Fermented foods are among the oldest processed foods and have formed a traditional part of the diet in almost all countries for millennia. Today they continue to form major sectors of the food processing industry, including baked products, alcoholic drinks, yoghurt, cheese and soy products among many others. 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 which increase the quality and value of raw materials. Today the preservative effect is supplemented by other unit operations (for example pasteurisation, chilling or modified atmosphere packaging (Chapters 11, 19 and 20). The main advantages of fermentation as a method of food processing are:

- the use of mild conditions of pH and temperature which maintain (and often improve) the nutritional properties and sensory characteristics of the food
- the production of foods which have flavours or textures that cannot be achieved by other methods
- low energy consumption due to the mild operating conditions
- relatively low capital and operating costs
- relatively simple technologies.

A more recent development is the separation and purification of enzymes from microbial cells, or from animal or plant sources for use in food processing. The enzymes are either added to foods as concentrated solutions or powders, or immobilised on support materials in a ‘reactor’ where they are re-used for extended periods. They are used to bring about specific reactions under mild conditions of temperature and pH and have found very wide applications in the food industry, for example, in the production of bakery products, fruit juices, glucose syrups and cheese.

The main advantages of technical enzymes are:

- they cause highly specific and controlled changes to foods
- there is minimal loss of nutritional quality at the moderate temperatures employed
- lower energy consumption than corresponding chemical reactions
the production of new foods, not achievable by other methods.

In this chapter, commercially important food fermentations and technical enzymes are described. The use of enzymes in food analysis is rapidly expanding and is discussed in detail by Guilbault (1984) and Allen (1990). The effects of naturally occurring enzymes on food quality are discussed in other chapters where their action relates specifically to the unit operation under consideration.

7.1 Fermentation
7.1.1 Theory
The main factors that control the growth and activity of micro-organisms in food fermentations are:

- availability of carbon and nitrogen sources, and any specific nutrients required by individual micro-organisms
- substrate pH
- moisture content
- incubation temperature
- redox potential
- stage of growth of micro-organisms
- presence of other competing micro-organisms.

These factors are discussed in greater detail in microbiological texts (for example Jay (1978) and Stanbury and Whitaker (1984)).

Batch culture
In batch culture the growth of micro-organisms can be described by a number of phases (Fig. 7.1). Cell growth during the logarithmic (or exponential) phase is at a constant rate which is shown by:

\[
\ln C_b = \ln c_0 + \mu t
\]

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 (Fig. 7.1).

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 (AB in Fig. 7.2). This indicates substrate limitation of cell growth, which is described by:

\[
c_b = Y(S_0 - S_r)
\]

where \(c_b\) = concentration of biomass, \(Y\) (dimensionless group – Appendix D) = 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. 7.2 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:

\[
\mu = \frac{\mu_{\text{max}} S_t}{(K_s + S_t)}
\]

where \(\mu_{\text{max}} (\text{h}^{-1})\) = maximum specific growth rate, \(K_s (\text{mg l}^{-1})\) = 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 \(K_s\)).

The rate of production of primary metabolic products (for example ethanol, amino acids and citric acid) is determined by the rate of cell growth, and is found using:

\[
q_p = Y_{p/s} \mu
\]

where \(q_p\) = specific rate of product formation, and \(Y_{p/s}\) = yield of product related to amount of substrate consumed.

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 (for example aromatic compounds and fatty acids)), which are produced

Fig. 7.1 Phases in the growth of micro-organisms.

Fig. 7.2 Effect of initial substrate concentration on cell concentration at the end of the logarithmic phase of growth.

(After Stanbury and Whitaker (1984).)
in the stationary growth phase, does not vary in this way and may remain constant or change in more complex ways.

The productivity of a culture is the amount of biomass produced in unit time (usually per hour) and is found using:

$$P_b = \frac{(c_{\text{max}} - c_0)}{t_1 - t_2}$$  \hspace{1cm} (7.5)

where $P_b$ (gl$^{-1}$ h$^{-1}$) = productivity, $c_{\text{max}}$ = 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.

### Sample problem 7.1

An inoculum containing $3.0 \times 10^4$ cells ml$^{-1}$ of *Saccharomyces cerevisiae* is grown on glucose in a batch culture for 20 h. Cell concentrations are measured at 4 h intervals and the results are plotted in Fig. 7.1. The total time taken for culture preparation and harvest is 1.5 h. Calculate the maximum specific growth rate and the productivity of the culture.

### Solution to Sample problem 7.1

From equation (7.1) for the logarithmic phase,

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

Therefore,

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

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

From equation (7.5),

$$P_b = \frac{2 \times 10^8 - 3 \times 10^4}{8.5 + [(20 - 8.5) + 1.5]}$$

$$= 9.3 \times 10^6 \text{ cells h}^{-1}$$

### Continuous culture

Cultures in which cell growth is limited by the 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:

$$D = \frac{F}{V}$$  \hspace{1cm} (7.6)

where $D$ (h$^{-1}$) = dilution rate, $F$ (l h$^{-1}$) = substrate flow rate and $V$ (l) = volume of the fermenter.
The steady-state cell concentration and residual substrate concentration respectively are found using:

\[
\bar{c} = Y(S_0 - \bar{S})
\]

\[
\bar{S} = \frac{K_s D}{\mu_{\text{max}} - d}
\]

where \(\bar{c}\) = steady-state cell concentration, \(Y\) = yield factor, \(\bar{S}\) = 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 \(\mu_{\text{max}}\) and is influenced by the substrate utilisation constant and yield factor (Fig. 7.3).

The productivity of a continuous culture is found using:

\[
P_c = D\bar{c} \left(1 - \frac{t_3}{t_4}\right)
\]

where \(P_c\) = productivity of continuous culture, \(t_3\) (h) = time before steady-state conditions are established, \(t_4\) (h) = duration of steady-state conditions.

Further details of the above equations are given by Frazier and Westhoff (1978), Stanbury and Whitaker (1984), Jay (1978) and other microbiological texts.

