2 Enzymes in the food industry

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2.1 Introduction

Enzymes play an important role in the food industry in both traditional and novel products. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. In brewing, the malting of grain develops amylase and protease activities, which then act on the starch and protein reserves of the grain during mashing to produce fermentable sugars and other nutrients on which yeast can grow. By a process of trial and error, brewers over the millennia have optimised conditions for malting and mashing despite their lack of knowledge of enzymes. In the same way, the slaughter of animals for meat was developed so that the quality of the meat was improved. The importance of resting animals prior to slaughter, killing them without trauma and hanging the meat for several days was recognised before the role of enzymes in the process was known. It is now known that resting animals prior to slaughter ensures that sufficient glycogen is present in the muscle to act as a substrate for enzymes *post mortem*. The role of *post mortem* glycolysis and natural muscle proteases in the conversion of muscle to meat is also understood and has led to attempts to improve further the process, and therefore the quality of the meat.

Other traditional products like yoghurt and fermented beverages owe their character to enzyme reactions but these are performed by whole organisms rather than isolated enzymes. The main reaction in yoghurt production is the conversion of lactose from milk to lactic acid. The decrease in pH then affects the casein proteins to give yoghurt its characteristic texture. Similarly in beer fermentation, the conversion of sugars to ethanol involves just a few of the enzymes present in yeast. It is even more convenient to carry out the reaction using the whole organism rather than isolated enzymes, and important flavour notes in the finished product are a result of other metabolic processes carried out by the yeast organism under anaerobic conditions.

These changes in traditional products are due to enzymes that are endogenous; that is they occur naturally in the tissues of the plant or animal or in the micro-organism. The activity of endogenous enzymes can be manipulated by optimising the conditions for enzymic activity (pH, temperature) or by altering the genetic control of enzyme expression using the genetic engineering techniques now available. However, there are limitations to the degree of manipulation that can be achieved by these means. The idea of adding

enzymes from other sources (exogenous enzymes), to improve existing reactions or to initiate new reactions, dates from the start of this century (Wolnak, 1980). Early work in the USA led to the development of enzymes for the leather industry and started the commercial production of papain for use in the beer industry.

The use and production of enzymes remained fairly static until biological washing powders were formulated in the late 1960s and early 1970s. The use of alkaline proteases with relatively high temperature optima created a large market, and biological washing powders still comprise a considerable sector of the enzyme market (Godfrey and Reichelt, 1983). With the availability of enzymes on a large scale, and at reasonable prices, the food industry reconsidered the use of enzymes in food processing. Around the same time, advances in biotechnology in the field of genetic manipulation created massive euphoria and extravagant claims about the new technology were made. Despite these technological advances and the thousands of potential applications, the use of enzymes in the food industry is limited. In 1980, Whitaker estimated that, of the world market for enzymes, just nine enzymes accounted for 65% of the total revenue. The situation in 1994 is similar. A typical enzyme producer will have studied thousands of enzymes but will have a commercial portfolio comprising typically thirty enzymes, of which a handful account for the vast majority of sales. The 1993 annual report of Novo Nordisk (which accounts for about 50% of world enzyme sales) showed that sales were divided between detergents (40%; 80% of this is protease), starch enzymes (12%; due to amylase, amyloglucosidase and glucose isomerase), textile processing (13%; 90% of this is cellulase) and others (includes animal feeds and human foods) accounted for 35%. Thus the majority of sales revenue can still be attributed to a small group of enzymes. The reasons for this are as follows.

2.2 Commercialisation of enzyme processes

When enzymes are considered for use in a food process, it is essential to ensure that they will confer some commercial benefit. There are several ways of defining this latter parameter. Enzymes may improve the conversion of a raw material to its constituent parts as in the hydrolysis of starch to glucose (see Chapter 8). Acid hydrolysis of starch gives limited conversion whereas enzymes can improve the yield. This example of starch hydrolysis also illustrates another beneficial effect of using enzymes, namely that the effluent from enzyme hydrolysis is less toxic and therefore cheaper in terms of waste disposal.

