydrophobic silica and polyalkylenes. Flowmeters are linked to the controller and adjustable electronic timers determine the length of the antifoam addition and the period of delay between doses.

*Medium addition*

In continuous fermentations, a solid state electronic controller operates a variable speed metering pump to control the addition of presterilised culture medium to the fermenter, and a medium flowmeter verifies the flowrate. An 'anti-growback' medium inlet tube provides a barrier against potential backgrowth of cultured micro-organisms into the medium feed pipe. Sterile air is directed through the anti-growback inlet tube as a further precaution. Medium and product reservoirs each have an exhaust air vent with autoclavable 0.3 $\mu$m air filters and a sampling device.

*Fermentation time*

The duration of the fermentation is controlled using a programmable timer with automatic shutdown of the fermenter.

*Displays and data logging*

Data logging and control software enable the fermentation cycle to be programmed from a computer or panel-mounted display screen, and different fermentation cycle programmes can be saved for future reference. A data plot screen (Fig. 6.4) has a graphical display of the entire process and a synoptic screen, updated every few minutes, displays the fermentation parameters and individual controls throughout the fermentation. All data can be printed and saved through a PC interface.

*Automatic control of fermenters*

Fermentation processes are becoming increasingly sophisticated and 'bioinformatics' is used to automate the entire operation and to obtain traceability of the process. A series of commands, designed to control a fermenter without operator intervention, are known as 'sequences' and they perform the actions and decisions that an operator would take if the fermenter were being operated manually (see also Chapter 27, section 27.2.2). These

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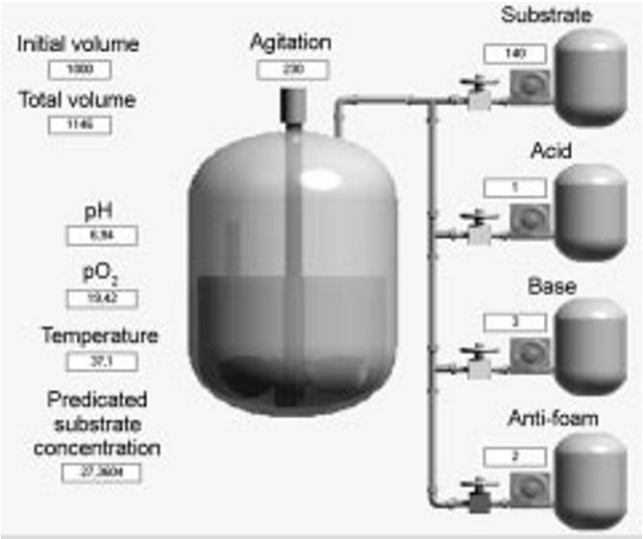


**Fig. 6.4** Fermenter control screen (courtesy of Foxy Logic at http://imsb.au.dk/~mha/toverview.htm).

include inoculating or stopping the fermentation process, driving actuators (pumps or valves), or modifying the timing ratios for an actuator to be active/non-active (Andersen 2005). Sequential programming first identifies the measured values that should be logged (e.g. temperature, agitator speed, dissolved oxygen, pH, air flow), and their set-points and alarm settings. Then predefined changes that should take place in these values are selected and these 'profiles' are programmed in as control loops for the fermentation to follow. They may include specified times for media addition, or specified changes in pH as the fermentation proceeds using the elapsed fermentation time as the cue for an action to be taken. All set-points are displayed by numerical settings, controlled by a keyboard or by graphical indicators on a display screen. The controller collects process data and displays it as scrollable graphs that can be printed for traceability and management and accounting procedures (Felezeu 2001).

Fermentations are biological systems that are often non-linear and difficult to model mathematically. More intelligent control is possible with 'fuzzy logic' control or neural network software (Chapter 27, section 27.2.3). Fuzzy logic control is empirically based without any mathematical model. It is equivalent to having the control computer reason as a human from prior experience and acting accordingly to it, but faster. It thus enables the control of systems that would not normally be suitable for automation. It uses imprecise inputs such as 'a little', 'hot' and 'bigger' which are formulated into simple rules. The fuzzy logic controller uses a set of parameters to evaluate these rules and decide what output it should apply to the fermentation process (Park *et al.* 1993). Neural networks can be used to forecast process values in a fermentation on the basis of inputs such as culture volume, pH, DO concentration or substrate feed rate. Before a neural network can be used to control a fermentation, it is 'trained' using historical process data obtained from previous fermentations to produce a parameter file. This is used by the fermenter control program in a network that has modelled the training data. Further information is given by Karim and Rivera (1992) and Linko *et al.* (1997). Neural networks are used in large-scale fermentation systems to aid the control and maximise the product output. For example, Jin *et al.* (1996) describe neural networks and fuzzy logic control of a batch fermentation.