### 7.1.2 Types of food fermentations

Micro-organisms that produce a single main by-product are termed *homofermentative* whereas those that produce mixed products are *heterofermentative*. Fermentations can be classified into those in which the main products are organic acids and those in which ethanol and carbon dioxide are the primary products. Lactic acid and ethanolic fermentations are among the most important commercial fermentations and details of the metabolic pathways that are used to produce these products are readily available (for example Stanier *et al.*, 1976). Many fermentations involve complex mixtures of micro-organisms or sequences of microbial populations which develop as changes take place in the pH, redox potential or substrate availability. These are described below.
Lactic acid fermentations

A selection of common lactic acid fermentations is shown in Table 7.1. The sequence of lactic acid bacteria in a fermentation is determined mainly by their acid tolerance. For example in milk, *Streptococcus liquefaciens*, *Lactococcus* (formerly *Streptococcus lactis*) or the closely related *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). Similarly, in vegetable fermentations, *Lactobacilli* spp. are stronger acid producers than *Streptococci* spp. Of the four main groups of lactic acid bacteria, *Streptococcus* spp. and *Pediococcus* spp. are homolactic, *Leuconostoc* spp. are heterolactic and *Lactobacillus* spp. vary according to the strain.

In some fermentations, particularly those that involve low-acid substrates (for example milk and meat), a starter culture is added to rapidly generate large numbers of the desired

---

**Sample problem 7.2**

Brewers’ yeast is grown continuously in a fermenter with an operating volume of 12 m³. The residence time is 20 h and the yeast has a doubling time of 3.2 h. A 2% inoculum, which contains 5% yeast cells is mixed with the substrate. Calculate the mass of yeast harvested from the fermenter per hour. (Assume that the density of the broth is 1010 kg m⁻³.)

**Solution to Sample problem 7.2**

\[
\text{Flow-rate} = \frac{\text{volume of fermenter}}{\text{residence time}}
\]

\[
= \frac{12}{20}
\]

\[
= 0.6 \text{ m}^3 \text{ h}^{-1}
\]

**Mass flow rate**

\[
= 0.6 \times 1010
\]

\[
= 606 \text{ kg h}^{-1}
\]

**Initial 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 \(\frac{20}{3.2} = 6.25\) 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,

\[
\text{mass of product} = \text{initial concentration} \times \text{growth} \times \text{mass flow-rate}
\]

\[
= 0.001 \times 76 \times 606
\]

\[
= 46 \text{ kg h}^{-1}
\]
micro-organism, and thus reduce fermentation times and inhibit growth of pathogens and spoilage bacteria. In other fermentations, the natural flora are sufficient to reduce the pH rapidly and to prevent the growth of undesirable micro-organisms.

Developments in biotechnology have produced lactic acid bacteria that also have stabilising and viscosity-forming 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* spp., *Lactobacillus* spp. and *Pediococcus* spp. produce a range of bacteriocins. An example is *Pediococcus acidilactici*, which when used in fermented meat, has the potential to inhibit spoilage bacteria and thus reduce the need for nitrate addition. A similar benefit has been found in the production of European cheeses using starter cultures of *Lactococcus lactis* which produces the bacteriocin, nisin. This prevents growth of *Clostridium tyrobutyricum*. 

<table>
<thead>
<tr>
<th>Food</th>
<th>Micro-organisms</th>
<th>Incubation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassava</td>
<td><em>Corynebacterium</em> species</td>
<td>Ambient 96 h</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Geotrichum</em> species</td>
<td>Ambient 3–12 months</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Bacillus pumilus</em></td>
<td>Fish to salt ratio between 3 to 1 and 5 to 1</td>
</tr>
<tr>
<td>Maize</td>
<td><em>Corynebacterium</em> species</td>
<td>Ambient 24–72 h</td>
</tr>
<tr>
<td>Maize</td>
<td><em>Aerobacter</em> species</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td><em>S. cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td><em>Lactobacillus</em> species</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td><em>Candida mycoderma</em></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td><em>Pediococcus</em> species</td>
<td>15–26 24 h 85–90% relative humidity</td>
</tr>
<tr>
<td>Meat</td>
<td><em>cervisiae</em></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td><em>Lactobacillus</em> species</td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td><em>curvatus</em></td>
<td></td>
</tr>
<tr>
<td>Milk (stirred yoghurt)</td>
<td><em>Streptococcus</em> species</td>
<td>40–45 2–3 h</td>
</tr>
<tr>
<td>Cheese</td>
<td><em>thermophilus</em></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>L. <em>bulgaricus</em></td>
<td></td>
</tr>
<tr>
<td>Cottage</td>
<td><em>Streptococcus</em></td>
<td>22 14–16</td>
</tr>
<tr>
<td>Cottage</td>
<td><em>diacetylactis</em></td>
<td></td>
</tr>
<tr>
<td>Camembert and Brie</td>
<td><em>S. cremoris</em></td>
<td>32 b</td>
</tr>
<tr>
<td>Camembert and Brie</td>
<td><em>S. lactis</em></td>
<td></td>
</tr>
<tr>
<td>Camembert and Brie</td>
<td><em>Penicillium</em></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td><em>caseicolum</em></td>
<td>32 b</td>
</tr>
<tr>
<td>Cheddar</td>
<td><em>S. cremoris</em></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td><em>S. lactis</em></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td><em>S. diacetylactis</em></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td>Lactobacilli</td>
<td></td>
</tr>
<tr>
<td>Vegetables (cucumber and cabbage)</td>
<td><em>Lactobacillus</em> sources</td>
<td>48–260 h 2.5–6% salt</td>
</tr>
<tr>
<td>Vegetables (cucumber and cabbage)</td>
<td><em>mesenteroides</em></td>
<td></td>
</tr>
<tr>
<td>Vegetables (cucumber and cabbage)</td>
<td><em>L. brevis</em></td>
<td></td>
</tr>
<tr>
<td>Vegetables (cucumber and cabbage)</td>
<td><em>P. cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td>Vegetables (cucumber and cabbage)</td>
<td><em>L. plantarum</em></td>
<td></td>
</tr>
</tbody>
</table>

* Prepared inocula used.

* Fermentation of cheeses continues for 1–12 months during ripening.

1. Naturally produced peptides that inhibit other micro-organisms, similar in effect to antibiotics.
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and thus prevents off-flavour development and ‘blowing’ of Swiss-type cheese during ripening. Nisin is 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 potentially dangerous food poisoning micro-organism from cheese. *Lactococcus lactis* is permitted for use in more than 45 countries and has also found application to inhibit the growth of *Cl. botulinum* in processed cheese, other dairy products, processed vegetables, soups, sauces and beer (Roller *et al.*, 1991). Natural production of nisin may also be used to reduce or avoid chemical preservatives such as nitrate, sorbic acid and benzoic acid (Mogensen, 1991) and to control the quality of wines (Daeschel *et al.*, 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.