In the brewing industry, savings on raw material costs can be achieved by the use of enzymes in the mashing process. The traditional mash process relies on the enzyme activities in the malt constituent to hydrolyse the macro-molecules of malt and barley into fermentable substrate. Malt is an expensive

commodity, however, and it is also variable in terms of enzyme activity. Since brewing is a complex process, the complete replacement of malt enzymes by commercial enzymes may have other effects on the quality of the final product. Rather than total replacement of malt enzymes, commercial enzymes are often used in conjunction with the malt enzymes so that brewers can standardise the processes and produce consistent quality beer, regardless of raw material fluctuations. Thus the commercial benefits of using enzymes may be expressed in different ways as: (1) improved conversion; (2) as an environmental benefit; (3) cost savings on raw material; or (4) standardisation of the process.

Given the fact that food is biological in nature and that food processing involves some type of conversion of raw materials to processed foods, it is surprising that enzymes are so little used in the industry. In their book *Food Biotechnology*, Angold *et al.* (1989) presented several reasons why biotechnology (which includes enzyme technology) has not found greater use in the food industry. They first differentiate between small-scale and large-scale biotechnology.

The pharmaceutical industry is typical of the small-scale operation where the high costs of research and development can be recouped by charging (relative) high prices for drugs. Indeed, it could be argued that pharmaceutical research and development creates markets, as without research into diseases and ailments, no cures could be found. In contrast, the food industry can be described as 'large-scale, commodity transformation characterised by a low-margin operation' (Angold *et al.*, 1989). Since food is a basic commodity, consumers expect it to be available at a reasonable cost. Moreover, it is difficult to improve food significantly so that it might attract a premium price. People in the Western World will pay a little extra for improved quality but, apart from specialities like caviar or truffles, food generally is cheap. In addition, the population in developed countries already consumes a sufficient variety and quantity of food to satisfy their nutritional requirements and over-consumption is now recognised as undesirable. There is therefore a limit to the market size and expansion can only be achieved by increasing the market share of a particular company.

Food is a traditional, craft-based industry and consumers are already suspicious about scientists 'messing about' with their food. The public has seen so many contradictory statements by food experts that the credibility of science as a whole has decreased. Improvements in the processing of food are more likely to be achieved through optimisation of existing processes or through advances in engineering to allow efficient production of novel products (e.g. co-extrusion machines that have produced tomato-filled sausages, Battenberg cakes or filled, reformed meat and poultry products such as chicken kievs).

The economic system of the consumer and producer is normally allowed to find its own balance in the so-called free market but food is such an important strategic commodity that there is sometimes political intervention. The story

of high-fructose syrup illustrates how a novel product, based on an enzyme process that was technically and economically viable in the free market of the USA, was undermined when it was applied to the controlled EC market and when a powerful lobby (European farmers) intervened. Their pressure on the EC in the mid 1970s resulted in the imposition of tight quotas on high-fructose syrup and its raw materials to protect the sugar-beet growers and processors of Europe. This made the production of high-fructose syrup in Europe un-economic and therefore unattractive. Angold *et al.* (1989) discussed the outcome of this political pressure and pointed out that, although the status of the European sucrose industry was maintained (which has obviously benefited those employed in the industry), the political intervention had discouraged other technological advances in the food area.

2.3 Alternative methods to the use of enzymes

There is often more than one solution to a problem, and the enzymic solution should always be considered alongside other methods. In the early days of enzyme technology, enzymes were suggested as the answer to many problems, although experience has shown that they were not always ideal. Whitaker (1980) cites the case of discoloration in egg whites as an example.

The discoloration of egg whites is due to a Maillard reaction between glucose and the egg protein, principally mucin. The enzymic solution to the problem is to convert the glucose in egg whites to gluconic acid. Since gluconic acid has no reducing group it cannot take part in the Maillard reaction and browning is avoided. The conditions (pH, temperature) are favourable for glucose oxidase (EC 1.1.3.4) and the other product of the reaction, hydrogen peroxide, has some use in reducing the microbial population of the egg white. Excess hydrogen peroxide can be removed using another enzyme, catalase (EC 1.11.1.6). While this approach achieves the objective, there are other alter-natives that may be equally effective depending on the circumstances. These are as follows[1]:

- Convert glucose to gluconic acid;
- Remove mucin, dry, adjust pH;
- Store dried egg whites at −20°C;
- Store aqueous egg whites at −4°C;
- Use egg white before serious problems develop.

