Enzymes in Food Technology – Introduction

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1 Enzymes

1.1 Introduction

Enzymes are proteins that enhance (or accelerate) chemical reactions. This process is called catalysis and enzymes thus catalyze chemical reactions [1]. In enzymatic reactions, the molecules present at the beginning of the reaction are called substrates. Enzymes convert substrates into different molecules, called products. All processes in nature require enzymes in order to occur at significant rates. Enzymes are selective for their substrates and therefore catalyze only a few reactions from among many possibilities.

Like all catalysts, enzymes work by lowering the activation energy for a reaction. This is illustrated in figure 1.1.

Figure 1.1. Lowering the activation energy of a reaction.

Catalysts, like enzymes, act by lowering the energy difference between the reactants (A, B) and the transition state. This lowers the activation barrier for the reaction, allowing it to proceed more rapidly. When the energy ΔG is lowered by a relatively small amount of 5.71 kJ/mol (a typical H-bond in water has an energy of 20 kJ/mol), a 10-fold rate enhancement can be obtained. Lowering of the activation energy ΔG by 34.25 kJ/mol leads to a 10^6-fold rate enhancement.
Since lowering of the kinetic barrier also accelerates the reverse reaction, the equilibrium of the reaction remains unchanged.

As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts by being much more specific. Although almost all enzymes are proteins, not all biochemical catalysts are enzymes, since some RNA molecules called ribozymes also catalyze reactions [2].

1.2 History

In the 19th century, Pasteur studied the fermentation of sugar to alcohol by yeast. He concluded that this fermentation was catalyzed by "ferments" within the yeast cells. Pasteur believed that these ferments displayed their activity only within living organisms.

Near the end of the 19th century, Kühne first used the term enzyme, which comes from Greek "in yeast", to describe the activity already investigated by Pasteur. The word enzyme was used later to distinguish nonliving substances such as the enzymes we know today from ferments, whereby “ferment” is used to describe chemical activity produced by living organisms.

Also at the end of the 19th century, Buchner made a significant contribution to enzymology by studying the ability of yeast extracts, lacking living yeast cells, to ferment sugar. He found that the sugar was fermented even when there were no living yeast cells present.

In 1926, Sumner was able to obtain the first enzyme in pure form. He isolated and crystallized the enzyme urease from jack beans.

In the middle of the 19th century Northrop and Stanley developed a complex procedure for isolating pepsin. Their precipitation technique has since been used to crystallize many enzymes. A few years later, for the first time, an enzyme (a protease) was produced by fermentation of Bacillus licheniformis. In this way large scale production of enzymes became possible thus facilitating the industrial application of enzymes.

In 1969 the first chemical synthesis of an enzyme was reported. Since then thousands of enzymes have been studied by X-ray crystallography and NMR. Application of genetic engineering techniques improved enzyme production efficiency and even allowed improvement of the properties of specific enzymes by means of protein engineering and evolutionary design. In 2004 the first computer designed enzymes was reported.

1.3 Nomenclature of enzymes

Enzymes are usually named according to the reaction they carry out. Typically the suffix *"ase"* is added to the name of the substrate (e.g. glucose-oxidase, an enzyme which oxidizes glucose) or the type of reaction (e.g. a polymerase or isomerase for a polymerization or isomerization reaction). The exceptions to this rule are some of the enzymes studied originally, such as pepsin, rennin and trypsin. The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommend that enzyme names indicate both the substrate acted upon and the type of reaction catalyzed. Detailed information on nomenclature can be found on the I.U.B. homepage [3].

Enzymes can be classified by the kind of chemical reaction catalyzed. Officially, 6 groups of enzymes have been classified:

- EC 1 Oxidoreductases: catalyze oxidation/reduction reactions which generally involve the transfer of electrons. Examples are: Oxidases or Dehydrogenases.
- EC 2 Transferases: transfer a functional group (e.g. a methyl or phosphate group) these generally involve the transfer of a radical. Examples are: Transglycosidases, e.g. of monosaccharides; Transphosphorylases and phosphomutases, e.g. of a phosphate group; Transaminases, e.g. of an amino group; Transmethylases, e.g. of a methyl group and Transacetylases, e.g. of an acetyl group.
- EC 3 Hydrolases: catalyze the hydrolysis of various bonds. The hydrolase reaction generally involves addition or removal of water. Examples are: Hydrolases, including esterases, carbohydrases, nucleases, deaminases amidases and proteases; Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase.
- EC 4 Lyases: cleave various bonds by means other than hydrolysis and oxidation This reaction involves the splitting or forming a C-C bond. Examples are Desmolases.
- EC 5 Isomerases: catalyze isomerization changes within a single molecule and involves changing the geometry or structure of a molecule. An example is Glucose-isomerase.
- EC 6 Ligases: join two molecules with covalent bonds.