**Meat and fish products**

Pieces of meat are fermented by *Bacillus* spp. and *Staphylococcus* spp. and dried in many parts of Africa as traditional foods. The fermentation causes flavour development and softening due to proteolysis, and preservation is by drying. Dirar (1993a) also describes a fermented fat in Sudan which he notes as being ‘possibly the most foul-smelling fermented food in the country, second only to sigda’ (presscake fermented after sesame-seed oil extraction).

Fermented sausages (for example salami, pepperoni, medwurst 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 and then pasteurised at 65–68°C for 4–8 h, dried and stored at 4–7°C. The technology of production is described in detail by Pederson (1971).

Preservation is due to:

- the antimicrobial action of nitrite-spice mixtures and to a lesser extent from added salt
- 0.84–1.2% lactic acid from the fermentation
- heat during pasteurisation and/or smoking (and antimicrobial components in smoke when the product is smoked)
- reduction in water activity due to salt and drying
- low storage temperature.

In Southeast Asia, small fish, shrimp or waste fish are mixed with dry salt and fermented by bacteria including *L. mesenteroides*, *P. cerevisiae* and *L. plantarum* to produce a range of sauces and pastes. Proteins in the fish are broken down by the combined action of bacterial enzymes, acidic conditions and autolytic action of the natural fish enzymes. Dirar (1993a) describes the production of similar fermented fish pastes and fermented mullet in Sudan and North Africa.

**Vegetables**

Cucumbers, olives and other vegetables are submerged in 2–6% w/w brine, which inhibits the growth of putrefactive spoilage bacteria. Air is excluded and a naturally occurring sequence of lactic acid bacteria grow in the anaerobic conditions to produce approximately 1% w/w lactic acid. The relative importance of each species depends on the initial cell numbers on the vegetable, the salt content and the pH (Fleming, 1982). In some countries, the fermentation of cucumbers is controlled by the addition of acetic acid to prevent growth of spoilage micro-organisms. The brine is then inoculated with either *L. plantarum* alone or a mixed culture with *P. cerevisiae*. Nitrogen gas is continuously
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purged through the vessel to remove carbon dioxide and to prevent splitting of the cucumbers.

Other methods of pickling involve different salt concentrations: for example in ‘dry salting’ to make sauerkraut from cabbage, alternate layers of vegetable and granular salt are packed into tanks. Juice is extracted from leaves by the salt to form a brine, and the fermentation follows a similar sequence to that described for cucumber pickles (Pederson, 1971). In each case preservation is achieved by the combination of acid, salt and in some cases pasteurisation.

Maize, cassava and sorghum

In tropical countries, cereals and root crops are fermented to a range of beverages and staple foods. These are reviewed by Odunfa (1985) and Stanton (1985). Fermented maize flour is a staple food in many African countries. Maize kernels are soaked for 1–3 days, milled and formed into a dough. Initially Corynebacterium spp. hydrolyse starch and initiate lactic acid production. Aerobacter spp. increase the rate of acid production and S. cerevisiae contributes to the flavour of the product. As the acidity increases, Lactobacillus spp. predominate and continue acid production. Finally Candida mycoderma outgrows S. cerevisiae and contributes to the final flavour of the fermented dough. It is cooked to form a thick porridge within 1–2 days. The fermentation is therefore used to impart flavour and have a temporary preservative effect.

Cassava is grated and the pressed pulp is fermented by Corynebacterium spp., as for maize, to produce lactic and formic acids and to reduce the pH from 5.9 to 4.0. The increased acidity promotes the growth of Geotrichum spp., and detoxifies the cassava by releasing gaseous hydrogen cyanide by hydrolysis of the cyanogenic glycosides present in the cassava. Aldehydes and esters produced by Geotrichum spp. give the characteristic aroma and taste to the product. The fermented cassava is dried to a granular flour with a shelf life of several months. The fermentation therefore alters the eating quality, and preservation is achieved by drying. Details are given by Abe and Lindsay (1979) and Akinrele (1964). The detailed production of porridges, dried granules, flakes and breads from sorghum, millet and cassava is described by Dirar (1993b).

Milk products

There are a large number of cultured milk products produced throughout the world (for example yoghurt, cheese, Kefir, Koumiss, buttermilk, sour cream and Leben). Differences in flavour are due to differences in the concentration of lactic acid, volatile aldehydes, ketones, organic acids and diacetyl (acetyl methyl carbinol). The last is produced by fermentation of citrate in milk, and gives the characteristic ‘buttery’ aroma to dairy products. Changes in texture are due to lactic acid, which causes a reduction in electrical charge on the casein micelles. They coagulate at the isoelectric point to form characteristic flocs. These changes are described in detail by Fox (1987) and Schmidt (1992). Modifications to the 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. Preservation is achieved by chilling and increased acidity (yoghurt and cultured milks) or reduced water activity (cheese).

Yoghurt

In mechanised production, skimmed milk is mixed with dried skimmed milk and heated at 82–93°C for 30–60 min to destroy contaminating micro-organisms and to destabilise
K-casein. It is inoculated with a mixed culture of initially *S. thermophilus* and *L. bulgaricus*. Initially *S. thermophilus* grows rapidly to produce diacetyl and lactic, acetic, and formic acids. *L. bulgaricus* possesses weak protease activity which releases peptides from the milk proteins. These stimulate the growth of *S. thermophilus*. The increased acidity then slows the growth of *S. thermophilus* and promotes *L. bulgaricus*, which is stimulated by formate produced in the initial stage. *L. bulgaricus* produces most of the lactic acid and also acetaldehyde which, together with diacetyl, gives the characteristic flavour and aroma in yoghurt. Details of the production are described by Davis (1975) and Tamime and Robinson (1999).

**Cheese**

More than 400 types of cheese are produced throughout the world, created by differences in fermentation, pressing and ripening conditions, described in detail by Kosikowski (1978) and Campbell-Platt (1987). 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 the manufacture of cheddar cheese, *S. lactis* is added to milk and fermented for 30 min. Rennet (Section 7.2.2) 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°C to shrink the curd and to 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 for several months. Enzymes from both the micro-organisms and the cheese (including proteases, peptidases, lipase, decarboxylase and deaminases) produce compounds which give characteristic aroma and flavour. The time and temperature of ripening determine whether the cheddar has a mild, medium or strong flavour. Details of the production of cheese are given by Fox (1993) and Banks (1992).