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*Solid substrate fermentations*

Solid substrate fermentation (SSF) involves the growth of micro-organisms (mainly fungi) on moist solid materials that have a large surface area per unit volume (typically $10^3$–$10^6$ $m^2$ $cm^{-3}$) (Raimbault 1998). Traditional SSFs include koji, Indonesian tempeh, sake and Indian ragi. SSF is also used to produce enzymes, (e.g. amylases, amyloglucosidase, cellulases, proteases, pectinases, xylanases and glucoamylases) organic acids, aroma compounds and flavours. Substrates include rice, tubers, wheat, millet, barley, beans, maize, sugar beet pulps and soybeans, and also food-processing residues (e.g. wheat bran and soy flakes remaining after oil extraction). The stages in a SSF are first to prepare a substrate by homogenisation, enzymatic hydrolysis, grinding or flaking, and sometimes heating to soften the material and remove contaminants. It is then inoculated and incubated with the micro-organism. Finally the product is either collected or leached from the fermented solids to obtain the required extracts. Some meat products are fermented in plastic or cellulose casings and SSF is also used to treat food and agricultural wastes. Pérez-Guerra *et al.* (2004) describe the advantages of SSF over submerged culture fermentations as:

- simple reactor design and smaller reactors required;
- similar or higher yields of products;
- low energy requirements (sterilisation, mechanical agitation and aeration are not always necessary);
- relatively simple culture media – the substrate usually provides all nutrients required;
- the low moisture availability may favour production of compounds that cannot be produced in submerged culture;
- smaller volumes of polluting effluents produced.

The main disadvantages when compared to submerged culture are that it is only suitable for micro-organisms that can grow at relatively low moisture contents; fermentation cycle times are longer because high inoculum volumes are required and inoculated spores have a lag time before germination, leading to a risk of contamination by undesirable fungi; and because of the nature of the substrate, it is more difficult to monitor process parameters (e.g. moisture content, oxygen and biomass produced).

For micro-organisms to grow on a solid substrate they should be able to tolerate lower water activities, penetrate the substrate and utilise complex mixtures of different polysaccharides. The hyphae of filamentous fungi grow into solid substrates and secrete enzymes that enable the utilisation of available nutrients at low water activity and high osmotic pressure/nutrient concentration. As a result these are the most commonly used micro-organisms and examples in food processing are shown in Appendix B.4. Bacteria (*Bacillus* spp.) are used for enzyme production, and for producing fermented products such as sausages, Japanese natto and fermented soybean paste. Yeasts are used for tapé production, fermented cassava and rice, ethanol production and protein enrichment of agricultural residues for feeds (Appendix B.4).

The factors that control the fermentation are:

- temperature;
- rates of gas and nutrient diffusion; and
- water activity of the substrate.

The complex chemical composition of most substrates has a buffering effect and pH control is usually not necessary. When it is needed, buffering solutions are added.

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Temperature control

Metabolic activity increases the temperature in SSFs if the rate of heat removal is inadequate, and this directly affects spore germination, growth and product formation. The temperature increase depends on the type of micro-organism, and the porosity, particle size and depth of the substrate bed. This determines the rate at which heat is transferred from the substrate surface to air, and the rate of diffusion of heated air from the substrate. Temperature control is therefore more difficult than in submerged fermentations and relies on mechanical agitation or forced aeration.

Aeration

Aeration has two functions: to supply oxygen for aerobic metabolism and to remove $CO_2$, heat and volatiles produced by cell metabolism. The rate of diffusion depends (in addition to the moisture content of the substrate) on the particle size and porosity of the substrate, the substrate depth, and any forced aeration or agitation that is used.

Moisture content

The water activity of the substrate affects biomass growth, metabolic reactions and mass transfer processes. Depending on the application, the optimum substrate moisture content (usually between 30% and 75%) allows maximum cell growth or production of metabolites (enzymes, organic acids, etc.). Lower values induce sporulation of fungi and higher levels can reduce the porosity of the substrate, limit oxygen transfer and increase the risk of bacterial contamination. Moisture loss during the fermentation is prevented by the use of humidifiers and water-saturated air.

Most solid substrates act as both a support material and a nutrient source. They may be supplemented with nutrients (phosphorus, nitrogen, salts) and the pH and/or moisture content adjusted. Less commonly, an inert support material (e.g. sugar cane, bagasse, hemp, inert fibres, resins, polyurethane foam or vermiculite) is impregnated with a liquid medium that contains the required nutrients. This approach enables better monitoring and control of the process, allows higher rates of oxygen and nutrient diffusion, and produces more reproducible fermentations, but it has higher costs.

SSF bioreactor systems are mostly unsophisticated. Batch processes are the most common and different types of bioreactors include stationary packed beds and trays, rotating drums, fluidised beds (similar to fluidised dryers, Chapter 16, section 16.2.1), stirred aerated beds and rocking drums. Filtration of the inlet and outlet air streams may be used to prevent contamination and the uncontrolled release of the process micro-organism into the environment. In agitated bioreactors, shear forces generated by the mixing can damage fungal hyphae. A more sophisticated bioreactor is described by Pérez-Guerra *et al.* (2004). It consists of a slowly rotating basket that contains a bed of substrate, and it is fitted with slowly rotating helical baffles for mixing the substrate with minimal damage to hyphae. Sterilisation and inoculation are automated. The slow rotation of the basket removes temperature gradients in the substrate bed. Forced aeration with humid air from the base of the basket maintains oxygen levels without evaporative moisture loss. In other designs, microprocessor-controlled chamber bioreactors that have automatic loading, stirring and unloading and capacities of up to 2 tonnes per batch are described by Raimbault (1998).

### 6.4.3 Commercial food fermentations

Throughout the world a very large number of fermented foods are important components of the diet (Appendix B.4). In this section a brief outline is given of selected commercial fermentations and further details are given by Steinkraus (1995, 2002, 2004).