1.4 Enzymology

Any living cell inside human beings, animals, microorganisms, plants, etc., is the site of enormous biochemical activity called metabolism. Metabolism is the process of chemical and physical change which goes on continually in a living organism. Build-up of new tissue, replacement of old tissue, conversion of food to energy, disposal of waste materials, reproduction - all the activities that we characterize as "life." However, hardly any of these biochemical reactions takes place spontaneously. Enzymatic catalysis is needed to makes these biochemical reactions possible. In this way enzymes and are responsible for bringing about all chemical reactions in living organisms. Without enzymes, chemical reactions would take place at a rate far too slowly for the pace of metabolism.

1.4.1 The function of enzymes in nature

Enzymes serve a wide variety of functions inside living organisms. They are indispensable for signal transduction and cell regulation, often via kinases and phosphatases [4]. They also generate movement, with myosin hydrolyzing ATP to generate muscle contraction and also moving cargo around the cell as part of the cytoskeleton [5]. Other ATPases in the cell membrane are ion pumps involved in active ion transport. Generally it can be said that the metabolic pathways in a cell are determined by the types and amount of enzymes present in that cell.

Enzymes have an important function in the ‘digestive systems’ of mammals and other animals. Enzymes such as amylases break down large starch molecules; proteases break down large protein molecules. The result of these breakdown reactions is the formation of smaller fragments, which can easier be absorbed by the intestines of animals. Starch molecules, for example, are too large to be absorbed as such, but enzymes hydrolyze starch chains into smaller molecules such as dextrins, maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances. In ruminants which have a herbivorous diets, microorganisms in the gut produces enzymes like cellulase to break down the cellulose cell walls of plant fibers [6].

Several enzymes can work together in a specific order, creating metabolic pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed on to another enzyme. Sometimes more than one enzyme can catalyze the same reaction in parallel, this can allow more complex regulation: with for example a low constant activity being provided by one enzyme but an inducible high activity from a second enzyme.

Enzymes determine what steps occur in these pathways. Without enzymes, metabolism would neither progress through the same steps, nor be fast enough to serve the needs of the cell. A metabolic pathway such as glycolysis could not exist without enzymes. Glucose, for example, can react directly with ATP to become phosphorylated at one or more of its carbons. In the absence of enzymes, phosphorylation is insignificant. However, if the enzyme hexokinase is added, phosphorylation at carbon 6 of the glucose molecule occurs extremely fast, leading to a large excess of glucose-6-phosphate versus the products formed by the slow non-catalyzed reactions. Consequently, the network of metabolic pathways within each cell depends on the set of functional enzymes that are present.

1.4.2 Chemistry of Enzymes

Enzymes are generally globular proteins, having a size range from just over 60 to more than 2500 amino acids, i.e. a MW of ± 6000 – 250.000. The activities of enzymes are determined by their three-dimensional structure [7]. Most enzymes are much larger than the substrates they act on. It is therefore even more remarkable that only a small part of the enzyme molecule is directly involved in catalysis [7]. This small section is called the active site and this site usually contains not more than a few (3-4) amino acids which are directly involved in the catalytic process. The substrate is normally bound by the enzyme in close proximity to, or even in the active site.

1.4.3 Specificity of Enzymes

One of the most relevant and also intriguing properties of enzymes is their specificity. Some enzymes exhibit absolute specificity. This means that these enzymes catalyze only one particular reaction. Other

enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

- Absolute specificity. Highly specific enzymes catalyze only one reaction.
- Group specificity. Group specific enzymes act only on molecules that have specific functional groups, such as amino, phosphate or methyl groups.
- Linkage specificity. Such enzymes act on chemical bonds of certain nature, regardless of the rest of the molecular structure.
- Stereochemical specificity. Stereospecific enzymes act only on a particular steric or optical isomer and not on their isomeric counterparts.

The specificity of enzymes is determined by complementary shape, charge, hydrophilic/hydrophobic characteristics of the substrates and their three dimensional organization [8]. The three dimensional interaction has been described in various interaction models. The two most relevant are described.

1.4.3.1 "Lock and key" model

Emil Fischer suggested as early as 1894 that enzyme specificity was caused by specific complementary geometric shapes of both the enzyme and the substrate (see figure 1.2).