**Alcoholic and mixed alcohol–acid fermentations**

Table 7.2 describes the conditions used in selected ethanolic and mixed acid–ethanol fermentations.

**Bread**

The fermentation and baking of cereal flours alter the texture and flavour of the flour and make it palatable as a staple food. Fermentation has no preservative effect and the main function is to produce carbon dioxide to leaven and condition the dough. Yeast and other micro-organisms (e.g. *Lactobacillus* spp.) present in the dough also contribute to the flavour of the bread. Carbon dioxide is retained within the loaf when the gluten structure is set by heat above 74°C. The heat treatment and reduction in water activity preserve the bread. Details of production and different types of bread are described by Matz (1972).

The two main commercial methods of dough preparation are the bulk fermentation process and the Chorleywood bread process, which are described in detail by Chamberlain *et al.* (1965) and Oura *et al.* (1982). A more recent development is a continuous liquid fermentation system for doughs (Fig. 7.4). Here, the growth of yeast and *Lactobacillus* spp. 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, 10% of the liquor is pumped to a storage vessel, ready for up to several weeks for use in the mixer. As it is used, it is replaced by fresh flour/water to allow the fermentation to continue. The computer-controlled process
### Table 7.2 Alcohol and mixed alcohol–acid fermentations

<table>
<thead>
<tr>
<th>Food</th>
<th>Micro-organisms</th>
<th>Incubation conditions</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ale</td>
<td><em>S. cerevisiae</em>&lt;sup&gt;a&lt;/sup&gt; ('top-yeast')</td>
<td></td>
<td>20</td>
<td>120–240 h</td>
<td></td>
</tr>
<tr>
<td>Lager</td>
<td><em>Saccharomyces carlsbergensis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>12–15</td>
<td>120–240 h</td>
<td></td>
</tr>
<tr>
<td>Millet</td>
<td><em>Saccharomycopsis fibuliger</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread</td>
<td><em>S. cerevisiae</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>26</td>
<td>0.5–1 h</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agave (cactus)</td>
<td><em>Saccharomyces carabidai</em></td>
<td></td>
<td>30</td>
<td>200 h</td>
<td></td>
</tr>
<tr>
<td>Bordeaux</td>
<td><em>Saccharomyces oviformis</em></td>
<td></td>
<td>25</td>
<td>360 h</td>
<td></td>
</tr>
<tr>
<td>Other grape</td>
<td><em>S. cerevisiae</em> var. <em>ellipoideus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm</td>
<td><em>Zymomonas</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td><em>Saccharomycysis sake</em></td>
<td></td>
<td>30</td>
<td>4–12 h</td>
<td>–</td>
</tr>
<tr>
<td>Mixed alcohol–acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td><em>Leuconostoc</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Bacillus</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Erwinia</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fusarium</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoa</td>
<td>Yeasts (see text)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. plantarum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus mali</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus fermentum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus collinoides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acetobacter rancens</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acetobacter aceti</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acetobacter oxydans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy sauce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First stage</td>
<td><em>Aspergillus sojae</em></td>
<td></td>
<td>30</td>
<td>48–72 h</td>
<td></td>
</tr>
<tr>
<td>Second stage</td>
<td><em>Mucor</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rhizopus</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pediococcus sojae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Saccharomyces rouxii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temppeh</td>
<td><em>Rhizopus oligosporus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>15–25</td>
<td>3–6 months</td>
<td>15–20% brine</td>
</tr>
<tr>
<td>Vinegar</td>
<td><em>S. cerevisiae</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>15–25</td>
<td>3–6 months</td>
<td>15–20% brine</td>
</tr>
<tr>
<td></td>
<td><em>A. aceti</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>25</td>
<td>72–168 h</td>
<td>First stage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72–120 h</td>
<td>Second stage</td>
</tr>
<tr>
<td>Citric acid</td>
<td><em>Acetobacter niger</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>27</td>
<td>168 h</td>
<td>Substrate limited</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inoculum used.
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.

**Alcoholic beverages**

Beer wort is produced by boiling malted grains (for example barley) to release maltose and other sugars and, in some beers, by adding hop flowers to produce bitterness. Developments in wort preparation, described by Atkinson (1987) and Hudson (1986), include the use of hop extracts and dextrose syrups to increase product uniformity, and higher-temperature shorter-time boiling to reduce energy consumption. Variation in the composition of the wort, the strain of yeast (*S. cerevisiae, S. carlsbergensis*), and the fermentation time and conditions, result in the wide range of beers produced. Other substrates including millet, sorghum and maize are also used where these are the staple crops.

Sugars present in grape juice (or ‘must’) are fermented to produce 6–14% ethanol in wines. Cells are removed by filtration or centrifugation (Chapter 6) 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. In these, 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. Details of grape wine production are given by Amerine et al. (1967). Other wines are produced throughout the world from many fruits, tree saps, honey and vegetable pods. For example palm sap is fermented by naturally occurring Zymomonas spp. to produce palm wine. Lactic acid bacteria produce small amounts of aldehydes and lactic and acetic acids, which give the product a characteristic aroma and flavour. Fermentation times in excess of 12 h produce an over-acidified product and it is therefore consumed on the day of preparation.

Vinegar and other food acids
Ethanolic fermentation of wine, cider or malt by yeast is the first of a two-part fermentation in the production of vinegar. In the second stage the ethanol is oxidised by A. aceti to acetic acid and a number of flavour compounds. This stage is sensitive to the concentration of dissolved oxygen, and fermenters are carefully designed to ensure that an adequate supply of air is maintained (Beaman, 1967). During maturing of vinegar, reactions between residual ethanol and acetic acid form ethyl acetate, which imparts the characteristic flavour to the product.

Citric acid is widely used as an acidulant in foods. It is produced by fermenting sugar using Aspergillus niger in submerged culture, under conditions of substrate limitation (Kapoor et al., 1982). The production of other important food acids, including glutamic acid, gluconic acid, lactic acid, propionic acid and tartaric acid, is described by Pederson (1971).