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*Alcohol production*

Beer wort is produced by boiling malted grains (e.g. barley) to release maltose and other sugars, and in some beers by adding hop extract to produce bitterness (Forster *et al.* 1995). Developments in wort production include the use of dextrose syrups to increase product uniformity, and higher-temperature shorter-time boiling to reduce energy consumption. Other substrates including millet, sorghum and maize are also used where these are the staple crops. Variation in the composition of the wort, the strain of yeast, and the fermentation time and conditions result in the wide range of beers produced. These differences are described in detail by Goldammer (2000). After fermentation, beers are filtered or centrifuged (Chapter 5, sections 5.1 and 5.2) and pasteurised (Chapter 12) before pouring into bottles, cans, kegs or bulk containers (Chapter 26, section 26.1).

Sugars in grape juice (or 'must') are fermented to produce 6–14% ethanol in wines. Cells are removed by filtration or centrifugation and the wine is aged to reduce the acidity and to develop a characteristic bouquet. The main acid in most wines is tartaric acid but, in some red wines, malic acid is present in a high concentration. A secondary malo-lactic fermentation by lactic acid bacteria converts malic acid to lactic acid, which reduces the acidity and improves the flavour and aroma. This is reviewed by Moreno-Arribas and Polo (2005). Details of grape wine production are given by Jackson (2000), Ribereau-Gayon *et al.* (2000), Boulton *et al.* (1996) and Fleet (1993). Other wines are produced throughout the world from many fruits, tree saps, honey and vegetable pods. Distillation of wines to produce spirits is described in Chapter 14 (section 14.2).

*Bakery products*

Leavened breads are produced in batch operations by first mixing ingredients in a Z-blade or planetary mixer (Chapter 4, section 4.1.3) to form a dough. This is then fermented in a proving cabinet by *Saccharomyces cerevisiae* to produce carbon dioxide, which leavens the dough and makes it anaerobic. Ethanol is produced in minor amounts, which are evaporated during baking. In sour dough fermentations, lactic acid bacteria and yeasts are used to ferment different cereal and legume mixtures using similar equipment. In a continuous liquid fermentation system for doughs, the growth of yeast and *Lactobacillus* sp. are separated and optimised. Yeast is mixed with flour and water and stored until it is needed. It is then activated by addition of dextrose and added to the dough mixer. Similarly a flour and water mixture is seeded with *Lactobacillus* culture and when the pH has dropped to around 3.8, part of the liquor is pumped to a storage vessel, to be used over several weeks' production. The liquor is pumped to a continuous mixer and as it is used, it is replaced by fresh flour/water to allow the fermentation to continue. The computer-controlled process is claimed to greatly improve fermentation efficiency, reduce labour, floor space, eliminate the need for dough-tubs and a fermentation room, and produce more consistent and hygienic doughs (Anon 1998). Other lactic acid fermentations produce leavened pancakes from a variety of cereals and legumes, including idli in India and injera in Ethiopia (Appendix B.4). In all products baking in an oven (Chapter 18, section 18.2) or on a griddle destroys the micro-organisms. Further details of bakery fermentations are given by Hui *et al.* (2004) and Cauvain and Young (2006).

*Dairy products*

There are a large number of cultured milk products produced throughout the world using lactic acid bacteria. Differences in flavour are due to differences in the concentrations of lactic acid, volatile aldehydes, ketones, organic acids and diacetyl (acetyl methyl carbinol), which give the characteristic 'buttery' aroma to dairy products, that are each

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produced by the fermentation. Changes in texture are due to coagulation of casein micelles to form characteristic flocs when lactic acid bacteria lower the pH to the isoelectric point. The sequence of lactic acid bacteria in a fermentation is determined mainly by their acid tolerance. In milk, *Streptococcus liquifaciens*, *Lactococcus lactis* or *Streptococcus cremoris* are inhibited when the lactic acid content reaches 0.7–1.0%. They are then outgrown by more acid-tolerant species including *Lactobacillus casei* (1.5–2.0% acid) and *Lactobacillus bulgaricus* (2.5–3.0% acid). These changes are described in detail by Fox *et al.* (2000), Hui (1993a–c) and Anon (2008f). Modifications to the type of starter culture, incubation conditions and subsequent processing conditions are used to control the size and texture of the coagulated protein flocs, and hence produce the many different textures encountered in fermented dairy products. Preservation is achieved by increased acidity (yoghurt and cultured milks) or reduced water activity (cheese) and by chilling (Chapter 21).

In yoghurt production, skimmed milk is mixed with dried skimmed milk and pasteurised at 82–93 ºC for 30–60 minutes. It is inoculated with a mixed culture of *S. thermophilus* and *L. bulgaricus* that act synergistically, with *L. bulgaricus* producing most of the lactic acid and also acetaldehyde and diacetyl, to give the characteristic flavour and aroma in yoghurt. Details of production are described by Tamime and Robinson (2007). Developments in biotechnology have produced lactic acid bacteria that also have stabilising and viscosity modifying properties (Mogensen 1991). These are used in a wide variety of fermented milks, dressings and breads to reduce or avoid the use of synthetic stabilisers and emulsifiers. Other lactic acid bacteria, including *Leuconostoc* sp., *Lactobacillus* sp. and *Pediococcus* sp. produce a range of bacteriocins (section 6.7).