Figure 1.2. Complementary geometric shapes.

Thanks to these shapes enzyme and substrate would fit exactly into one another [9].This is often referred to as "the lock and key" model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve. The "lock and key" model has proven inaccurate and the induced fit model is the most currently accepted model for enzyme-substrate-coenzyme interaction.

1.4.3.2 Induced fit model

In 1958 Daniel Koshland [10] suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme . As a result, the substrate does not simply bind to a rigid active site, the amino acid side chains which make up the active site are moulded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, *such* as glycosidases, the substrate molecule also changes shape slightly as it enters the active site [11].The active site continues to change until the substrate is completely bound, at which point the final shape and charge is determined [12].

1.4.4 Mechanisms

Enzymes can act in several ways, whereby each way lowers the energy needed for the reaction to occur or to proceed. These mechanisms are described briefly as:

- Lowering the activation energy by creating an environment in which the transition state is stabilized. This can be achieved by binding and thus stabilizing the transition-state conformation of the substrate/product molecules.
- Lowering the energy of the transition state, but without distorting the substrate, by creating an environment with the opposite charge distribution to that of the transition state.
- Providing an alternative pathway. For example, temporarily reacting with the substrate to form an intermediate ES complex, which would be impossible in the absence of the enzyme.
- Reducing the reaction entropy change by bringing substrates together in the correct orientation to react. Considering an energy effect ($\Delta H^{\ddagger}$) alone overlooks this effect.

1.4.5 The Enzyme Substrate Complex

A theory to explain the catalytic action of enzymes was proposed by Arrhenius at the end of the 19^{th} century [19]. He proposed that the substrate and enzyme formed some intermediate transition state which is known as the enzyme substrate complex. This can be schematically represented as shown in Equation 1.

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \qquad (1)$$

The existence of intermediate enzyme-substrate complexes has been demonstrated in the laboratory, for example, using catalase and a hydrogen peroxide derivative.

From this intermediate ES complex, the reaction proceeds with the formation of the product(s) and the enzyme then returns to its original form after the reaction is concluded.

1.4.6 Chemical Equilibrium

Many chemical reactions do not go to true completion. Enzyme catalyzed reactions do not form an exception to that chemical 'law', which is due to the reversibility of most (enzyme catalyzed) reactions. Equilibrium is a sort of steady state condition, which is reached when the forward reaction rates equal the backward rates. Enzyme activity studies are always based on the principle of equilibrium reactions.

1.5 Enzyme Kinetics

Enzyme kinetics is a fundamental way of describing, predicting and calculating how enzymes bind substrates, turn these into products and also how fast and efficiently this is happening.

Early last century a quantitative theory of enzyme kinetics was proposed [13], but the experimental data were not useful since the logarithmic pH-scale was not known yet. This scale was introduced a little later [14] together with the concept of buffering. Later on, when these early experiments were repeated, the equations were confirmed and referred to as (Henri-) Michaelis-Menten kinetics [15]. This work was further developed and resulted in kinetic equations which are still in use [16].

The model for enzyme action, as first elucidated by Michaelis and Menten [15], suggests the binding of free enzyme to the reactant forming a enzyme-reactant complex. This complex undergoes a transformation, releasing product and free enzyme. This is schematically shown in Equation 2.

$$ES \xrightarrow{K_2} E + P \qquad (2)$$

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P \qquad (3)$$

When reactions 1) and 2) are combined into reaction 3), a model for enzyme catalysis is obtained. First, the enzyme (E) and substrate (S) come together to form an enzyme-substrate complex (ES); the reaction occurs by which the substrate is converted into the product of the reaction and then the enzyme-substrate complex is broken apart, yielding enzyme (E) plus product (P).

The Michaelis-Menten model assumes that only a negligible amount of enzyme-substrate complex reverts to reactants (*i.e.*, $k_1 >> k_{-1}$ in Equation 1). The rate of formation of product (shown in Equation 4) can be determined from Equation 2 in the mechanism written above.