Cocoa and coffee
Cocoa and coffee berries contain mucilage around the beans, which is removed by fermentation. Cocoa beans are either heaped or placed in slatted fermentation bins (‘sweat boxes’) and initial fermentation by yeasts (including S. ellipsoideus, Saccharomyces apiculata, Hansenula spp., Kloeckera spp., Debaromyces spp., Schizosaccharomyces spp. and Candida spp.), produces ethanol from sugars in the pulp and raises the temperature in the box. Lactic acid bacteria then predominate in the anaerobic conditions. They reduce the pH and further raise the temperature. Pulp is hydrolysed and solubilised during this period and drains away to allow air to penetrate the bean mass. Ethanol is then oxidised to acetic acid by acetic acid bacteria which also cause the temperature to rise to 45–60°C, and destroy the yeast population. The combination of heat and up to 2% w/w acetic acid kills the beans. They are then dried to 7% moisture to preserve the product and roasted to produce the characteristic chocolate flavour and aroma (Carr, 1985). The manufacture of cocoa powder and chocolate are described by Meursing (1987). Coffee berries are soaked, pulped and fermented in slatted tanks where microbial and naturally occurring pectic enzymes solubilise the mucilage. Details of chemical changes during coffee fermentation are described by Arunga (1982).

Soy products
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, α-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. sojae* lowers the pH to 5.0, and an alcoholic fermentation by *S. rouxii* takes place. Finally the temperature is gradually returned to 15°C 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 is preserved by 2.5% ethanol and 18% salt (Pederson, 1971). Details of the biochemistry of flavour and aroma production are described by Yokotsuka (1960), Yong and Wood (1974) and Wood (1982).

In the production of tempeh, soy beans are soaked, deskinne, steamed for 30–120 min and fermented. Enzyme activity by *Rhizopus oligosporus* 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 no preservative effect. The product is either consumed within a few days or preserved by chilling.

### 7.1.3 Equipment

Solid substrates are incubated in trays or tanks, contained in rooms that have temperature and humidity control. Some meat products are filled into plastic or cellulose casings prior to fermentation. Liquid substrates are incubated in either stainless steel tanks or in cylindrical stirred fermenters (Fig. 7.5). Fermenter design and operation is discussed in detail by Stanbury and Whitaker (1984).

![Batch fermenter showing controls and instrumentation](image)

**Fig. 7.5** Batch fermenter showing controls and instrumentation: S, steam sterilising points.
7.1.4 Effect on foods

The mild conditions used in food fermentations produce few of the deleterious changes to nutritional quality and sensory characteristics that are found with many other unit operations. Complex changes to proteins and carbohydrates soften the texture of fermented products. Changes in flavour and aroma are also complex and in general poorly documented. Flavour changes include reduction in sweetness and increase in acidity due to fermentation of sugars to organic acids, 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 aroma of fermented foods is due to a large number of volatile chemical components (for example amines, fatty acids, aldehydes, esters and ketones) and products from interactions of these compounds during fermentation and maturation. In bread and cocoa, the subsequent unit operations of baking and roasting produce the characteristic aromas. 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 to formation of brown pigments by proteolytic activity, degradation of chlorophyll and enzymic browning.

Microbial growth causes complex changes to the nutritive value of fermented foods by changing the composition of proteins, fats and carbohydrates, and by the utilisation or secretion of vitamins. Micro-organisms absorb fatty acids, amino acids, sugars and vitamins from the food. However, in many fermentations, micro-organisms also secrete vitamins into the food and improve nutritive value (Table 7.3) (Dworschak, 1982). Micro-organisms also hydrolyse polymeric compounds to produce substrates for cell growth, which may increase the digestibility of proteins and polysaccharides.

7.2 Enzyme technology

Only 1% of the enzymes so far identified are produced commercially and the largest volume (35%) are proteases for use in detergent manufacture. However, advances in biotechnology have had a significant effect on the number and type of new enzymes that are available for use in food processing or production of specialist ingredients. There has also been rapid growth in recent years 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 (Table 7.4). The main advantages in using enzymes instead of chemical modifications are that 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. Selection of the precise enzyme for a particular application can be difficult and guidelines on methods to do this are given by West (1988).

Enzymes are active at low concentrations and the rates of reaction are easily controlled by adjustment of incubation conditions. Details of the factors that influence enzyme activity and reaction rates are described by Whitaker (1972). 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 are usually coated or immobilised on carrier materials to reduce the risk of inhalation of enzyme dust by operators.
Table 7.3 Changes in vitamin content of selected foods during fermentation

<table>
<thead>
<tr>
<th>Product</th>
<th>Thiamin (mg)</th>
<th>Riboflavin (mg)</th>
<th>Niacin (mg)</th>
<th>Vitamin C (mg)</th>
<th>Pantothenic acid (mg)</th>
<th>Vitamin B6 (mg)</th>
<th>Vitamin B12 (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>0.04</td>
<td>0.18</td>
<td>0.1</td>
<td>1</td>
<td>0.37</td>
<td>0.042</td>
<td>0.4</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>0.04</td>
<td>0.18</td>
<td>0.1</td>
<td>1</td>
<td>–</td>
<td>0.040</td>
<td>–</td>
</tr>
<tr>
<td>Cheese (Cheddar)</td>
<td>0.03</td>
<td>0.46</td>
<td>0.1</td>
<td>0</td>
<td>0.50</td>
<td>0.08</td>
<td>1.0</td>
</tr>
<tr>
<td>Grapes</td>
<td>0.05</td>
<td>0.03</td>
<td>0.3</td>
<td>4</td>
<td>0.075</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>Wine (table)</td>
<td>Trace</td>
<td>0.01</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0.05</td>
<td>0.07</td>
<td>0.3</td>
<td>51</td>
<td>0.21</td>
<td>0.16</td>
<td>0</td>
</tr>
<tr>
<td>Sauerkraut\textsuperscript{a}</td>
<td>0.07</td>
<td>0.03</td>
<td>0.2</td>
<td>14</td>
<td>0.09</td>
<td>0.13</td>
<td>0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0.03</td>
<td>0.04</td>
<td>0.2</td>
<td>11</td>
<td>0.25</td>
<td>0.042</td>
<td>0</td>
</tr>
<tr>
<td>Dill pickle</td>
<td>Trace</td>
<td>0.02</td>
<td>Trace</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Soy bean (unfermented)</td>
<td>0.22</td>
<td>0.06</td>
<td>0.90</td>
<td>–</td>
<td>–</td>
<td>0.08</td>
<td>–</td>
</tr>
<tr>
<td>Tempeh</td>
<td>0.13</td>
<td>0.49</td>
<td>4.39</td>
<td>–</td>
<td>–</td>
<td>0.35</td>
<td>–</td>
</tr>
<tr>
<td>Soy sauce</td>
<td>0.88</td>
<td>0.37</td>
<td>6.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Loss due to canning and storage.
Adapted from Murata \textit{et al.} (1967), Watt and Merrill (1975) and Orr (1969).
7.2.1 Enzyme production from micro-organisms