More than 400 types of cheese are produced throughout the world, created by differences in bacteria and fermentation conditions, pressing and ripening conditions, and are described in detail by Fox (1999). The fermentation of cottage cheese is stopped once casein precipitation has occurred and the flocs are removed along with some of the whey, but most other cheeses are pressed and allowed to ripen for several weeks or months. In Cheddar cheese manufacture, rennet (section 6.5) is added and the culture is incubated for 1.5–2 h until the curd is firm enough to cut into small cubes. It is then heated to 38 ºC to shrink the curd and expel whey. The curd is recut and drained several times, milled, salted and placed in hoops (press frames). It is pressed to remove air and excess whey, and the cheese is then ripened in a cool room. It is matured for different periods of time, and the flavour gradually changes from a mellow creamy taste after 2–8 months, to a tangy flavour of mature cheese after 8–12 months, and then to a strong, more bitter flavour of vintage cheese after >12 months. The changes are due to enzymes from both the micro-organisms and the cheese (including proteases, peptidases, lipase, decarboxylase and deaminases) that produce compounds with characteristic aromas and flavours. Details of cheese production are given by Mullan (2001), Fox *et al.* (2000), Law (1997) and Banks (1992).

*Mycoprotein*

*Fusarium venenatum* (PTA-2684) is used to make mycoprotein, a meat substitute that is flavoured and textured to resemble chicken or beef and formed into patties, pie ingredients, sausages, 'deli' slices and cutlets. It is marketed as being a healthy food, a good source of protein and fibre, lower in fat and saturated fat than meat equivalents and with no cholesterol. Manufacturers also claim improved taste and texture over soy-based equivalent products (Wilson 2001). It contains dietary fibre in the form of $\beta$-1.3 and -1,6 glucans and chitin (Appendix A.5), which may also act as a prebiotic (section 6.3.3).

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Mycoprotein has been shown to significantly reduce total and LDL cholesterol levels and also reduce glycaemia (Turnbull and Ward 1995). An evaluation of the safety and nutritional value of mycoprotein is reported by Miller and Dwyer (2001).

Production of the fungal mycelia by continuous axenic fermentation is described by Rodger (2001). The fermentation medium is continuously removed from the fermenter and is heated rapidly to kill the mycelia, coagulate cellular proteins and cause the cell RNA to leak through cell walls into the supernatant. This reduces the RNA content of the mycelia from 10% to <2%, which is necessary to prevent the formation of serum uric acid when consumed (RNA is metabolised to uric acid). The biomass is collected as a paste that contains approximately 75% moisture using a dewatering centrifuge (Chapter 5, section 5.1.2).

The mycelial hyphae measure 400–700 $\mu$m by 3–5 $\mu$m in diameter and this high length : diameter ratio means that they are similar in morphology to animal muscle cells. The hyphae are mixed with an egg albumin or milk protein binder, vegetable fat, flavourings and colourants, in a ratio of approximately 90% hyphae : 10% added ingredients, and formed into the required shape. The pieces of mycoprotein are then heated to cause the binder to gel and hold the hyphae together in a similar way that connective tissue holds myofibril cells together in meat. The texture of mycoprotein can be adjusted by varying the amounts of binder and/or hyphae, which influence the firmness, chewiness and fibrousness of the product, or by adjusting the amount of added fat to control juiciness. Other applications of mycoprotein, including use as a fat replacer in dairy products, and in extruded cereals and snacks are under development (Roger 2001).

*Pickling and curing*

Vegetables (e.g. olives, cucumbers) are submerged in 2–6% brine, which inhibits the growth of putrefactive spoilage bacteria. The brine is inoculated with either *L. plantarum* alone or a mixed culture with *P. cerevisiae*. Alternatively a naturally occurring sequence of lactic acid bacteria grows in the anaerobic conditions to produce approximately 1% lactic acid. Nitrogen gas is continuously purged through the vessel to remove carbon dioxide and to prevent cucumbers splitting. Other methods of pickling involve different salt concentrations: for example in 'dry salting' to make sauerkraut or kim-chi, alternate layers of vegetable and granular salt are packed into tanks. Juice is extracted by the salt to form a brine and the fermentation takes place as described above. In each case preservation is achieved by the combination of acid and salt, and, where products are packed into jars, by pasteurisation.

Fermented sausages (e.g. salami, pepperoni and bologna) are produced from a mixture of finely chopped meats, spice mixtures, curing salts (sodium nitrite/nitrate), salt and sugar. The meat is filled into sausage casings, fermented to cause the pH to fall from ≈6.0 to 4.5–4.6, and then pasteurised at 65–68 ºC for 4–8 h, dried and/or smoked and stored at 4–7 ºC. The fermentation causes flavour development and softening of meat tissues due to proteolysis. The technology of production is described in detail by Campbell-Platt and Cook (1995) and Hutkins (2006). Preservation is due to the antimicrobial action of nitrite–spice mixtures and salt; 0.84–1.2% lactic acid from the fermentation; heat during pasteurisation and/or antimicrobial components in smoke when the product is smoked; reduction in water activity due to salt and drying; and low storage temperature (see also hurdle technologies in Chapter 1, section 1.3.1). In Southeast Asia, small fish, shrimp or waste fish are mixed with dry salt and fermented to produce a range of sauces and pastes (Appendix B.4). Proteins in the fish are broken down by the

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combined action of bacterial enzymes, acidic conditions and autolytic action of the natural fish enzymes. Dirar (1993) describes the production of similar fermented fish pastes and fermented mullet in Sudan and North Africa. Other fermented porridges are described by Steinkraus (1995, 2002, 2004).