$$\text{Rate of formation of product is } K2\,[S] \qquad (4)$$

and the rate of formation of the intermediate ES (Equation 5) can be determined from the equations 1 and 2 in the mechanism written above:

$$\text{Rate of formation of ES} = K_1[E][S] - (K_{-1} + K_2)[ES] \qquad (5)$$

Using the steady-state approximation, i.e. the assumption that the concentration of intermediates (ES) stays constant while the concentrations of reactants and products change, the equation for the rate of formation of the product can be calculated as follows (Equation 6),

$$\frac{\delta[P]}{\delta t} = \frac{K_2[E_o][S]}{[S] + K_m} \qquad (6)$$

where $[E_o]$ is the initial concentration of free enzyme, [S] is the substrate concentration, and K_m is a constant specific to a given enzyme known as the Michaelis-Menten constant. The value of K_m relates to the rate constants shown in Equations 1 and 2, as given by Equation 7:

$$K = \frac{K_{;} + K_{.}}{K_{.}} \qquad (7)$$

The Michaelis-Menten constant (K_m) is very important, because it can be determined experimentally and describes the catalytic power of an enzyme. K_m can also be used to predict the rate of an enzyme catalyzed reaction when the starting conditions (enzyme and substrate concentration) are known.

The major contribution of Henri [13] was to think of enzyme reactions in two stages. In the first, the substrate binds reversibly to the enzyme, forming the enzyme-substrate complex. In the second reaction, the enzyme catalyzes the chemical step and releases the product.

Enzymes can catalyze up to several million reactions per second. Enzyme rates depend on solution conditions and substrate concentration. Conditions that denature the protein reduce or eliminate enzyme activity. Such conditions are high temperature, extreme pH or high salt concentrations. Raising substrate concentration tends to increase activity. To find the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is seen. Saturation happens because, as substrate concentration increases, more and more of the free enzyme is converted into the substrate-bound ES form. At the maximum velocity (V_{max}) of the enzyme, all the enzyme active sites are bound to substrate, and the amount of ES complex is the same as the total amount of enzyme. However, V_{max} is only one kinetic constant of enzymes. The amount of substrate needed to achieve a given rate of reaction is also important. This is given by the Michaelis-Menten constant (K_m), which is the substrate concentration required for an enzyme to reach one-half its maximum velocity. Each enzyme has a characteristic K_m for a given substrate, and this can show how tight the binding of the substrate is to the enzyme. Another useful constant is k_{cat}, which is the number of substrate molecules handled by one active site per second.

The efficiency of an enzyme can be expressed in terms of k_{cat}/K_m. This is also called the specificity constant and incorporates the rate constants for all steps in the reaction. Because the specificity constant reflects both affinity and catalytic ability, it is useful for comparing different enzymes against each other, or the

same enzyme with different substrates. The theoretical maximum for the specificity constant is called the diffusion limit and is about 10^8 to 10^9 (M^{-1} s^{-1}). At this point every collision of the enzyme with its substrate will result in catalysis, and the rate of product formation is not limited by the reaction rate but by the diffusion rate. Enzymes with this property are called catalytically perfect or kinetically perfect.

1.6 Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in order to understand the basic enzymatic reaction mechanism and to select methods for enzyme analysis.

Several factors affect the rate at which enzymatic reactions proceed, such as temperature, pH, enzyme concentration, substrate concentration and the presence of any inhibitors or activators.

1.6.1 Enzyme Concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. These reactions are said to be "zero order" because the rates are independent of substrate concentration and are equal to some constant k. The formation of product proceeds at a rate which is linear with time. The addition of more substrate does not serve to increase the rate. In zero order kinetics, allowing the assay to run for double time results in double the amount of product.

The amount of enzyme present in a reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, etc. Highest enzyme activity is generally measured when substrate concentration is unlimiting.

1.6.2 Substrate Concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum.

After this point, increases in substrate concentration will not increase the velocity. It is theorized that when this maximum velocity had been reached, all of the available enzyme has been converted to ES, the enzyme substrate complex. Michaelis constants have been determined for many of the commonly used enzymes. The size of Km tells us several things about a particular enzyme.

1) A small Km indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations.

2) A large Km indicates the need for high substrate concentrations to achieve maximum reaction velocity.

3) The substrate with the lowest Km upon which the enzyme acts as a catalyst is frequently assumed to be enzyme's natural substrate, though this is not true for all enzymes.

1.6.3 Allostery

Allostery or allosteric regulation is the regulation of an enzyme or other protein by binding an effector molecule at the protein's allosteric site. The allosteric site is a site other than the active site of the enzyme. Effectors that enhance the enzyme's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors. Following this mechanism, allosteric inhibition is a form of non-competitive inhibition (see 1.6.6.3).

The term allostery comes from the Greek allos, "other," and stereos, "space," referring to the regulatory site of an allosteric protein's being separate from its active site. Allosteric regulation is a natural example of feedback control.