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

Table 7.4 Enzymes used in food processing

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Major source</th>
<th>pH range</th>
<th>Temperature (°C)</th>
<th>Type of culture or application</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-amylases</td>
<td>Aspergillus oryzae</td>
<td>4.0–5.0</td>
<td>50–70</td>
<td>Surf., B, I</td>
</tr>
<tr>
<td></td>
<td>Bacillus subtilis</td>
<td>6.0–7.0</td>
<td>80–90</td>
<td>Sub., B, I</td>
</tr>
<tr>
<td></td>
<td>B.licheniformis</td>
<td>6.0–7.0</td>
<td>95–105</td>
<td>Sub., B, I</td>
</tr>
<tr>
<td>Amyloglucosidase</td>
<td>A. niger</td>
<td>3.5–5.0</td>
<td>55–65</td>
<td>B, I</td>
</tr>
<tr>
<td>Bromelain</td>
<td>Pineapple (Ananas comosus)</td>
<td>4.0–9.0</td>
<td>20–65</td>
<td>SA</td>
</tr>
<tr>
<td>Catalase</td>
<td>Beef liver</td>
<td>6.5–7.5</td>
<td>5–45</td>
<td>–</td>
</tr>
<tr>
<td>Cellulases</td>
<td>A. niger</td>
<td>3.0–5.0</td>
<td>20–60</td>
<td>Sub./Surf., B</td>
</tr>
<tr>
<td></td>
<td>Trichoderma viride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T. reesei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ficin</td>
<td>6.5–7.0</td>
<td>25–60</td>
<td>B, SA</td>
</tr>
<tr>
<td>Glucoamylases</td>
<td>A. niger</td>
<td>3.5–5.0</td>
<td>30–60</td>
<td>Sub., B, I</td>
</tr>
<tr>
<td></td>
<td>Rhizopus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose isomerase</td>
<td>Escherichia spp.</td>
<td>7.0–7.5</td>
<td>60–70</td>
<td>Sub., I</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacillus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>A. niger</td>
<td>4.5–7.0</td>
<td>30–60</td>
<td>Sub., Surf, B</td>
</tr>
<tr>
<td>Hemi-cellulases</td>
<td>Fungi</td>
<td>3.5–6.0</td>
<td>30–65</td>
<td>Sub.</td>
</tr>
<tr>
<td>Invertase</td>
<td>Kluyveromyces fragilis</td>
<td>4.5–5.5</td>
<td>55</td>
<td>Sub., B, I</td>
</tr>
<tr>
<td></td>
<td>S. cerevisiae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactase</td>
<td>K. fragilis</td>
<td>7</td>
<td>40</td>
<td>Sub., B, I</td>
</tr>
<tr>
<td></td>
<td>Aspergillus spp.</td>
<td>4.5–5.0</td>
<td>50–60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chaetomium spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucor pusillus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactococcus (formerly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus) lactis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Candida pseudotropicalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td>Porcine pancreas</td>
<td>5.5–9.5</td>
<td>20–50</td>
<td>B</td>
</tr>
<tr>
<td>Papain</td>
<td>Papaya (Carica papaya)</td>
<td>6.0–8.0</td>
<td>20–75</td>
<td>B, SA</td>
</tr>
<tr>
<td>Pectic enzymes</td>
<td>A. niger</td>
<td>2.5–5.5</td>
<td>25–65</td>
<td>Surf./Sub. B</td>
</tr>
<tr>
<td>Pepsin</td>
<td></td>
<td>1.5–4.0</td>
<td>40</td>
<td>B</td>
</tr>
<tr>
<td>Proteases</td>
<td>B. subtilis</td>
<td>6.0–8.5</td>
<td>20–55</td>
<td>Sub.</td>
</tr>
<tr>
<td></td>
<td>A. oryzae</td>
<td>4.0–7.5</td>
<td>20–50</td>
<td>Surf.</td>
</tr>
<tr>
<td></td>
<td>Rhizopus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
<td>7.0–8.0</td>
<td>20–50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. polymyxa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacillus spp.</td>
<td>9.0–11.0</td>
<td>20–50</td>
<td></td>
</tr>
<tr>
<td>Pullulanase</td>
<td>Aerobacter aerogenes</td>
<td>3.5–5.0</td>
<td>55–65</td>
<td></td>
</tr>
<tr>
<td>Rennet</td>
<td>Bovine (chymosin)</td>
<td>3.5–6.0</td>
<td>40</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>M. pusillus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. miehei</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Surf = surface culture, sub = submerged culture, B = batch application, SA = surface application, I = immobilised.

7.2.1 Enzyme production from micro-organisms

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 only released into the medium when cells undergo lysis in the stationary or decline phases (Fig. 7.1). 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
- methods for enzyme recovery should be simple and inexpensive
- the enzyme preparation should be stable.

Enzymes are produced by either surface culture on solid substrates (for example rice hulls, fruit peels, soy bean meal or wheat flour) or by submerged culture using liquid substrates (for example molasses, starch hydrolysate or corn steep liquor). Specific minerals may have to be added to substrates to maximise enzyme production. Submerged cultures have lower handling costs and a lower risk of contamination and are more suited to automation than are solid substrates. In batch methods, the inoculum is added to sterile substrate at 3–10% of the substrate volume and cells are grown under controlled conditions for 30–150 h in capacities ranging from 1000 to 100 000 l. Microprocessors are used to automatically control pH, dissolved oxygen, carbon dioxide and temperature (Armiger and Humphrey, 1979).

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, fractional precipitation, chromatographic separation, electrophoresis, membrane separation, freeze drying or a combination of these methods (Skinner, 1975). Intracellular enzymes are extracted by disruption of cells in a homogeniser or mill. 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 6).