Soy sauce and similar products are made by a two-stage fermentation in which one or more fungal species are grown on a mixture of ground cereals and soy beans. Fungal proteases, $\alpha$-amylases and invertase act on the soy beans to produce a substrate for the second fermentation stage. The fermenting mixture is transferred to brine and the temperature is slowly increased. Acid production by *P. soyae* lowers the pH to 5.0, and an alcoholic fermentation by *S. rouxii* takes place. Finally the temperature is gradually returned to 15 ºC and the characteristic flavour of soy sauce develops over a period of 6 months to 3 years. The process is described in detail by Fukushima (1985). The liquid fraction is separated, clarified, pasteurised and bottled. The final product has a pH of 4.4–5.4 and is preserved by 2.5% ethanol and 18% salt. In the production of tempeh, soy beans are soaked, deskinned, steamed for 30–120 min and fermented to reduce the pH to 4.0–5.0. Fungal enzyme activity softens the beans, and mycelial growth binds the bean mass to form a solid cake. The fermentation changes the texture and flavour of soy beans but has little preservative effect. The product is either consumed within a few days or preserved by chilling.

### 6.4.4 Effects on foods

The mild conditions used in food fermentations produce few of the deleterious changes to sensory characteristics and nutritional quality that are found with many other unit operations. In fact fermentation can improve food quality indices, including texture, odour, flavour, appearance, nutrition and safety.

*Sensory characteristics*

Fermenting foods are complex systems that have enzymes from ingredients interacting with metabolic activities of fermentation micro-organisms. Factors such as particle sizes, temperature, and DO concentration also have important effects on biochemical changes that occur during the fermentation. Fermentation improves the palatability and acceptability of raw materials by introducing flavour and aroma components and modifying the food texture. Flavour changes are complex (see Voilley 2006) and in general poorly documented for fermented foods. One example is Chaven and Kadam (1989), who describe flavour compounds formed in fermented seasonings and condiments. Flavour changes during fermentation have been reviewed by McFeeters (2004). The aroma of fermented foods is due to a large number of volatile chemical components (e.g. amines, fatty acids, aldehydes, esters and ketones) and products from interactions of these compounds during fermentation and maturation. In bread, coffee and cocoa, the subsequent unit operations of baking and roasting produce the characteristic aromas. The taste and texture of fermented foods are altered by the production of acidulants and include for example a reduction in sweetness and increase in acidity due to fermentation of sugars to organic acids. The acids in turn alter milk proteins to produce the characteristic yoghurt gel (Sodini *et al.* 2004) or cheese flocs. There is an increase in saltiness in some foods (pickles, soy sauce, fish and meat products) due to salt addition and reduction in bitterness of some foods due to the action of debittering enzymes.

The colour of many fermented foods is retained owing to the minimal heat treatment and/or a suitable pH range for pigment stability. Changes in colour may also occur owing

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to formation of brown pigments by proteolytic activity in fermented meats, degradation of chlorophyll and enzymic browning in pickles.

*Nutritional value*

Microbial growth causes complex changes to the nutritive value of fermented foods by changing the composition of proteins, fats and carbohydrates. Some micro-organisms hydrolyse polymeric compounds to produce substrates for cell growth, which may improve the digestibility of proteins and polysaccharides. Fermentation of cereals improves the nutritive value by increasing the amount and quality of proteins (Chavan *et al.* 1988) and available lysine. Micro-organisms also utilise or secrete sugars, vitamins, or essential fatty acids and amino acids, and remove antinutrients, natural toxins and mycotoxins. Changes in the vitamin content of foods vary according to the types of micro-organism and the raw material used, but there is usually an increase in B-vitamins (Chavan and Kadam 1989) and riboflavin and niacin contents may significantly increase (Table 6.3) (Steinkraus 1994). Although in general fermentations do not alter the mineral content of a food, hydrolysis of chelating agents (e.g. phytic acid) during fermentation may improve their bioavailability. The effect of fermentation on toxins and antinutritional components in plant foods is reviewed by Reddy and Pierson (1994). These include reductions in trypsin inhibitor, phytates and flatus producing oligosaccharides. However, fermentation of cereals by *Rhizopus oligosporus* has been reported to release bound trypsin inhibitor, thus increasing its activity (Haard *et al.* 1999).

*Safety*

The safety of fermented foods is reviewed by Nout (1994) and different pathogens that may infect fermented foods are described in Chapter 1 (section 1.2.4). Fungal and lactic acid fermentations reduce aflatoxin $B_1$, and lactic acid bacteria inhibit pathogenic micro-organisms by the production of bacteriocins (section 6.7). Cases of foodborne infection, or intoxication due to microbial metabolites such as mycotoxins, ethyl carbamate and biogenic amines may occur due to contaminated raw materials, lack of pasteurisation and inadequately controlled fermentation conditions (Haard *et al.* 1999).