1.6.4 Cofactors

Many enzymes require the presence of other compounds, called cofactors, which are needed in order to demonstrate their catalytic activity. This entire active complex is referred to as the holoenzyme; i.e., the apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) together is called the holoenzyme.

A cofactor may be:

1) A coenzyme - a non-protein organic substance which is dialyzable, thermostable and

loosely attached to the protein part.

2) A prosthetic group - an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.

3) A metal-ion-activator - these include K^{+}, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++},and Mo^{+++}.

Enzymes usually bind these cofactors in or close by the active site. Furthermore, some enzymes can additionally bind other molecules than substrates or cofactors and these are often molecules inhibiting the enzyme or are interfering with the catalytic process. In this way the enzymatic reaction can be regulated by the cell. Some enzymes do not need any additional components to show full activity. However, others require non-protein molecules called cofactors to be bound for activity. Cofactors can be either inorganic (e.g., metal ions or iron- sulphur clusters) or organic compounds, (e.g., flavin or heme). Organic cofactors can be either prosthetic groups, which are tightly bound to an enzyme, or coenzymes, which are released from the enzyme's active site during the reaction. Coenzymes include NADH, NADPH and adenosine triphosphate. These molecules act to transfer chemical groups between enzymes.

The tightly-bound cofactors are usually found in the active site and are involved in catalysis. For example, flavin and heme cofactors are often involved in redox reactions.

1.6.5 Coenzymes

Coenzymes are small organic molecules that transport chemical groups from one enzyme to another [17]. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins. Such compounds cannot be made in the body and must be acquired from the diet. The chemical groups carried include the hydride ion (H^{-}) carried by NAD or $NADP^{+}$, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosylmethionine.

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 700 enzymes are known to use coenzyme NADH [18].

Coenzymes are usually regenerated and their concentrations maintained at a steady level inside the cell.

1.6.6 Inhibitors

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis.

Most theories concerning inhibition mechanisms are based on the existence of the enzyme- substrate complex ES. Substrate inhibition will sometimes occur when excessive amounts of substrate are present. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition. Besides these inhibitor types, a mixed-inhibition exists as well.

1.6.6.1 Competitive inhibitors

Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. The "lock-key theory" of enzyme catalysts is used to explain why inhibition occurs. The concept holds that one particular portion of the enzyme surface has a strong affinity for the substrate. The substrate is held in such a way that its conversion to the reaction products is more favourable. However, when an inhibitor which resembles the substrate is present, it will compete with the substrate for the position in the active site. When the inhibitor is bound to the active site of the enzyme, it blocks this position in the active site, but is not converted by the enzyme. In this way, the active site remains blocked. Hence, the observed reaction is slowed down because a part of the available enzyme sites are occupied by the inhibitor. If a dissimilar substance which does not fit the site is present, the enzyme rejects it, accepts the substrate, and the reaction proceeds normally.

In competitive inhibition the maximal velocity of the reaction is not changed, but higher substrate concentrations are required to reach a given velocity, increasing the apparent K_m.

1.6.6.2 Uncompetitive inhibition

In uncompetitive inhibition the inhibitor can not bind to the free enzyme, but only to the ES-complex. The EIS-complex thus formed is enzymatically inactive. This type of inhibition is rare, but may occur in multimeric enzymes.

1.6.6.3 Non-competitive inhibition

Non-competitive inhibitors are considered to be substances which when added to the enzyme alter the enzyme in a way that it cannot accept the substrate.

Non-competitive inhibitors can bind to the enzyme at the same time as the substrate, i.e. they never bind to the active site. In this way a complex is formed of enzyme, substrate and inhibitor (EIS). Both the EI and EIS complexes are enzymatically inactive. Because the inhibitor cannot be driven from the enzyme by higher substrate concentration (in contrast to competitive inhibition), the apparent V_{max} changes. But because the substrate can still bind to the enzyme, the K_m stays the same.

1.6.6.4 Mixed inhibition

This type of inhibition resembles the non-competitive, except that the EIS-complex has residual enzymatic activity.

In many organisms inhibitors may act as part of a feedback mechanism. If an enzyme produces too much of one substance in the organism, that substance may act as an inhibitor for the enzyme at the beginning of the pathway that produces it, causing production of the substance to slow down or stop when there is sufficient amount. This is a form of negative feedback. Enzymes which are subject to this form of regulation are often multimeric and have allosteric binding sites for regulatory substances. Irreversible inhibitors react with the enzyme and form a covalent adduct with the protein.

