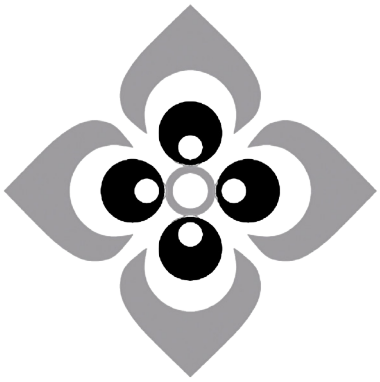

Consortium for Educational Communication

Module

on

Enzymes and their applications in food processing

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Text

Enzymes are proteins with catalytic activity due to their power of specific activation and conversion of substrates to products:

$$\text{Substrate(s)} \xrightarrow{\text{enzyme}} \text{product(s)}$$

Some of the enzymes are composed only of amino acids covalently linked via peptide bonds to give proteins that range in size from about 12,000 MW to those that are near 1,000,000 MW. Other enzymes contain additional components, such as carbohydrate, phosphate, and cofactor groups. Enzymes have all the chemical and physical characteristics of other proteins. Composition-wise, enzymes are not different from all other proteins found in nature and they comprise a small part of our daily protein intake in our foods. However, unlike other groups of proteins, they are highly specific catalysis for the thousands of chemical reactions required by living organisms. Enzymes are found in all living systems and make life possible, whether the organisms are adapted to growing near 0°C, at 37°C

(humans), or near 100°C (in microorganisms found in some hot springs). Enzymes accelerate reactions by factors of 10^3 to 10^{11} times that of non-enzyme-catalyzed reactions (10^8 to 10^{20} over uncatalyzed reactions). In addition, they are highly selective for a limited number of substrates, since the substrate(s) must bind stereospecifically and correctly into the active site before any catalysis occurs. Enzymes also control the direction of reactions, leading to stereospecific product(s) that can be very valuable by-products for foods, nutrition, and health or the essential compounds of life.

Enzyme nomenclature

Any particular enzyme only catalyses reactions between one type (or a narrow range) of chemical compound (its substrate). This defines its

'specificity' and provides the basis of its classification and name. The first enzyme to be named was catalase, which converts hydrogen peroxide to water and O_2 (Eq. 1).

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \text{ ---------- 1}$$

Its name was derived by using the stem of catalyst and adding ase. This generic type name is unfortunate considering the many enzymes that have since been discovered. The name of the second enzyme, diastase, was derived from the Greek word diastasis, meaning to separate. The name of the third enzyme, peroxidase, was based on one of the substrates being peroxide. Polyphenol oxidase was so named because it oxidizes numerous phenols, while invertase inverts the optical rotation of a solution of sucrose ([a] =+66.5°) to [a] =-19.7° due to formation of equimolar concentrations of glucose ([a] =+52.7°) and fructose ([a] =-92°). Note that two general principles began to be used in naming some enzymes: *-ase* to designate an enzyme, while in two cases the stem was derived from the name of one of the substrates (polyphenol oxidase from phenols and peroxidase from peroxide). In the case of polyphenol oxidase, it is clear the enzyme catalyzes oxidation of phenols.

The Enzyme Commission

An International Commission on Enzymes of the International Union of Biochemistry was established and its charge was "to consider the classification and nomenclature of enzymes and coenzymes, their units of activity and standard methods of assays, together with the symbols used in the description of enzyme kinetics."

Rules for Naming

The basis for the classification adopted by the Enzyme Commission was to divide the enzymes into groups on the basis of the type of reaction catalyzed, and this, together with the name(s) of the substrate(s), provided a basis for naming individual enzymes. There are six types of reactions catalyzed by enzymes: (a) oxidoreduction, (b) transfer, (c) hydrolysis, (d) formation

of double bonds without hydrolysis, (e) isomerization, and (f) ligation. The names of the type of enzymes are formed by adding -ase to the stem of the type of reaction catalyzed. Thus the corresponding six groups of enzymes are (a) oxidoreductases, (b) transferases, (c) hydrolases, (d) lyases, (e) isomerases, and (f) ligases.

Six Main Types of Enzymes

The six main types of enzymes, based on the chemical reaction catalyzed, are further explained in this section.