## 6.5 Microbial enzymes

Advances in biotechnology have had a significant effect on the number and type of new enzymes that are available for use in food processing, especially bakery products, fruit juices, glucose syrups and cheese, or for production of specialist ingredients, which have been reviewed by Pszczola (1999) (GMM enzymes are described in section 6.1.3). There has been rapid growth in the use of enzymes to reduce processing costs; to increase yields of extracts from raw materials; to improve handling of materials; and to improve the shelf-life and sensory characteristics of foods (Appendix B.3, Anon 2004d and Table 6.4). They are separated and purified from microbial cells, or less commonly from animal or plant sources, and are either added to foods as concentrated solutions or powders, or immobilised on support materials in a 'bioreactor'. The main advantages in using enzymes instead of chemical modifications are:

- Enzymic reactions are carried out under mild conditions of temperature and pH, and are highly specific, thus reducing the number of side reactions and by-products.
- Enzymes are active at low concentrations and the rates of reaction are easily controlled by adjustment of incubation conditions.

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**Table 6.3** Nutritional improvement of selected foods by fermentation

| Food | Thiamin ($\mu g\, g^{-1}$) | | Food | Lysine ($mg\, g^{-1}$ N) | | Food | Riboflavin ($mg\, 100\, g^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | Unfermented | Fermented | | Unfermented | Fermented | | Unfermented | Fermented |
| Finger millet | 0.30 | 0.47 | Barley | 19 | 75 | Whole milk | 0.18 | 0.50 (cheese) |
| Maize | 0.37 | 0.86 | Maize | 18 | 46 | Soybean | 0.06 | 0.37 (soy sauce) |
| Pearl millet | 0.37 | 0.64 | Millet | 2 | 37 | | | 0.49 (tempeh) |
| Sorghum | 0.20 | 0.47 | Oats | 18 | 104 | | | |
| Soybean | 0.22 | 0.88 | Rice | 4 | 46 | | | |
| | | (soy sauce) | Wheat | 24 | 65 | | | |

Adapted from Chaven and Kadam (1989), Hamad and Fields (1979) and Paul and Southgate (1991)

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**Table 6.4** Examples of enzymes used in food processing

| Enzyme | Operating conditions | |
|---|---|---|
| | pH range | Temperature (ºC) |
| Microbial sources | | |
| $\alpha$-amylases | 4.0–5.0 | 50–70 |
| Amyloglucosidase | 3.5–5.0 | 55–65 |
| Cellulases | 3.0–5.0 | 20–60 |
| Glucoamylases | 3.5–5.0 | 30–60 |
| Glucose isomerase | 7.0–7.5 | 60–70 |
| Glucose oxidase | 4.5–7.0 | 30–60 |
| Hemi-cellulases | 3.5–6.0 | 30–65 |
| Invertase | 4.5–5.0 | 50–60 |
| Pectic enzymes | 2.5–5.0 | 25–65 |
| Proteases (acid) | 4.5–7.5 | 20–50 |
| Proteases (neutral) | 7.0–8.0 | 20–50 |
| Proteases (alkaline) | 9.0–11.0 | 20–50 |
| Pullanase | 3.5–5.0 | 55–65 |
| Plant sources | | |
| Bromelain (from pineapple) | 4.0–9.0 | 20–65 |
| Ficin (from fig) | 6.5–7.0 | 25–60 |
| Papain (from papaya) | 6.0–8.0 | 20–75 |
| Animal sources | | |
| Catalase (from beef liver) | 6.5–7.5 | 5–45 |
| Lipase (from porcine pancreas) | 5.5–9.5 | 20–50 |
| Rennet (from calf stomach) | 3.5–6.0 | 40 |

- There is minimal loss of nutritional quality at the moderate temperatures employed.
- Lower energy consumption than corresponding chemical reactions.
- They enable the production of new foods, not achievable by other methods.

The use of enzymes in food analysis is also rapidly expanding and is discussed in detail by Pomeranz and Meloan (2000) and Schwedt and Stein (1994).

However, the cost of many enzymes is high and, in some products enzymes must be inactivated or removed after processing, which adds to the cost of the product. Like other proteins, enzymes may cause allergic responses in some people, and they may be microencapsulated or immobilised on carrier materials to reduce the risk of inhalation of enzyme dust by operators. Selection of the precise enzyme for a particular application can be difficult and guidelines on methods to do this are given by West (1988). Details of the factors that influence enzyme activity and reaction rates are described in standard biochemistry texts and by Whitehurst and Law (2002).

Microbial enzymes have optimum activity under similar conditions to those that permit optimum cell growth. They are either secreted by the cells into the surrounding medium ('extracellular' production) or retained within the cell ('intracellular' enzymes). Extracellular enzyme production occurs in either the logarithmic phase or the stationary phase of growth, whereas intracellular enzymes are produced during logarithmic growth but are released into the medium only when cells undergo lysis in the stationary or decline phases. The requirements of commercial enzyme production from micro-organisms are as follows:

- Micro-organisms must grow well on an inexpensive substrate.
- Substrates should be readily available in adequate quantities, with a uniform quality.
- Micro-organisms should produce a constant high yield of enzyme in a short time.

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- Methods for enzyme recovery should be simple and inexpensive.
- The enzyme preparation should be stable.