1.7 Industrial enzymes

For centuries, enzymes have been employed in a variety of applications such as beer and cheese production. Both in the past and currently enzymes have been derived from natural sources such as the tissue of plants and animals; Table 1.1 summarises these. However, over the years, advancements in biotechnology have resulted in newer and more highly efficient varieties of enzymes.

Table 1.1

The industrial success of enzymes can be attributed to certain key benefits that enzymes offer in comparison with chemicals. The combination of catalytic function, specificity and the ability to work under reasonably mild conditions, make enzymes the preferred catalyst in a variety of applications.

Industrial enzymes are prepared and commercialized as partly purified or `bulk' enzymes, as opposed to highly purified enzymes for analytical or diagnostic use. Industrial enzymes may be derived from a wide variety of plant, animal or microbial sources, although most production processes rely on microbial sources. Microbial enzymes are either extracellular, such as the proteases and carbohydrates, which account for a large proportion of total sales, or intracellular, such as glucose oxidase. Intracellular enzymes usually remain associated with the cell and therefore have to be released, unless the microorganism itself is used as the catalyst.

The real break through of enzymes occurred with the introduction of microbial proteases into washing powders. The first commercial bacterial Bacillus protease was marketed in 1959 and the first major detergent manufacturer started to use it around 1963.

The industrial enzyme producers sell enzymes for a wide variety of applications. The estimated value of world market is presently about US $ 2.2 billion. Detergents (30%) , textiles (12 %), starch (12 %), baking (11 %), biofuel (9 %) and animal feed (8 %) are the main industrial applications, which use about >80 % of industrially produced enzymes.

Industrial enzymes represent the heart of biotechnology. Advancements in biotechnology and genomics have aided the discovery of fresh enzyme sources and production strains for commercialization. The operating conditions and performance of enzyme candidates can be tuned to provide the desired performance.

Enzymes can be used not only for chemical processes, but also for mechanical and physical processes. An example of a chemical reaction is the use of amylases to replace acid in the hydrolysis of starch. The use of cellulose degrading or modifying enzymes instead of pumice stone for the abrasion of denim is a perfect example of enzymes replacing a mechanical process. Employing protease enzymes can easily perform physical processes such as high temperature resistance for laundry cleaning.

With advances in biotechnology, the horizon of enzyme applications is getting broader day by day. Enzymes are now being used in newer processes that could compete with synthetic processes which were previously not commercially viable. For example, several companies are nowadays developing newer enzymes that could convert cellulosic biomass into ethanol to be blended in fuels. Other examples include the use of enzyme technology when making sugars from starch, which helped turn high fructose corn syrup production into a multi-billion dollar industry.

Most industrial enzymes are produced by modified microorganisms (by recombinant DNA techniques) for the following reasons:

1) Higher expression levels
2) Higher purity (% enzyme protein versus : % other components)
3) Cheaper production due to the above
4) Recombinant DNA techniques open the door to engineering the enzyme protein
5) Enzymes can be expressed which originate from organisms which have low expression levels or which are pathogenic.

Protein engineering (item 4 in the list above) can improve enzymes with regards to e.g. oxidation resistance, improved processing tolerance, changed substrate specificity, improved thermostability and improved storage stability, e.g. in detergent systems containing bleach agents.

Recombinant DNA techniques may open the door to the application of enzymes from so-called extremophiles. These are microorganisms which can, in contrast to mesophiles, grow under extreme conditions. Such organisms grow under the following conditions:

- Thermophiles (high temperature > 90^{o}C stability)
- Psycrophiles (Extreme Low temperatures 0^{o}C or lower)
- Thermoacidophiles (high temperature, low pH)
- Barophiles (high pressure)
- Halophiles (high concentrations of salt)
- Alkaliphiles (high pH)

- Acidophiles (low pH)

It can be imagined that such organisms either produce a different range of enzymes than mesophiles, or produce enzymes with extreme characteristics, such as temperature or stability and activity at extreme pH values.

1.8. Food enzymes

1.8.1. Food Biotechnology

The food industry is using a wide variety of crop plants and animal products as basis for their manufacturing processes, leading to an even wider variety of consumer products. Biotechnology, which has been used to manufacture food products for more than 8,000 years , offers ways to improve the processing of raw materials into final products. Bread, alcoholic beverages, vinegar, cheese and yogurt and many other foods have been made using enzymes which were found in various microorganisms. Today biotechnology is still affecting the food industry by providing new products, lowering costs and improving processes on which food producers have long relied. Without a doubt, this will continue into the future.