Discoloration due to the Maillard reaction can also be prevented by removing mucin from the egg whites as this protein is the most reactive. Storage of dried egg white at −20°C is also effective but requires frozen storage, which is not available in all parts of the world. Similarly, the storage of the aqueous egg

[1] After Beck and Scott (1974).

white at $-4°C$ is effective but demands more space than the dried product. Lastly, discoloration may be avoided by rapid stock rotation and good housekeeping. In many cases the latter alternative is optimal as it avoids extra processing of the egg white and does not involve the addition of any 'chemicals' to the food, which might prejudice consumer acceptance.

2.4 Accessibility of substrate to enzyme

In tissues some enzymes are found free in the cytoplasm of the cell but many are bound to membranes and are often in direct contact with the substrate. If exogenous enzymes are to be used to effect changes in whole cells or tissues, then the enzyme has to be able to cross membranes to contact the substrate. Since intact membranes are normally impermeable to large molecules this use of exogenous enzymes is extremely limited.

An example of this is the tenderisation of meat using proteases. Although the process is extremely attractive in commercial terms, success has been limited. The toughness of meat is normally due to excessive connective tissue, which is intimately associated with the contractile proteins. To tenderise meat therefore, an enzyme is required to selectively hydrolyse the connective tissue and to gain access to the substrate. Unfortunately, most of the protease enzymes available are relatively non-specific and will attack both connective tissue and contractile proteins. Since native collagen is resistant to proteolytic attack, most procedures rely on action during cooking when the collagen denatures.

Attempts to coat meat with proteases or to inject proteases into meat using multiple injectors certainly help distribution, but the enzymes tend to attack muscle protein as well as the collagen of the connective tissue and localised softening rather than tenderisation often results. The most effective way of introducing proteolytic enzymes into meat is by pre-slaughter intravenous injection (Lawrie, 1985). A dose related to the live weight of the animal is administered and allowed to circulate throughout the body for 5 to 30 min. The enzymes are largely inactive while the animal is still alive due to the adverse conditions of pH and redox potential. After *post mortem* glycolysis. however, the pH changes and when collagen is denatured during cooking, the enzymes are able to act (Partridge, 1959). There are side-effects on organs like the tongue and the liver. which also accumulate the enzyme on account of their substantial blood network. On cooking, these organs can literally fall apart as the connective tissue fails to hold the cells together. The technique has been used commercially, although it is difficult to estimate how widespread the practice is. In the UK. the current trend among meat producers for the production of tender, high-quality meat is the maturation of meat at 4°C for a period of 3 to 5 days. During this time, the endogenous proteolytic enzymes

that are *in situ* have sufficient time to work on the meat, producing tenderisation and contributing to the flavour as well.

In products where there is no cell structure, for example milk, enzymes are more readily applicable and enzymes have been successfully incorporated into products like cheese during manufacture to improve or accelerate ripening.

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2.5 Types of reaction

Although there are thousands of different enzymes (Trevan *et al.*, 1987), the mechanisms of enzyme action can be classified into six types as shown in Table 1.1. The reactions catalysed by each type are shown in Table 2.1. The first three types require no co-factors such as ATP or NAD(P)H and are relatively simple reactions. As in all classifications, there are always exceptions and some of the oxidative reactions that use molecular oxygen as the hydrogen acceptor could also be considered simple. Reactions that depend on co-factors are more difficult to apply to the food industry as the co-factors need to be regenerated by another enzyme system and the co-factors themselves are expensive. Estimates of co-factor cost are given by Whitesides (1980) as $2 500 to $250 000 mole^{-1}.