Enzymes are produced by either solid substrate fermentations (e.g. rice hulls, fruit peels, soybean meal or wheat flour) or by submerged culture using liquid substrates in fermenters (e.g. molasses, starch hydrolysate or corn steep liquor (section 6.4.2)). Submerged cultures have lower handling costs and a lower risk of contamination and are more suited to automation than are solid substrates. The success of commercial enzyme production depends on maximising the activity of the micro-organism and minimising the costs of the substrate and incubation and recovery procedures. Extracellular enzymes are recovered from the fermentation medium by centrifugation, filtration (Chapter 5, sections 5.1 and 5.2), fractional precipitation, chromatographic separation, electrophoresis, membrane separation (Chapter 5, section 5.5), freeze drying (Chapter 23, section 23.1) or a combination of these methods. Intracellular enzymes are extracted by disruption of cells in a homogeniser or mill (Chapter 3). Recovery is more difficult and the yield is lower than for extracellular enzymes, because some enzymes are retained within the cell mass. If required, the specific activity of the enzyme is increased by precipitation using acetone, alcohols or ammonium sulphate or by ultrafiltration (Chapter 5, section 5.5.3).

In batch operation the enzyme is mixed with food, allowed to catalyse the required reaction, and then either retained within the food or inactivated by heat. This is used when the cost of the enzyme is low. In continuous operation, immobilised enzymes are either mixed with a liquid substrate and removed by centrifugation or filtration and re-used, or the feed liquor is passed over an immobilised bed of enzyme in a bioreactor. Different methods include the following:

- Micro-encapsulation in membranes that retain the enzyme but permit passage of substrates and products.
- Electrostatic attachment to ion exchange resins.
- Adsorption onto colloidal silica, charcoal, polyacrylamide or glass, and/or cross linking with glutaraldehyde. Chitin and its derivatives are suitable as supports in immobilised enzyme reactors, for example by coating magnetic beads that are easily removed from the reactor (Synowiecki and Al-Khateeb 2003).
- Entrapment in polymer fibres (e.g. cellulose triactetate or starches).
- Covalent bonding to organic polymers or co-polymerisation with maleic anhydride.

Immobilised enzymes should have the following characteristics: short residence times for a reaction; stability to variations in temperature and other operating conditions over a period of time (e.g. glucose isomerase is used for ≈1000 h at 60–65 ºC) and suitability for regeneration. The main advantages of enzyme immobilisation are that enzymes are re-used, so reducing costs, and they allow continuous processing and closer control of pH and temperature to achieve optimum activity. Immobilisation is used when an enzyme is difficult to isolate or expensive to prepare. The main limitations are increased costs of carriers, equipment and process control, changes to the reaction kinetics of enzymes, loss of activity and risk of microbial contamination. A summary of the food applications of the main enzyme groups is shown in Appendix B.3 and information on enzymes on the Internet has been compiled by Swadling (2001).

### 6.5.1 Novel enzyme technologies

Extreme thermophilic and hyperthermophilic micro-organisms (also ‘extremophiles’) of the *Pyrococcus* spp. and *Thermococcus* spp. produce enzymes that have optimum activity

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between 80 and 100 ºC and are more resistant than mesophilic equivalents to environmental conditions such as low or high pH. Their reactions can be terminated by simply lowering the temperature and the lower viscosity of concentrated substrates at high temperatures allows improved mixing and pumping. However, commercial applications may be limited because these micro-organisms produce enzymes in amounts that are insufficient for large-scale enzyme production, and they may also produce toxic or corrosive metabolites. Synowiecki *et al.* (2006) describe studies of recombinant $\alpha$-amylase from *Pyrococcus woesei* and a thermostable $\alpha$-glucosidase from *Thermococcus thermophilus* that were expressed in an *E. coli* host. $\alpha$-amylase had optimum activity at pH 5.6 and 93 ºC and the $\alpha$-glucosidase retained 80% of its activity between pH 5.8 and 6.9. The authors concluded that these enzymes were suitable for starch processing. They also reported a recombinant $\beta$-galactosidase from *P. woesei* that was active in the pH range 4.3–6.6 with high thermostability. It would be suitable for the production of low-lactose milk or whey at temperatures that restrict microbial growth in continuous-flow immobilised enzyme reactors. Details of the applications of novel enzyme technologies are described by Rastall (2007).

## 6.6 Microencapsulation and controlled release technologies

Microencapsulation (also Chapter 24, section 24.3) and liposomes have been used to protect enzymes from adverse environmental conditions during processing (e.g. heat or pH) and enable their delayed or controlled activity within the processed food. Liposomes are spherical vesicles, varying in size from <30 to >300 nm, which have one or more bilayer membranes of polar phospholipids. The two layers are formed by the hydrophobic ends of the lipids being oriented towards each other, thus presenting hydrophilic ends of the molecules to both the inside and outside of the vesicle (Fig. 6.5). Liposomes were originally developed for the pharmaceutical industry but have found applications in food processing for the controlled delivery of functional ingredients, including proteins, enzymes and flavours. These applications are reviewed by Gibbs *et al.* (1999). They are

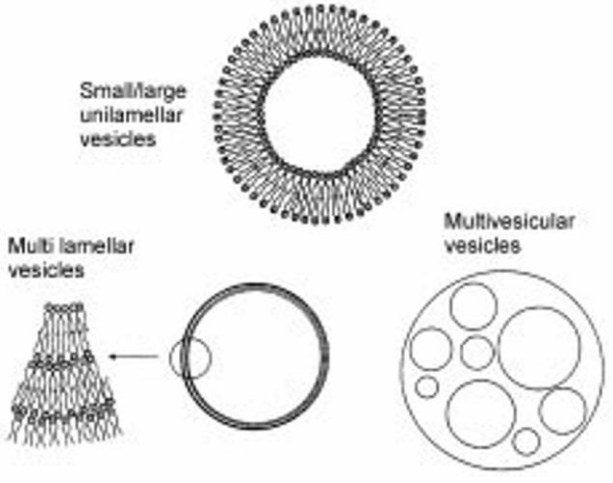


**Fig. 6.5** Structure of liposome vesicle (reprinted by permission of the publisher, Taylor and Francis Ltd (http://www.tandf.co.uk/journals) (from Taylor *et al.* 2005).