Using biotechnology, improvements in functionality, nutritional value, sensoric properties, like flavor and texture have been achieved as well as improvements in the processing itself, using new tools, such as enzymes, emulsifiers and improved starter cultures. Biotechnology also offers improved ways to deal with waste problems, food safety problems, packaging issues, etc.

1.8.2 Food enzyme application

Enzymes can modify and improve the functional, nutritional and sensoric properties of ingredients and products and therefore enzymes have found widespread applications in processing and production of all kind of food products.

In food production, enzymes have a number of advantages. First and most important is that enzymes are used as alternatives to traditional chemical based technology. Enzymes can thus replace synthetic

chemicals in a wide range of processes. This allows advantages in environmental performance of processes by lowering energy consumption levels and biodegradability of products. Furthermore, since enzymes are more specific in their action than chemical reactants, enzyme catalyzed processes have fewer side reactions and by-products (waste products). The result is higher quality products and less pollution. Enzymes can catalyze reactions under very mild conditions, allowing mild processing conditions which do not destroy valuable attributes of foods and food components. Finally, enzymes allow processes to be carried out which would be otherwise impossible.

The first commercial food product produced by biotechnology was an enzyme used in cheese making. Prior to biotech techniques, this enzyme had to be extracted from the stomach of calves, lambs and baby goats, but it is now produced by microorganisms that were given the gene for this enzyme.

The food industry uses more than 55 different enzyme products in food processing. This number will increase as we discover how to capitalize on the extraordinary diversity of the microbial world and obtain new enzymes that will prove important in food processing. A brief summary of enzymes used in various food applications is shown in table 1.2

Table 1.2

1.9 Genetic engineering

Since the early 1980s, companies which produce enzymes have been using genetic engineering techniques to improve production efficiency and quality and to develop new products. There are clear advantages here for both industry and consumers, with major improvements in enzyme production giving better products and processes. However, progress is being slowed down because the debate on some other, more controversial applications of biotechnology - such as genetic engineering in animals - is continuing throughout Europe.

1.10 Enzyme allergy

To date, there have been no reports of consumer allergies to enzyme residues in food. The levels of enzyme residues appearing in foods are so low that they are highly unlikely ever to cause allergies. Like all proteins, enzymes can cause allergic reactions when people have been sensitised through exposure to large quantities. For this reason, enzyme companies take a variety of protective measures and some enzymes are produced as liquids, granules, in capsules or as immobilised preparations to limit worker exposure.

1.11 Summary and Conclusions

From the earliest application of enzymes derived from plant or animal origin enzymologists and biotechnologists continue to develop an increasingly sophisticated range of enzymes for specific food applications. To name but a few examples; their products enable the removal or minimisation of chemicals used for food processing, minimisation of waste, facilitate processes which may otherwise be impractical or uneconomic, enhance nutrition, texture and extend shelf life. The more recent techniques of protein engineering of enzymes will enable technologists to drive these valuable tools into the future.

Table 1.1 Enzymes widely sourced from animals and plants used in food manufacturing technology

Enzyme	Source	Action in food	Food applications
alpha-Amylase	Cereal seeds e.g. wheat, barley	Starch hydrolysis to oligosaccharides	Bread making brewing (malting)
beta-Amylase	Sweet potato	Starch hydrolysis to maltose	Production of high malt syrups
Papain	Latex of unripe papaya fruit	Food and beverage protein hydrolysis	Meat tenderisation chill haze prevention in beer
Bromelain	Pineapple juice and stem	Muscle and connective tissue protein hydrolysis	Meat tenderisation
Ficin	Fig fruit latex	As bromelain	As bromelain and papain but not widely used due to cost
Trypsin	Bovine/porcine pancreas	Food protein hydrolysis	Production of hydrolysates for food flavouring (mostly replaced now by microbial proteinases)
Chymosin (rennet)	Calf abomasum	*kappa*-Casein hydrolysis	Coagulation of milk in Cheese making
Pepsin	Bovine abomasum	As chymosin + more general casein hydrolysis in cheese	Usually present with chymosin as part of 'rennet'
Lipase/esterase	Gullet of goat and lamb; calf abomasum pig pancreas	Triglyceride (fat) hydrolysis	Flavour enhancement in; cheese products; fat function modification by interesterification
Lipoxygenase	Soy bean	Oxidation of unsaturated fatty acids in flour	Bread dough improvement
Lysozyme	Hen egg white	Hydrolysis of bacterial cell wall polysaccharides	Prevention of late blowing defects in cheese by spore-forming bacteria
Lactoperoxidase	Cheese whey; bovine colostrum	Oxidation of thiocyanate ion to bactericidal hypothiocyanate	Cold sterilisation of milk