Figure 2.1 shows a reaction requiring ATP which is regenerated using acetate kinase (EC 2.7.2.1) and acetyl phosphate (produced by chemical means from acetone). These reactions are inefficient if performed in free solution as the transfer of ADP to the regenerating enzyme is random and large amounts of co-factor are required to obtain reasonable reaction rates. In nature, the kinase and the regenerating enzymes are thought to be in close proximity— probably membrane bound. If the two enzymes are immobilised to mimic this effect, even in a simple gel, reaction efficiency is improved. The regeneration of co-enzymes has been reviewed (Wandrey and Wichmann, 1985). There are still problems with the stability of the co-factors and, generally, these reactions are limited to high-cost products. Many of the raw materials used in the food

Table 2.1 Types of enzyme reactions

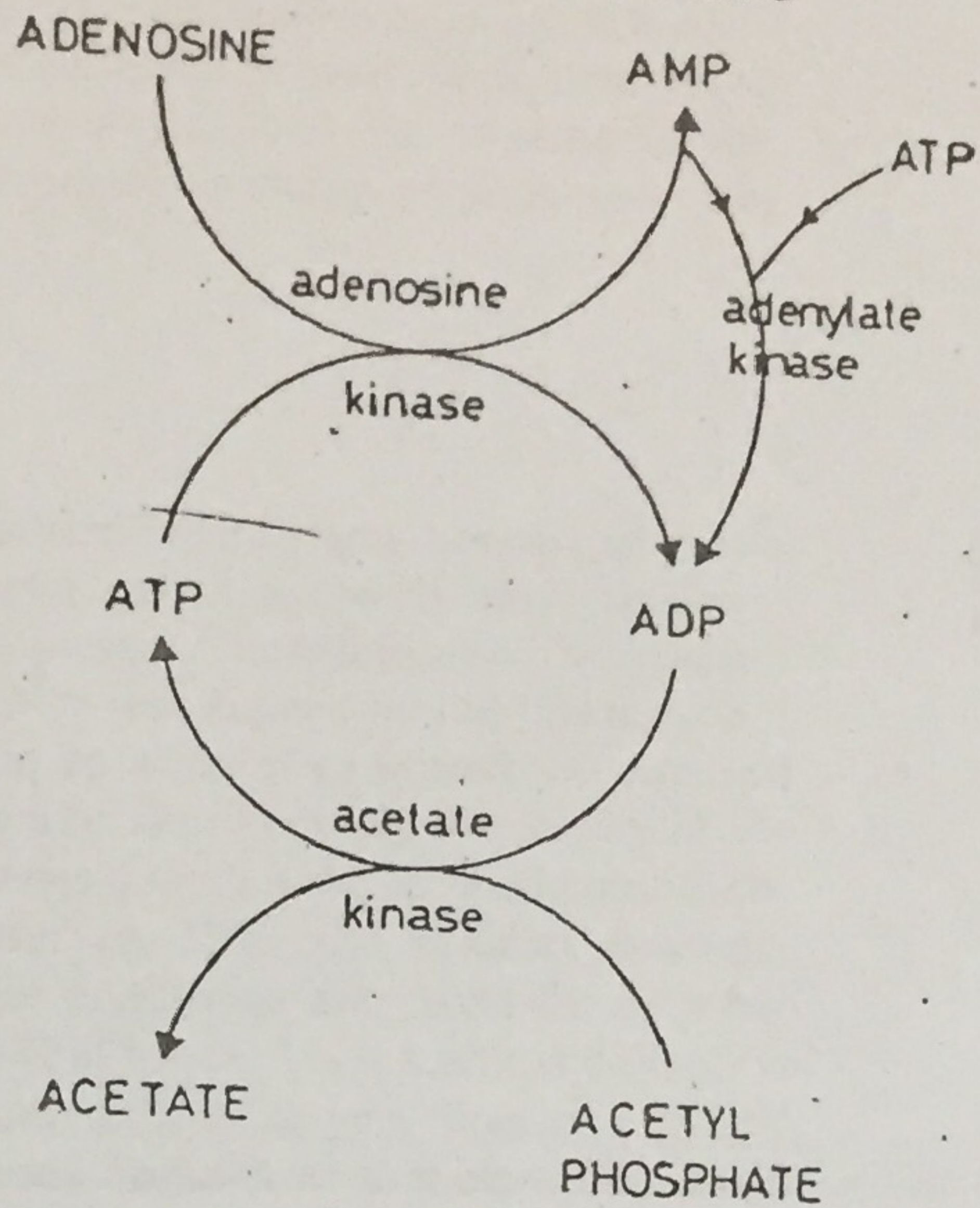| Enzyme	Type of reaction
Hydrolytic enzymes	Addition of water from across a bond
Lyases	Non-hydrolytic cleavage of bonds
Transferases	Transfer of a group from one molecule to another
Oxidoreductases	Oxidation or reduction of molecules
Isomerases	Conversion of one isomer to another
Lyases	Joining of two molecules with ATP