Oxidoreductases

Oxidoreductases are enzymes that oxidize or reduce substrates by transfer of hydrogens or electrons or by use of oxygen. The systematic name is formed as "donor:acceptor oxidoreductase." An example, including systematic name followed by trivial name and EC number in parenthesis, is:

H2O2 + H2O2 = O2 + 2H2O

which gives hydrogen peroxide:hydrogen peroxide oxidoreductase (catalase, EC 1.11.1.6).

Transferases

Transferases are enzymes that remove groups (not including H) from substrates and transfer them to acceptor molecules (not including water). The systematic name is formed as "donor:acceptor group-transferred-transferase." An example is:

ATP + D-glucose = ADP + D-glucose 6-phosphate

which gives ATP:D-glucose 6-phosphotransferase (glucokinase, EC 2.7.1.2). Note that the position to which the group is transferred is given in the systematic name when more than one possibility exists.

Hydrolases

Hydrolases are enzymes in which water participates in the breakage of

covalent bonds of the substrate, with concurrent addition of the elements of water to the principles of those bonds. The systematic name is formed as "substrate hydrolase." Water is not listed as a substrate, even though it is, because it is 55.6 *M* and the concentration does not change significantly during the reaction. When the enzyme specificity is limited to removal of a single group, the group is named as a prefix, for example, "adenosine aminohydrolase." Another example is

Triacylglycerol + H2O = diacylglycerol + a fatty acid anion

catalyzed by triacylglycerol acylhydrolase (triacylglycerol lipase, EC 3.1.1.3).

Lyases

Lyases are enzymes that remove groups from their substrates (not by hydrolysis) to leave a double bond, or which conversely add groups to double bonds. The systematic name is formed as "substrate prefix-lyase." Prefixes such as "hydro-" and "ammonia-" are used to indicate the type of reaction—for example, "L-malate hydro-lyase" (EC 4.2.1.2). Decarboxylases are named as carboxy-lyases. A hyphen is always written before "lyase" to avoid confusion with hydrolases, carboxlases, etc. An example is:

(*S*)-Malate = fumarate + H2O

using the enzyme (*S*)-malate hydro-lyase (fumarate hydratase, EC 4.2.1.2; formerly known as fumarase).

Isomerases

Isomerases are enzymes that bring about an isomerization of substrate. The systematic name is formed as "substrate prefixisomerase." The prefix indicates the types of isomerization involved, for example, "maleate *cis-trans*- isomerase" (EC 5.2.1.) or "phenylpyruvate keto-enolisomerase" (EC 5.3.2.1). Enzymes that catalyze and aldose-ketose interconversion are known as "ketol-isomerases," for example, "L-arabinose ketol-isomerase" (EC 5.3.1.4). When the isomerization consists of an intramolecular transfer of a group, such as 2-phospho-D-glycerate = 3-phospho-D-glycerate, the enzyme is named a "mutase," for example, "D-phosphoglycerate 2,

3-phosphomutase" (EC 5.4.2.1). Isomerases that catalyze inversions of asymmetric groups are termed "racemases" or "epimerases," depending on whether the substrate contains one or more than one center of asymmetry, respectively. A numerical prefix is attached to the word "epimerase" to show the position of inverstion. An example is

L-Alanine = D-alanine

with alanine racemase (alanine recemase, EC 5.1.1.1).

Ligases

Ligases are enzymes that catalyze the covalent linking together of two molecules, coupled with the breaking of a pyrophosphate bond as in ATP. This group of enzymes has previously been referred to as the "synthetases." The systematic name is formed as "X:Y ligase (Z)," where X and Y are the two molecules to be joined together. The compound Z is the product formed from the triphosphate during the reaction. An example is:

ATP + L-aspartate + NH3 = AMP + pyrophosphate + L-asparagine

with L-aspartate:ammonia ligase (AMP-forming) (aspartate-ammonia ligase, EC 6.3.1.1).

Enzyme Kinetics and Reaction Order:

Arrhenius first pointed out that, all the molecules in a given population do not have the same kinetic energy some molecules are energy poor and other are energy rich. Higher is the energy barrier the grater is the inactiveness of reaction. This energy barrier can be overcome by the enzymes and making the molecule active with available energy level.