Enzyme	**Source**	**Action in food**	**Application in food technology**
alpha-Amylase	*Aspergillus* spp. *Bacillus* spp *Microbacterium imperiale*	Wheat starch hydrolysis	Amylase Dough softening, increased bread volume, aid production of sugars for yeast fermentation
alpha-Acetolactate	*Bacillus subtilis*	Converts acetolactate to acetoin	Reduction of wine maturation time by circumventing need decarboxylase for secondary fermentation of diacetyl to acetoin
Amyloglucosidase	*Aspergillus niger* *Rhizopus* spp.	Hydrolyses starch dextrins to glucose (saccharification)	One stage of high fructose corn syrup production; production of 'lite' beers
Aminopeptidase	*Lactococcus lactis* *Aspergillus* spp. *Rhizopus oryzae*	Releases free amino acids from N-terminus of proteins and peptides	Debittering protein hydrolysates accelerating cheese maturation
Catalase	*Aspergillus niger* *Micrococcus luteus*	Breaks down hydrogen peroxide to water and oxygen	Oxygen removal technology, combined with glucose oxidase
Cellulase	*Aspergillus niger* *Trichoderma* spp	Hydrolyses cellulose	Fruit liquefaction in juice production
Chymosin	*Aspergillus awamori* *Kluyveromyces lactis*	Hydrolyses *kappa*-casein	Coagulation of milk for cheese making
Cyclodextrin glucanotransferase	*Bacillus* spp	Synthesise cyclodextrins from liquefied starch	Cyclodextrins are food-grade micro-encapsulants for colours, flavours and vitamins
beta-Galactosidase(lactase)	*Aspergillus* spp. *Kluyveromyces* spp.	Hydrolyses milk lactose to glucose and galactose	Sweetening milk and whey; products for lactose-intolerant individuals; reduction of crystallisation in ice cream containing whey; improving functionality of whey protein concentrates; manufacture of lactulose
beta-Glucanase	*Aspergillus* spp. *Bacillus subtilis*	Hydrolyses beta-glucans in beer mashes	Filtration aids, haze prevention in beer production
Glucose isomerase	*Actinplanes missouriensis* *Bacillus coagulans*	Converts glucose to fructose	Production of high fructose corn syrup (beverage sweetener)

	Streptomyces lividans *Streptomyces rubiginosus*		
Glucose oxidase	*Aspergillus niger* *Penicillium chrysogenum*	Oxidises glucose to gluconic acid	Oxygen removal from food packaging; removal of glucose from egg white to prevent browning
Hemicellulase and xylanase	*Aspergillus* spp *Bacillus subtilis* *Trichoderma reesei*	Hydrolyses hemicelluloses(insoluble non-starch polysaccharides in flour)	Bread improvement through improved crumb structure
Lipase and esterase	*Aspergillus* spp *Candida* spp *Rhizomucor miehei* *Penicillium roqueforti* *Rhizopus* spp. *Bacillus subtilis*	Hydrolyses triglycerides to fatty . acids and glycerol; hydrolyses alkyl esters to fatty acids and alcohol	Flavour enhancement in cheese products; fat function modification by interesterification; synthesis of flavour esters
Pectinase(polygalacturonase)	*Aspergillus* spp. *Penicillium funiculosum*	Hydrolyses pectin	Clarification of fruit juices by depectinisation
Pectinesterase	*Aspergillus* spp	Removes methyl groups from galactose units in pectin	With pectinase in depectinisation technology
Pentosanase	*Humicola insolens* *Trichoderma reesei*	Hydrolyses pentosans (soluble non-starch polysaccharides in wheat flours)	Part of bread dough improvement technology
Pullulanase	*Bacillus* spp *Klebsiella* spp.	Hydrolyses 1-6 bonds that form branches' in starch structure	Starch saccharification (improves efficiency)
Protease(proteinase)	*Aspergillus* spp *Rhizomucor miehei* *Cryphonectria parasitica* *Penicillium citrinum Rhizopus niveus Bacillus* spp.	Hydrolysis of *kappa*-casein; hydrolysis of animal and vegetable food proteins; hydrolysis of wheat glutens	Milk coagulation for cheese making; hydrolysate production for soups and savoury foods; bread dough improvement

Table 1.2