Figure 2.1 ATP regeneration using acetyl phosphate. (Redrawn after Whitesides, 1980).

industry are low-cost items (Table 2.2) so the use of co-factor-requiring enzymes could only be justified for high-value products such as flavourings or some hydrocolloid thickeners.

Most commercial enzymes are hydrolytic with proteases (46%) and carbohydrase (47%) being the most common (Righelato and Rodgers, 1987). So far, little, if any, progress has been made on a commercial anabolic process, although there is considerable potential in producing new designer proteins or polysaccharides with specific properties. The synthesis of polysaccharides has been documented and it is known that the chains are formed by the addition of

Table 2.2 Approximate costs of some food ingredients

Ingredient	£ per tonne
Milling wheat	100–120
Soya grits	850
Artificial colourant (tartrazine)	6–8000
Milk powder	1300
Whey powder	320
Emulsifier (glycerol monostearate)	1400–2000
Potato starch	350

a nucleoside diphosphate of a sugar (e.g. UDP-glucose) to an oligosaccharide catalysed by a synthase enzyme. Besides the technical problems in developing large-scale processes, the high production cost and the expense of the toxicological testing required for a new protein or polysaccharide also deter research.

2.6 Reaction conditions

Classical enzyme studies, like those reported in Chapter 1, are carried out in dilute aqueous solutions under optimal conditions with only substrate, enzyme, buffer and necessary co-factors present. The efficiency of the reaction is measured by the enzyme activity, which was defined by the International Union of Biochemistry in the 1960s in an attempt to produce a standard system. One Unit (U) is defined as 'the amount of enzyme that catalyses the transformation of 1 µmole of substrate per minute under defined conditions'. The defined conditions normally refer to 25°C and optimal substrate concentration and pH; however, these conditions are rarely found when enzymes are used in the food industry (Fullbrook, 1983) and it is difficult to predict activity and therefore the amount of enzyme that is required. Fullbrook also raises difficult questions about how a mole of industrial substrate, e.g. corn starch, can be defined when the molecular weight varies and the fact that enzyme activity in industry may be measured, not in terms of µmoles of substrate transformed but in terms of reduced viscosity or a related chemical value, e.g. a colour standard. Although there is a great deal of published information about enzymes, the application of these data to the industrial context is not always straightforward.

Other problems in applying pure biochemical criteria to the food situation are associated with substrate concentration, which is rarely optimal, and normally governed by other factors such as solubility. The optimum temperature of commercial enzymes (typically around 50 to 100°C) is also far removed from the standard temperature of 25°C and the pH optimum may be temperature dependent (Fullbrook, 1983). Physical factors also affect the enzymes and there are certainly differences between reaction rates in aqueous solution and when enzymes are membrane bound. When enzymes are immobilised or encapsulated for convenience in food processing, the properties of the enzyme will also change. Reactions at low water activity or in fat/water mixtures (e.g. in the modifications of lipids where the lipid/water interface is important) are also outside the classical enzyme studies. Application of these reactions has been hindered by a lack of understanding of the basic chemistry, although the enzymic modification of lipids has considerable commercial potential (Critchley, 1987), as shown in Chapter 9.