### 7.2.2 Application of enzymes in food processing

Batch operation is widely used when the cost of the enzyme is low. The enzyme is mixed with food, allowed to catalyse the required reaction, and then either retained within the food or inactivated by heat. In continuous operation, enzymes are immobilised on support materials by:

- micro-encapsulation in polymer membranes which retain the enzyme but permit the passage of substrates and products
- electrostatic attachment to ion exchange resins
- adsorption onto colloidal silica and/or cross linking with glutaraldehyde
- covalent bonding to organic polymers
- entrapment in polymer fibres (for example cellulose triacetate or starches)
- co-polymerisation with maleic anhydride
- adsorption onto charcoal, polyacrylamide, or glass (Konecny, 1977).

The main advantages of enzyme immobilisation are that enzymes are re-used, there is continuous processing and closer control of pH and temperature to achieve optimum activity. Immobilisation is at present used when an enzyme is difficult to isolate or expensive to prepare. The main limitations are:
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- the higher cost of carriers, equipment and process control
- changes to the pH profiles and reaction kinetics of enzymes
- loss of activity (25–60% loss)
- risk of microbial contamination.

In use, either immobilised enzymes are mixed with a liquid substrate and then removed by centrifugation or filtration and re-used, or the feed liquor is passed over an immobilised bed of enzyme fixed into a reactor. 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 (for example glucose isomerase is used for 1000 h at 60–65ºC)
- suitability for regeneration.

A summary of the food applications of the main enzyme groups is shown in Table 7.5.

Carbohydrases

This group of enzymes hydrolyse polysaccharides or oligosaccharides. The commercially important types are:
- α-amylase
- glucoamylase
- invertase
- lactase
- glucose isomerase.

\(\alpha\)-amylases randomly hydrolyse \(\alpha-(1\rightarrow4)\) linkages to liquefy starch and produce maltose. They do not hydrolyse \(\alpha-(1\rightarrow6)\) linkages and therefore leave low-molecular-weight dextrins and oligosaccharides unhydrolysed. The products are used as bland tasting, functional ingredients in dried soups, infant foods and sauces. The enzymes are also used for the limited hydrolysis of starch to produce fat mimetics.\(^2\) \(\beta\)-amylases are exo-enzymes that remove maltose units from non-reducing ends of amylose chains. In amylopectin, the cleavage stops a few units away from the \(\alpha\)-1,6-branches. Products are maltose syrups from barley or soybean starches which have wide applications in, for example, brewing, confectionery and bakery products.

Glucoamylase (or amyloglucosidase) is a saccharifying enzyme which removes successive glucose units from the non-reducing ends of starch molecules by rapid cleavage of the \(1,4\)-\(\alpha\)-linkages and slower cleavage of the \(\alpha\)-1,6-linkages. Unlike \(\alpha\)-amylases, it therefore hydrolyses linear and branched chains to form glucose. The products are sweet tasting, fermentable hydrolysates with special functional properties, which are used in the production of alcohol, ascorbic acid and penicillin. The most commonly used starches are maize, wheat, tapioca and potato (Olsen, 1993).

Commercial fungal amylase preparations, which contain smaller quantities of phosphatase, glucoamylase and protease, saccharify starch to a greater extent than a single amylase does. They produce substantial quantities of maltose without significant quantities of glucose and are used in the following applications:
- to eliminate starch hazes and to reduce the viscosity of fruit juices

\(^2\) Low calorie fat substitutes.
<table>
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<th>Table 7.5 Uses of enzymes in the food industry</th>
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<td>Food sector</td>
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<td>Starch modification</td>
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<td>Protein modification</td>
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<td>Alcohol removal</td>
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<td>Extraction of plant components</td>
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Adapted from Law (1990).
• to convert cocoa starch to dextrins to reduce viscosity and to improve the stability of chocolate syrups
• to produce glucose syrups (Delrue, 1987)
• to reduce dough viscosity and to accelerate fermentation by yeast
• to increase loaf volume by breaking down gelatinised starch at a temperature above that survivable by indigenous enzymes (Law, 1990).

Bacterial amylases liquefy starch at higher temperatures (Table 7.4). They are used to produce glucose and maltose syrups and to replace malted grain for brewing. In baking they are used to improve gas production, crust colour, shelf life and toasting characteristics of bread. Glucoamylase is used to increase the alcohol content and to reduce the carbohydrate content of beer, by converting dextrins to fermentable sugars during fermentation. It is also used in the production of vinegar and yeast from starch-based products.

Invertase hydrolyses sucrose to glucose and fructose. It is used to remove sucrose from foods, to prevent crystallisation in molasses, and in the production of invert sugar, confectionery, liquers and frozen desserts. Lactase (β-D-galactosidase) hydrolyses lactose to D-glucose and D-galactose. It is used to prevent lactose crystallisation in ice cream and frozen milk concentrates, in starter cultures for cheese, and to prepare foods for those suffering from lactose intolerance (Bauer, 1986).

Glucose isomerase is used for the production of high-fructose corn syrup (HFCS) sweeteners from glucose. Different proportions of fructose and glucose are prepared by adjustment of the incubation time with the enzyme. Two products are widely used as an alternative to sucrose in the bakery and confectionery industries (Law, 1990): HFCS-42 and HFCS-55, containing respectively 42% and 55% fructose (dry weight basis). Other applications include the production of dextrose from corn starch, clarification of fruit juice and wine, removal of glucose from egg white, inversion of sucrose and chillproofing of beer (Hultin, 1983).

The degree of hydrolysis of starch is measured by the dextrose equivalent (DE). Maltodextrin (DE 15-25) is valuable for its rheological properties and is used as fillers, stabilisers, thickeners and pastes in a wide variety of foods. When further hydrolysed, a variety of sweeteners can be made, having dextrose equivalents of 40–45 (maltose), 50–55 (high maltose) and 55–70 (high conversion syrup). Cyclodextrins are molecules containing 6–8 glucose units, linked together in a ring. They can bind other molecules in their cavities and therefore stabilise, solubilise or precipitate other compounds. They are also widely used to encapsulate flavours and aromas. They are produced from starch using the enzyme cyclodextrin glycosyl transferase (CGT-ase) (Pszczola, 1988; Nielsen, 1991).