Reaction order is determined from the dependence of velocity (*dP/dt,* or *-dS/dt*) on the concentration(s) of the reactant(s). For an enzyme appropriate kinetic description is given by Equation 2, where E is enzyme, S is substrate, E.S is the enzyme substrate complex, and P is product.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E + P \quad \text{---------- 2}$$

The velocity of formation of E.S (d[E.S]/dt) is*k*1 [E][S], while the velocity of disappearance of E.S (-d[E.S]/*dt*) is *k*-1(E.S) + *k*2 [E.S]. Steady state conditions exist when ever d(E.S)/dt =-d(E.S)/dt for ~5 msec. The Michaelis-Menten equation (Eq. 3), derived from Equation 2, is based on the following assumptions:

$$v_o = \frac{V_{max}[S]_o}{K_m + [S]_o} = \frac{k_2[E]_o[S]_o}{K_m + [S]_o} \text{ ---------- 3}$$

1. Initial velocity, no, is used so that [S]o [S]. As discussed earlier, this is common practice.

2. [S]o>>[E]o, so that there is little change is [S] o. Practically, this is the case with most enzymes where [S]o is of the order of 10^{-4} to $10^{-2}M$ since *K*m is generally within this range and [E]o is on the order of 10^{-8} -$10^{-6}M$.

3. The step controlled by *k*2 (Eq. 2) is irreversible in reality or because no is used in ([P] is essentially zero). Therefore, *k*-2 is ~0.

4. *d*(E.S/*dt*=-*d*(E.S)/*dt,* so steady-state conditions prevail.

5. *k*2 controls the velocity of formation of product (*dP/dt*). If *k*2 > *k*1 then *k*1 controls velocity of product formation. Note that

Vmax is not really a constant in Equation 3, since it is dependent on [E] o (Vmax = *k*2 [E]o); *k*2 is a constant, but Vmax will change when [E]o is changed.

6. If any one of these assumptions is not true then the form of the Michaelis-Menten equation will be more complex, even through a plot of no versus [S]o will be hyperbolic. For example, if there is an additional intermediate in the reaction, such as an acylenzyme (Eq. 4); then Equation 4 applies:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \xrightarrow{k_2} E - X \xrightarrow{k_3} E + P \text{ ---------- 4}$$

and the Michaelis-Menten equation is

$$v_o = \frac{V_{max}[S]_o}{K_m + [S]_o} = \frac{[k_2k_3/(k_2 + k_3)][E]_o[S]_o}{(k_{-1} + k_2)k_3/k_1(k_2 + k_3) + [S]_o} \text{ ---------- 6}$$

In the preceding equations, [E]o is total enzyme concentration, [E] is free enzyme concentration, [E.S] is enzyme-substrate concentration, [S]o is initial substrate concentration, [S] is substrate concentration at any time *t,* and Km = ($k2$ + k-1)/k-1 for Equation 3 and (k-1 + $k2$)$k3$/$k1$ ($k2$ + $k3$) for Equation 6.

Several theories have been put forwarded by different biochemists to explain the mechanism of the enzyme action.

(i) Lock and key theory:

According to Fildes only a specific substrate can combine with the active site of a particular enzyme as a specific key fits to open a specific lock. In this enzyme molecule posses an active site to fit correctly with the substrate forming ES complex. When reaction completed ES complex breaks into products and enzymes. Enzymes remain intact.

(II) Inducted fit Theory:

According to Koshland, when a suitable substrate approaches the active site of an enzyme, the substrate inducts some conformational changes in the enzyme as a result the attractive groups and buttressing groups form a complementary structure so that the catalytic group of the active site is in proximity of the bonds to be broken.

After the suitable enzyme substrate complex has been formed, the subtract molecule is held by hydrogen bonds while a strain nucleophilic attack of the charged catalytic groups of the active site. The strain weaker the bond which is ultimately broken and the products are formed.

Food Modification by Enzymes

Enzymes have a very important impact on the quality of our foods. In fact, without enzymes there would be no food. But then there would be no need for food, since no organism could live without enzymes. They are the catalysts that make life possible, as we know it. Following maturation,

the harvesting, storage, and processing conditions can markedly affect the rate of food deterioration. Enzymes can also be added to foods during processing to change their characteristics. Microbial enzymes, left after destruction of the microorganisms, continue to affect the quality of processed and reformulated foods. For example, starch-based sauces can undergo undesirable changes in consistency because of heat-stable microbial α-amylases that survive a heat treatment sufficient to destroy the microorganisms. Because of their high specificity, enzymes are also the ideal catalysts for the biosynthesis of highly complex chemicals.