There are many food enzymes available from different companies that originate from different sources and therefore have different pH and tempera-

ture characteristics. It is worth testing a number of these to see if there are significant differences in performance or not. In the case of the proteases, there is a wide range available with pH optima from 2.5 to 9 although they do have different affinities for certain amino-acid bonds. Other types of enzymes generally have narrower ranges of optimum pH and some properties of common commercial enzymes are given in Chapter 1 and by Godfrey (1983). Recent advances in genetic engineering have provided the means for improving the stability of enzymes; this is achieved by altering the structure at vulnerable points by substitution of a different amino acid. These developments are discussed in Chapter 3.

Another factor that may limit the usefulness of an enzyme in the industrial context is product inhibition. In normal metabolism, this property is useful as it helps regulate metabolic pathways but if the enzyme is required to effect complete conversion of a substrate, the product needs to be removed to increase the percentage conversion. Enzyme processes need to be designed so that the desired changes can occur. The product may be removed to increase conversion and the design of enzyme reactors is critical.

When enzymes are used over relatively long periods and at elevated temperature, there is a decline in enzyme activity. In some applications, this is welcome as active enzyme may be unacceptable in the final food product. In other applications, it leads to decreased conversion rate and loss of efficiency. Again, design of the process can overcome these problems so that a constant degree of conversion is achieved.

2.7 Source of enzymes

Most organisms have certain 'core' enzymes in common. For instance, enzymes of the Embden–Meyerhof pathway can be found in microbes, plants and animals. Similarly, amylase activity is found widely in human saliva, in plant seedlings and in many microbes that use starch as an energy source. For enzymes like these, there are many potential sources. Other enzymes are specific to an organism or even give that organism its characteristic features. Examples are the specialised enzyme systems in nitrogen-fixing bacteria and the enzyme alliinase (EC 4.4.1.4), in onion and related plants, which catalyses the breakdown of a peptide precursor to liberate sulphur-containing volatiles that give the characteristic aroma. In cases like these, the source is limited as well as obvious. Techniques of genetic manipulation where genes can be removed from one species and transferred to a microbe, which then produces the protein (enzyme) on a commercial scale, have removed the technical problems of securing adequate sources of raw materials. The legal and safety status of engineered organisms is not totally clear, however, as discussed in Section 2.8.

Animals have traditionally produced some enzymes and products for food

and medical use. The best-known food enzyme obtained from animals is rennin (EC 3.4.4.3) found in the stomachs of calves before they are weaned. The slaughter of young calves to produce rennet, however, is both emotional and economically wasteful. This has led to the development of a microbially derived alternative. In general, animals are poor sources of enzymes as they are slow-growing and expensive. Large-scale production of enzyme from animals therefore requires large numbers of animals and large capital outlay; and animal production lacks the flexibility if enzyme production needs to be suddenly decreased or increased. Extraction of enzymes from animal tissues can also be difficult, further adding to the production cost of the enzymes.

Plants grow more quickly than most animals and can be produced in quantity on an annual basis. Again, this time scale is too long for enzyme manufacturers and the only commercially important plant enzymes are proteases obtained from crops such as pineapple and papaya, which are important in their own right. For these reasons, enzyme production from microbes is preferred as they are fast growing, can be easily controlled during growth and produce enzymes that are easy to extract. In some cases, microbes produce extracellular enzymes making extraction and purification even simpler. The production and uses of microbial enzymes has been reviewed by Fogarty (1983).

2.8 Legal and safety implications

Enzymes are used in different ways in the processing of food, and their legal status depends on the application. In the manufacture of high-fructose syrup, hydrolysis is effected by free enzymes, whereas isomerisation is catalysed by immobilised enzymes. There is the possibility, therefore, that some amylase may find its way into the finished product but it is unlikely that any isomerase will be present. In the former case, the enzyme might be considered as an additive and subjected to the statutory additive safety testing programme. There is a subtle difference, however, in that an additive like a coal-tar dye shows colour properties over a wide range of conditions but an enzyme is more restricted and can be denatured in an irreversible manner. What tests are then appropriate and what labelling requirements are needed?