Pectic enzymes
There are three types of pectic enzymes: pectin esterase, polygalacturonase and pectin lyase although the last is not used commercially. Pectin esterase hydrolyses the methoxyl group from pectin molecules to form low-methoxyl pectin and polygalacturonic (pectic) acid. Polygalacturonase hydrolyses α-(1→4) linkages of polygalacturonic acid to produce oligogalacturonomans and galacturonic acid. Polygalacturonase has both endo- and exo-forms which respectively hydrolyse the polymer randomly and sequentially from the ends. Commercially the endo-polygalacturonase is more useful as it produces more rapid depolymerisation of pectin molecules. Fungal pectic enzyme preparations, particularly from Aspergillus spp., consisting of polygalacturonase, pectin methylesterase, cellulase, hemicellulase and protease, are used to:
• accelerate rates of filtration of fruit juices
• remove pectin from fruit base prior to gel standardisation in jam manufacture
• prevent undesirable gel formation in fruit and vegetable extracts and purées
• standardise the characteristics of pectin for the varied uses as a thickener
• recover citrus oils
• stabilise cloud in fruit juices.
(Rombouts and Pilnik (1978), King (1991) and Faigh (1995)).

Cellulases and hemicellulases

Fungal cellulase preparations, which contain smaller quantities of hemicellulase and pectinase, act on the \( \alpha-(1\rightarrow6) \) linkages of glucose units in soluble forms of cellulose. They are used to:
• improve filtration of vanilla extracts
• tenderise vegetables prior to cooking
• degrade nut shells prior to oil extraction to save energy
• extract flavour compounds from vegetables which was previously difficult and expensive.

Hemicellulases reduce the viscosity of several plant gums by conversion of D-xylans to xylo-oligosaccharides, D-xylose and L-arabinose. They are used:
• to reduce the viscosity of coffee concentrates
• in the extraction and clarification of citrus juices
• in the hydrolysis of apple and grape pomace to fermentable sugars
• to increase the yield of essential oils, spices and other plant extracts.

Proteases

Proteases are classified according to their pH optima into acid, neutral and alkaline types. Acid proteases are produced mostly by fungi, and neutral and alkaline proteases are produced by both fungi and bacteria (Table 7.4). Neutral bacterial proteases, which also have significant \( \alpha \)-amylase and alkaline protease activity, are used to hydrolyse plant and animal proteins and to improve the flavour of crackers and the handling of pizza doughs. Acid fungal proteases which have significant \( \alpha \)-amylase activity are used to:
• hydrolyse gluten to reduce mixing times, to make dough more pliable and to improve the loaf volume and texture
• tenderise meat
• prepare liquid meat products
• reduce the viscosity and prevent gelation of concentrated soluble fish products
• reduce the setting time for gelatin without affecting the gel strength.

Bromelain is a mixture of proteases which hydrolyse plant and animal proteins to amino acids and peptides. It is used to:
• tenderise meat
• improve the handling of pizza doughs
• chillproof beer
• produce waffles, pancakes and wafers.

Papain and ficin are proteases that have broad substrate specificities and stability at higher temperatures. They are used to produce savoury flavourings for soups, to
chillproof beer and as meat tenderisers. Details of the action of papain are given by Law (1990).

Rennet partially coagulates milk proteins to form casein curds in cheese production (Section 7.2.1). A low proteolytic activity is required to prevent solubilisation of the casein and to achieve an adequate yield of correctly flavoured cheese. Calf rennet is expensive and the use of microbial proteases is therefore increasing. Fungal proteases have a lower activity than bacterial proteases and the protease from *M. meihei* or *M. pusillus* is used for short and medium fermentation times. Developments in recombinant deoxyribonucleic acid (DNA) technology applied to cheese manufacture are described by Law (1986). *Chymosin* was the first enzyme to become commercially available from genetically engineered *Kluyveromyces lactis* and *Escherichia coli* (Roller et al., 1991).

**Oxidases**

*Glucose oxidase* oxidises glucose in the presence of oxygen to form gluconic acid. It is used to de-sugar, and hence to stabilise egg products, and to increase the shelf life of bottled beer, soft drinks and other oxygen sensitive foods. It has advantages over chemical anti-oxidants because it does not lose its activity over time as it is not itself oxidised. *Catalase* decomposes hydrogen peroxide to form water and oxygen. It is used to provide oxygen for de-sugaring egg products by glucose oxidase.

**Lipases**

Lipases hydrolyse fats and fatty acid esters to form diglycerides and monoglycerides. Preparations which contain smaller quantities of *α*-amylase and protease are used to:

- improve whipping properties of egg albumin
- modify or solubilise fats
- break down emulsions
- improve the flavour of dairy products
- produce free fatty acids from butterfat.

The fatty acids are used in small amounts to enhance natural flavours, in larger amounts to give a buttery flavour and in large amounts to give a cheesy flavour. They are used in cheese flavoured dips, sauces and snackfoods, processed cheese, soups, baked products and chocolate confections. Lipases from *Candida cylindracea* and *Mucor meihei* have been used to inter-esterify triglycerides in palm oil to change the melting curve to one that is similar to cocoa butter and thus increase its value. *Phospholipases* are used to improve the emulsifying properties of phospholipids and can be used in inter-esterification to change their physical properties (Law, 1990).

**Other enzymes**

*Diacetyl reductase* converts the flavour compound diacetyl to flavourless acetoin to improve the flavour in beer (Eckett, 1985). Fungal *phosphodiesterases* can be used to make 5′-nucleotide flavour enhancers which accentuate ‘meaty’ flavours in soups, sauces and gravies. Enzymes are also used for debittering of fruit products; for example *limoninase* which hydrolyses the bitter component ‘limonin’ in orange juice or *nariniginase* which breaks down naringin in grapefruit juice. *Pentosanase* converts pentosans to D-xylose and L-arabinose to reduce bread staling, to reduce dough viscosity, to lighten the crumb of rye bread and to prevent it from separating from the crust during baking. *β-glucanases* converts β-glucans to β-D-glucose to facilitate filtration of barley wort in brewing. *Stachyase* converts stachyose and rafinnose to monosaccharides to
reduce flatulence produced by leguminous foods. The protease trypsin retards the development of oxidised flavours in milk and collagenase and elastase soften and tenderise connective tissues in meat. Other examples of enzyme applications in food processing are given by Whitaker (1990).

7.3 Acknowledgements

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7.4 References


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