Role of Endogenous Enzymes in Food Quality

Color

Color is probably the first attribute the consumer associates with quality and acceptability of foods. A steak must be red, not purple or brown. Redness is due only to oxymyoglobin, the main pigment in meat. Deoxymyoglobin is responsible for the purple color of meat. Oxidation of the Fe(II) present in oxymyoglobin and deoxymyoglobin, to Fe(III) producing metmyoglobin, is responsible for the brown color of meat. Enzyme-catalyzed reactions in meat can compete for oxygen, can produce compounds that alter the oxidation-reduction state and water content, and can thereby influence the color of meat.

The quality of many fresh vegetables and fruits is judged on the basis of their "greenness." On ripening, the green color of many of our fruits decreases and is replaced with red, orange, yellow, and black colors. In green beans and English green peas, maturity leads to a decrease in chlorophyll level. All of these changes are a result of enzyme action. Three key enzymes responsible for chemical alterations of pigments in fruits and vegetables are lipoxygenase, chlorophyllase, and polyphenol oxidase.

Lipoxygenase

Lipoxygenase (lineoleate:oxygen oxidoreductase; EC 1.13.11.12) has six important effects on foods, some desirable and others undesirable. The two desirable functions are (a) bleaching of wheat and soybean flours and (b)

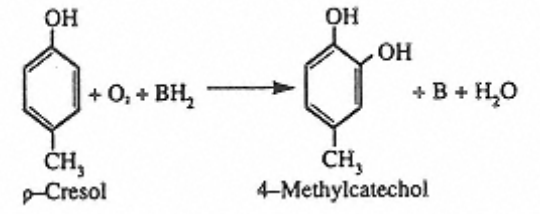
formation of disulfide bonds in gluten during dough formation (eliminates the need to add chemical oxidizers, such as potassium bromate). The four undesirable actions of lipoxygenase in food are (a) destruction of chlorophyll and carotenes, (b) development of oxidative off flavors and aromas, often characterized as hay like, (c) oxidative damage to compounds such as vitamins and proteins, and (d) oxidation of the essential fatty acids, lineoleic, linolenic, and arachidonic acids. All six of these reactions result from the direct action of lipoxygenase in oxidation of polyunsaturated fatty acids (free and lipidbound) to form free radical intermediates.

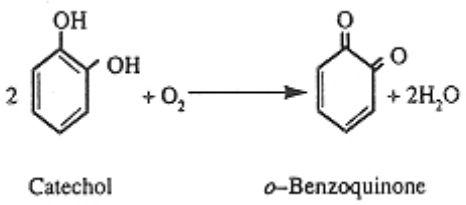
Chlorophyllase

Chlorophyllase (chlorophyll chlorophyllido-hydrolase, EC 3.1.1.14) is found in plants and chlorophyll-containing microorganisms. It hydrolyzes the phytyl group from chlorophyll to give phytol and chlorophyllide. Although this reaction has been attributed to a loss of green color, there is no evidence to support this as chlorophyllide is green. Furthermore, there is no evidence that the chlorophyllide is any less stable to color loss (loss of Mg^{2+}) than is chlorophyll. The role of chlorophyllase in vivo in plants is not known. Very few studies have been done on chlorophyllase-catalyzed hydrolysis of chlorophyll during storage of raw plant foods.

Polyphenol Oxidase

Polyphenol oxidase (1,2-benzenediol:oxygen oxidoreductase; EC 1.10.3.1) is frequently called tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, or catecholase, depending on the substrate used in its assay or found in the greatest concentration in the plant that serves as a source of the enzyme. Polyphenol oxidase is found in plants, animals and some microorganisms, especially the fungi. It catalyzes two quite different reactions with a large number of phenols.





Catechol *o*–Benzoquinone

The 4-methyl-*o*-benzoquinone is unstable and undergoes further non-enzyme-catalyzed oxidation by O_2, and polymerization, to give melanins. The latter is responsible for the undesirable brown discoloration of bananas, apples, peaches, potatoes, mushrooms, shrimp, and humans (freckles), and the desirable brown and black colors of tea, coffee, raisins, prunes, and human skin pigmentation.