As a result of the consumers' view that food should be totally safe, these scientific and moral questions have to be addressed. The public's perception of food safety can be illustrated using analogies. Denner (1983) expresses it thus: 'When a traveller purchases an airline ticket he takes a positive decision to accept a small but quantifiable risk that the plane will crash, but when that same traveller enters the airport restaurant and purchases food, his expectation of the exposure to risk in consuming that food approaches zero.' A comparison between the relative dangers of motor vehicles and canned food was used by Angold et al. (1989) to illustrate the point: 'The major

hazards in our diet are natural bacteria and fungal toxins... When a failure in processing occurred as in canned salmon in 1978. killing two people, sales were halved and the company lost £2 million. Motor vehicles kill about 100 people in Britain every week. Being run over by a lorry load of canned salmon is an acceptable hazard; being poisoned by it is not.' The consumers' current conception of food is that it should be absolutely safe. Furthermore. 'natural' foods are considered good and safe while processed foods have the 'goodness' taken out of them and are somehow perceived as inferior in safety and quality terms. While these perceptions are not backed by scientific evidence. manufacturers have to take note of consumer views and they have to adopt a pragmatic attitude, combining science and public opinion. Any discussion on safety testing is complicated by the many different types of legislation that vary country by country. Although the EC is working towards harmonisation in such matters. agreement has not yet been reached.

Enzymes may constitute a safety hazard on several grounds. Firstly. they are foreign proteins and may set off the immune response. Experience with biological washing powders highlighted this effect as certain individuals reacted when they inhaled the enzyme powder during the manufacturing process. It was recognised very quickly that powdered enzymes were a health hazard, thus most preparations are now in the form of solutions or suspensions. Minor allergenic responses associated with washing powders were also noted but it was established that enzymes were not the primary cause of this disorder (Denner, 1983). Generally, the consumer will not experience enzymes in the concentrated form but this only reduces the risk and does not completely remove it.

Secondly, the activity of enzymes may be injurious to humans. If enzymes remain active in the digestive tract. can they cause problems by attacking human tissue? The body's defences. however. are designed to cope with a wide range of active enzymes consumed in food and there is no evidence that enzymes added to food are an exception.

Thirdly, the source of enzyme has given some cause for concern as toxins may be incorporated into the crude enzyme preparations. Expert committees in the UK that considered the problem. reasoned that enzymes from plant and animal sources that are normally consumed by man did not require toxicological testing. Enzymes from microbial sources presented more problems as bacterial and fungal toxins are recognised as extremely toxic to humans and may also be carcinogenic. In the UK, minimum testing requirements have been defined together with guidelines for good manufacturing practice to avoid contamination with other pathogens or toxins.

In the UK, the Department of Health and the Ministry of Agriculture, Fisheries and Food (MAFF) have set up a committee to study the use of enzymes and the introduction of novel foods (Advisory Committee on Novel Food Products; ACNFP). ACNFP has produced several reports (e.g. Department of Health, 1991; ACNFP Annual Report, 1993) and consultation

documents (MAFF, 1994) that indicate the thinking that is shaping legislation. Generally, the European Union (EU) guidelines are being implemented and the use of enzymes in foods will be regulated by considering each case using decision trees, the latest versions of which are described in the MAFF (1994) discussion document. Briefly, the decision tree defines a novel food as 'a food or food ingredient which has not hitherto been used for human consumption to a significant degree in the EC'. By asking a series of structured questions, the decision tree determines what information needs to be supplied by manufacturers who wish a novel food to be considered for general use. The key questions involve establishing the source of the enzyme, the presence and stability of any altered genetic material and whether the enzyme is active in the food. If an enzyme is used to effect a change during processing it is considered as a processing aid but if it has a function in finished product then it is considered as an additive. This shows the concepts behind any decision to permit the use of an enzyme in a food product and determines the typical information that must be supplied to satisfy concerns about safety. The situation is still under discussion and the guidelines remain flexible to allow new information or experience from other schemes to be included in future safety assessments. Up-to-date information on the current status can be obtained from MAFF who have a duty to consult interested parties and consider comments on the draft regulations. At the time of writing (August 1994), the MAFF telephone helpline was available on 01645 33 55 77. Advice can also be sought through trade associations like Leatherhead Food RA which offers help on legislation world wide. The industry seems generally satisfied with the current regulatory approach although there are other pieces of UK legislation that refer to particular foods (e.g. baked goods and cheese) which will need amendment to avoid conflict with any ACNFP recommendations.