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produced by high-intensity ultrasound, high-pressure homogenisation or membrane homogenisation (Chapter 3, section 3.2.3). The functionality of liposomes depends on their interfacial properties, especially the surface charge, the phospholipid concentration, the concentration of entrapped material, as well as the pH, ionic strength and temperature of the medium in which they are contained. Details of the structure and properties of different types of liposomes are reviewed by Taylor *et al.* (2005).

Examples of enzymes that have been microencapsulated or entrapped in liposomes include proteinases that accelerate cheese ripening and increase levels of flavour compounds such as diacetyl, acetoin and methyl ketones, and lipases that reduce the firmness of cheese while increasing the elasticity and cohesiveness and improving the flavour profile (Kheadr *et al.* 2002). The microcapsules or liposomes protect the enzymes from the low pH and high ionic strength in the cheese (el Soda *et al.* 1989). Liposome-entrapped $\beta$-galactosidase has been added to dairy products to aid digestion by lactose-intolerant consumers (Rao *et al.* 1995). Other studies indicate that entrapment of enzymes, including $\alpha$-amylase and glucose oxidase, can restrict their access to substrate and delay their activity, and also protect them from adverse environmental conditions of pH and temperature. Research using liposome-entrapped vitamins has also shown that there is potential to protect these nutrients against degradation and develop new methods for fortifying foods (Taylor *et al.* 2005).

## 6.7 Bacteriocins and antimicrobial ingredients

Bacteriocins are biologically active peptides that are produced by some stains of lactic acid bacteria and which inhibit the growth of spoilage or pathogenic bacteria. An example is nisin, produced by *Lactococcus lactis* in European cheeses, which prevents growth of *Clostridium tyrobutyricum* and thus prevents off-flavour development and 'blowing' of Swiss-type cheese during ripening. Nisin is also effective against *Listeria monocytogenes* and, although it has been added to cultures in the past, its production by *Lactococcus lactis* is a cheaper and more effective method of removing this pathogen from cheese. *L. lactis* has also been used to inhibit the growth of *Cl. botulinum* in processed cheese and other dairy products, processed vegetables, soups, sauces and beer (Roller *et al.* 1991). Liposomes containing entrapped nisin have been found to withstand the temperatures used in cheese making and remain active against *L. monocytogenes* during storage (Were *et al.* 2003, 2004). The advantage of using bacteriocins such as nisin is the reduction or avoidance of chemical preservatives such as nitrate, sorbic acid and benzoic acid (Mogensen 1991). Other applications of nisin have been reviewed by de Vuyst and Vandamme (1994) and other inhibitory metabolites of lactic acid bacteria are described by Breidt and Fleming (1997) in their application to minimally processed fruits and vegetables. Roller (2003) describes the use of natural antimicrobial chemicals for minimal processing. The use of antimicrobial chemicals to improve the safety of fresh foods is reviewed by Cherry (1999). The action of pediocin PA-1 and its applications in food systems are reviewed by Rodriguez *et al.* (2002) and the use of 'Microgard', reuterin and lactoferrin are described by Mahapatra *et al.* (2005). *Pediococcus acidilactici*, when used in fermented meat, also has the potential to inhibit spoilage bacteria and thus reduce the need for nitrate addition.

*Chitin and chitosan*

After cellulose, chitin is the most abundant natural polysaccharide, synthesised by insects, crustaceans, molluscs, algae, fungi and yeasts. Chitosans (deacetylated chitin)

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inhibit phyto-pathogenic fungi and bacteria and induce natural resistance in plants to fungal or viral infections (Pospieszny and Maćkowiak (1997)). Microcrystalline chitosan and its salts also show strong antiviral activity (Synowiecki and Al-Khateeb 2003). Sulphobenzoyl chitin prevents the growth of *Pseudomonas* spp., *Salmonella* spp., *Aeromonas* spp. and *Vibrio* spp. (Chapter 1, section 1.2.4). Treatment of potatoes with chitosan solution protects against pathogens such as those causing soft rot by inactivating polygalacturonase, pectate lyase and pectin methylesterase that are secreted by the pathogens. Chitosan with added calcium ions creates semipermeable membranes on the surface of treated fruits, tomatoes and cucumbers, which alters the rate of oxygen and carbon dioxide permeation and significantly extends the shelf-life (Li and Chung 1997). Chitosan coatings and coated packaging paper also inhibit microbial growth (also Chapter 24, section 24.3, and Chapter 25, section 25.4.1). Wastes from the processing of crabs, lobsters, shrimps, oysters and clams are a rich source of chitin. Chitosan is produced by deacetylation of chitin using concentrated NaOH solution, but this produces variable degrees of deacetylation and loss of nutritionally valuable proteins. Synowiecki and Al-Khateeb (2003) report mild enzymic methods that can deproteinise shrimp shells and deacetylate chitin, and Tharanathan and Kittur (2003) have reviewed the food applications of chitin.

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