Texture

Texture is a very important quality attribute in foods. In fruits and vegetables, texture is due primarily to the complex carbohydrates: pectic substances, cellulose, hemicelluloses, starch, and lignin. There are one or more enzymes that act on each of the complex carbohydrates that are important in food texture. Proteases are important in the softening of animal tissues and high-protein plant foods.

Pectic Enzymes

Three types of pectic enzymes that act on pectic substances are well described. Two (pectin methylesterase and polygalacturonase) are found in higher plants and microorganisms and one type (the pectate lyases) is found in microorganisms, especially certain pathogenic microorganisms that infect plants. The pectic enzymes cause the softening of fruits and vegetables during storage.

Cellulases

Cellulose is abundant in trees and cotton. Fruits and vegetables contain small amounts of cellulose, which has a role in the structure of cells. Whether cellulases are important in the softening of green beans and English green pea pods is still a matter of controversy. Abundant information is available on the microbial cellulases because of their potential importance in converting

insoluble cellulosic waste to glucose.

Pentosanases

Hemicelluloses, which are polymers of xylose (xylans), arabinose (arabans), or xylose and arabinose (arabinoxylans), with small amounts of other pentoses or hexoses, are found in higher plants. Pentosanases in microorganisms, and in some higher plants, hydrolyze the xylans, arabans, and arabinoxylans to smaller compounds. The microbial pentosanases are better characterized than those in higher plants.

Several *exo*- and *endo*-hydrolyzing pentosanases also exist in wheat at very low concentrations, but little is known about their properties. It is important that these pentosanases receive more attention from food scientists.

Amylases

Amylases, the enzymes that hydrolyze starches, are found not only in animals, but also in higher plants and microorganisms. Therefore, it is not surprising that some starch degradation occurs during maturation, storage, and processing of our foods. Since starch contributes in a major way to viscosity and texture of foods, its hydrolysis during storage and processing is a matter of importance. There are three major types of amylases: α-amylases, β-amylases, and glucoamylases. They act primarily on both starch and glycogen. The α-amylases, found in all organisms, hydrolyze the interior α-1, 4-glucosidic bonds of starch (both amylose and amylopectin), glycogen, and cyclodextrins with retention of the α-configuration of the anomeric carbon. Since the enzyme is *endo*-splitting, its action has a major effect on the viscosity of starch-based foods, such as puddings, cream sauces, etc**.** β-amylases, found in higher plants, hydrolyze the α-1,4-glucosidic bonds of starch at the non-reducing end to give β-maltose. Since they are *exo*-splitting enzymes, many bonds must be hydrolyzed before an appreciable effect on viscosity of starch paste is observed.

Proteases

Texture of food products is changed by hydrolysis of proteins by endogenous

and exogenous proteases. Gelatin will not gel when raw pine apples is added, because the pineapples contains bromelain, a protease. Chymosin causes milk to gel, as a result of its hydrolysis. This specific hydrolysis of k-casein destabilizes the casein micelle, causing it to aggregate to form a curd (cottage cheese). Action of intentionally added microbial proteases during aging of brick cheeses assists in development of flavors (flavors in Cheddar cheese vs. blue cheese, for example). Protease activity on the gluten proteins of wheat bread doughs during rising is important not only in the mixing characteristics and energy requirements but also in the quality of the baked breads.

The effect of proteases in the tenderization of meat is perhaps best known and is economically most important. After death, muscle becomes rigid due to rigor mortis (caused by extensive interaction of myosin and actin). Through action of endogenous proteases (Ca^{2+}-activated proteases, and perhaps cathepsins) on the myosin-action complex during storage (7–21 days) the muscle becomes more tender and juicy. Exogenous enzymes, such as papain and ficin, are added to some less choice meats to tenderize them, primarily due to partial hydrolysis of elastin and collagen.

Flavor and Aroma Changes in Foods

Chemical compounds contributing to the flavor and aroma of foods are numerous, and the critical combinations of compounds are not easy to determine. It is equally difficult to identify the enzymes instrumental in the biosynthesis of flavors typical of food flavors and in the development of undesirable flavors. Enzymes cause off flavors and off aromas in foods, particularly during storage. Improperly blanched foods, such as green beans, English green peas, corn, broccoli, and cauliflower, develop very noticeable off flavors and off aromas during frozen storage. Peroxidase, a relatively heat-resistant enzyme not usually associated with development of defects in food, is generally used as the indicator for adequate heat treatment of these foods. It is clear now that a higher quality product can be produced by using the primary enzyme involved in off flavor and off aroma development as the indicator enzyme. Evidence to support the

important role of lipoxygenase as a catalyst of off-flavor development in green beans. Naringin is responsible for the bitter taste of grapefruit and grapefruit juice. Naringin can be destroyed by treating the juice with naraginase. Some research is underway to eliminate naringin biosynthesis by recombinant DNA techniques.