The situation in other countries is different and has complicated the export and import of food between countries. Lists of national requirements tend to change quite quickly and manufacturers often rely on experts in trade associations or research associations for the latest information. At present, there is no evidence to link consumption of added enzymes in food with any deleterious effects in humans.

2.9 Use of enzymes

The specific applications of enzymes are considered in the following chapters but some general points can be made about the ways in which enzymes are used in the food industry. Examples from the brewing industry, where enzymes are an important part of the process, illustrate the ways in which enzymes are used. Since the enzyme activities for mashing are derived from the raw material malt, there are variations in the amount and activity of the enzymes

due to seasonal and processing variations in the production of malt. The variation makes it difficult to standardise processes such as mashing, and addition of commercial enzymes can provide this standardisation at a reasonable cost (Bass and Cayle, 1980).

Enzymes can also be used in a 'First Aid' capacity to prevent wastage when accidents occur. Again, using the brewing industry as an example, it sometimes happens that mashing fails due to incorrect temperature control or a poor batch of malt. Rather than reject the whole batch, commercial enzymes can sometimes be used to rectify the situation and effect conversion to an acceptable level.

Raw material costs can sometimes be reduced by using enzymes in the process. Malt is an expensive way of adding enzymes to the brewing process and it is possible to replace malt with a suitable mixture of enzymes and use cheap starch sources thus reducing raw material costs significantly. Legislation in certain countries, however, specifies ingredients from which beer may be brewed and there are implications for quality if malt is replaced totally.

Enzymes have also been used to produce raw materials such as glucose syrups and protein hydrolysates, and this has involved a substantial amount of chemical engineering to achieve the required conversion. While these techniques will continue to be used in the food industry, the difficulties described above limit the number of such applications. Thus attention has now been focused on the control of enzyme activity in whole organisms.

Selective breeding of plants and animals by farmers has been used for generations to produce improved materials, e.g. low erucic acid rape seed or leaner carcasses. With the advances in genetic engineering, scientists now have the means to achieve directly what breeding programmes approached in a rather roundabout fashion. This approach requires an understanding of basic metabolic processes like fruit ripening or texture loss. Modifications to key enzymes can then be attempted to induce the desired change. It seems that a genetically engineered plant or animal stands a better chance of being accepted by the public than an isolated enzyme used in processing and this acceptance is essential if the technique is to have a commercial future.

One area in which the genetic engineering of plants may be beneficial to the food industry is the use of antisense RNA technology to manipulate pectin degradation during tomato fruit processing. The quality of processed tomato products, such as purée or ketchup often depends on the extent of pectin degradation during processing. The use of 'hot break' processes are beneficial in that they inactivate endogenous polygalacturonase (PG) (EC 3.2.1.15) and pectinesterase (EC 3.1.1.11) enzymes, the combined action of which results in pectin degradation. However, 'hot break' procedures are expensive and can be detrimental to the final flavour and aroma of the paste.

The isolation of a cDNA for tomato PG (Grierson et al., 1986) enabled Smith et al. (1988) to generate tomato lines containing antisense genes for this

enzyme. The expression of antisense RNA in these tomato fruit resulted in a reduction of endogenous PG levels to less than 1% of normal and the pectin isolated from these engineered fruit was less degraded (Smith *et al.*, 1990). More importantly, for the food industry, the use of these fruit in 'cold break' processing resulted in tomato pastes whose viscosity, as measured by the standard Bostwick test, was greater than that obtainable from the traditional 'hot break' processing of normal fruit (Schuch *et al.*, 1990).

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