Nutritional Quality

There is relatively little data available with respect to the effects of enzymes on nutritional quality of foods. Lipoxygenase oxidation of linoleic, linolenic, and arachidonic acids certainly decreases the amounts of these essential fatty acids in foods. The free radicals produced by lipoxygenase-catalyzed oxidation of polyunsaturated fatty acids decrease the carotenoid (vitamin A precursors), tocopherols (vitamin E), vitamin C, and folate content of foods. The free radicals also are damaging to cysteine, tyrosine, tryptophan, and histidine residues of proteins. Ascorbic acid is destroyed by ascorbic acid oxidase found in some vegetables such as squash. Thiaminase destroys thiamine, an essential cofactor involved in amino acid metabolism. Riboflavin hydrolase, found in some microorganisms, can degrade riboflavin. Polyphenol oxidase-caused browning decreases the available lysine content of proteins.

Enzymes Used as Processing Aids and Ingredients

Enzymes are ideal for producing key changes in the functional properties of food, for removal of toxic constituents, and for producing new ingredients. This is because they are highly specific, act at low temperatures (25–45°C), and do not produce side reactions. The sources and range of enzymes for food processing are shown in Table 1.

Table 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
Chymosin (rennet)	Calfabomasum	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 pan of 'rennet'

Lipase/esterase	Gullet of goat and lamb: calf abomasum: pig pancreas	Triglyceride (fat) hvdrolvsis	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

Table 2: Enzymes derived from microorganisms and used in food manufacturing technology

Enzyme	Source	Action in food	Application in food technology
alpha-Amylase	*Axpergillux* spp. *Bacillus* spp. *Micmhacterium irnperiale*	Wheat starch hydrolysis	Dough softening, increased bread volume, aid production of sugars for yeast fermentation
alpha-Acetoluctute decarboxylase	*Bacillus subtilis**	Converts acetolactate to acetoin	Reduction of wine maturation time by circumventing need for secondary fermentation of diacetyl to acetoin
	Axpergillux niger *Rhizopux* spp.	Hydrolyses starch dextrins to glucose (saccharitication)	One stage of high fructose corn syrup production; production of Mite' beers
Aminopeptidase	*Lcictococcus lactix* *Axpergillux* spp. *Rhizopux oryzae*	Releases free amino acids from N-terminus of proteins and peptides	De-bittering protein hydrolysates accelerating cheese maturation
Catalase	*Aspergillux niger** *Micrococcux lute us*	Breaks down hydrogen peroxide to water and oxygen	Oxygen removal technology, combined with glucose oxidase
Cellulase	*Axpergillux niger* *Trichodernw* spp.	Hydrolyses cellulose	Fruit liquifaction in juice production

Chymosin	*Axpergillux awtunori** *Kluyvemmycex lactix*	Hydrolyses *kappa-casein*	Coagulation of milk for cheese making
Cyclodextrin	*Bacillus* spp.*	Synthesise cyclodextrins from liquified starch	Cyclodextrins are food-grade micro-encapsulants for colours, flavours and vitamins
beta - Galactosidase (lactase)	*Axpergillux* spp. *Kluyvennnvcex* 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	*Aspergilltix* spp. *Bacillus xuhtilix**	Hydrolyses beta-glucans in beer mashes	Filtration aids, haze prevention in beer production
Glucose isomerase	*Actinplani's missouriensis* *Bacillus coagulanst* *Streptomyces lividanx** *Streptomyces rubiginosus*	Converts glucose to fructose	Production of high fructose corn syrup (beverage sweetener)

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	*Aspergillus* spp. *Penicillium funiculosum*	Hydrolyses pectin	Clarification of fruit juices by depectinisation
Pectinesterase	*Aspergillus* spp.	Removes methyl groups from galacose units in pectin	With pectinase in depectinisation technology

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 forcheesemaking; hydrolysate production for soups and savoury foods; bread dough improvement