CHAPTER

# 48

# Enzymes Used in the Food Industry: Friends or Foes?

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## 48.1 INTRODUCTION

Enzymes play an important role in the food industry in both traditional and novel products. Since ancient times, enzymes found in nature have been used in the production of food products such as cheese, beer, wine, and vinegar (Kirk et al., 2002). Enzymes, the natural catalysts for chemical reactions, are produced by all living cells. Their role in food processing has also been recognized for many centuries. Even before the knowledge about enzymes, they have been used in a number of processes, such as tenderization of meat using papaya leaves, soy sauce preparation, curd or cheese making, baking, brewing, etc. Enzymes provide a powerful, varied set of specialized tools for food and beverage preparation. Besides their catalytic ability, enzymes can enhance reactions by >1010 times. They can function exceptionally well to control process time, enrich flavor, improve texture, increase shelf life, and decrease the use of chemical food additives. One advantage of enzymes is their high activity, which makes them the best cost-effective choice of ingredients in addition to their reputation. Enzymes decompose complex molecules into smaller units such as carbohydrates into sugar. They are natural substances involved in all biochemical reactions. Because of the enzyme specifications, each substratum has corresponding enzymes. Enzymes are produced by all living cells and act as catalysts for specific chemical reactions. Enzymes are present in all foods at some time, and have played an important role in food processing practices for thousands of years (Dewdney, 1973). Adding exogenous enzymes to enhance reaction processes or to create new products has been going on since the early 20th century; gradually, it became a significant part of the food industry. From animals to plants to microbial sources, enzymes may be extracted from any living organism. Of the hundred or so enzymes being used in industries, more than half are of microbial origin. In the food industry, microbial enzymes have been extensively used to increase the diversity, variety, and quality of food. Microorganisms as enzyme sources are always preferred over other sources as large amounts of enzymes can be produced from them in a controlled manner that is also faster and cheaper. The chances of


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other potentially harmful content (phenolics in plants or endogenous enzyme inhibitors and proteases in animals) are also minimized. Some of the important microbial enzymes used in the food processing industry are lipases, amylases, proteases, rennet, pectinases, invertases, cellulases, and glucose oxidase. Apart from these, many other enzymes such as raffinase, pullulanase, catalase, and lactase that have specific roles in the food industry are also being produced from microbial sources. Fungi *Aspergillus niger, Aspergillus oryzae, Saccharomyces cervisae*, and *Bacillus subtilis* bacteria are some examples of potential microbial sources that have been harnessed for production of many commercially important enzymes. This chapter deals with the types of microbial enzymes used in food processing and the food industry as well as their physicochemical and biological properties and industrial applications. The chapter also covers recent developments in this area.

## 48.2 MAJOR MICROBIAL ENZYMES AND THEIR APPLICATIONS IN THE FOOD INDUSTRY

The use of microorganisms in food preparation such as bread, curd, cheese, and alcoholic beverages has been done since ancient times. Some yeast, mold, and bacterial species have been highly explored and were found to be highly useful in the fermentation and mass-scale production of substances. Species of *Aspergilus* (*A. niger*), *Saccharomyces* (*S. cerevisiae*), *Mucor, Serratia*, *Bacillus* (*B. subtilis, B. amyloliquefaciens, B. licheniformis*), *Lactobacillus* (*L. casei, L. acidophilus*, *L. delbruekii*), *Corynebacterium* (*C. glutamicum)*, and *Rhizopus* (*R. oryzae*) are some significant examples of microbial species that are being extensively used commercially. Production of organic acids (citric acid, lactic acid, ascorbic acid, gluconic acid), enzymes (rennet, α-amylase, glucoamylase, glucose isomerase), amino acids (glutamate, methionine, glycine, lysine, arpartate, phenyl alanine), dairy products (yogurt, cheese, fermented foods such as idli, pickled vegetables), alcoholic beverages (beer, wine), and specialized microbial products (single cell proteins—SCP, flavor enhancers) is being done primarily with the use of microorganisms. The largest application of enzymes is in the food industry, and almost 50% of all enzymes produced for industrial purposes are used in food processing. Microbial enzymes predominate in this, comprising more than $2 billion in the world market. In the food industry, enzymes have been used to yield and to enhance the quality and variety of food. Ancient traditional knowledge of brewing, cheese making, meat tenderization with papaya leaves, and condiment preparation (e.g., soy sauce and fish sauce) depended on proteolysis, although the methods were established prior to our knowledge of enzymes.

### 48.2.1 Alpha-Amylases

Alpha-amylases belong to the family of *endo*-amylases and act on starch to yield glucose and maltose. The first starch-degrading enzyme was discovered in 1811, then named α-amylases by Kühn in 1925. Later, many other forms including β-amylases have been isolated and described. It belongs to two classes, namely, *endo*-amylases and *exo*-amylases, on the basis of the sessile glycosidic bond. Among the various extracellular enzymes, α-amylase is one of the most important industrial enzymes; it has been extensively used commercially. It has successfully replaced the chemical hydrolysis of starch in the starch-processing industries (Pandey et al., 2000; Reddy et al., 2003).

#### 48.2.1.1 Sources of α-Amylase

Although α-amylases is widely distributed in various bacteria, fungi, plants, and animals as well as human beings, commercial production has been limited to only a few selected strains of fungi and bacteria. In animals, the pancreas and salivary glands are the main sources of α-amylase. In plants, it is generally present in the green parts, but grains and starchy parts have its maximum concentrations. β-Amylase is found only in plants and yields maltose as its major product. Among microorganisms, α-amylase is produced by several fungi and bacteria. The most widely used bacterial species is the mesophilic *Bacillus* spp., namely, *B. amyloliquefaciens* and *B. licheniformis,* which are extensively used for commercial production of the enzyme. *B. subtilis, B. cereus, B. stearothermophilus,* and *B. licheniformis* are also much explored as good producers of thermostable α-amylase (Coronado et al., 2000; Konsoula and Liakopoulou-Kyriakides, 2007). Halophilic amylases from halophilic bacteria such as *Chromohalobacter* sp., *Halobacillus* sp., *Haloarcula hispanica*, *Halomonas meridiana* (Kathiresan and Manivannan, 2006), and *Bacillus dipsosauri* have been reported with promising advantages in many harsh industrial processes where concentrated salt solutions are used. Fungal sources of α-Amylase are mostly the *Aspergillus* species and few species of *Penicillium*, such as *P. brunneum*, *P. fellutanum* (Erdal and Taskin, 2010).

#### 48.2.1.2 Applications of α-Amylases in the Food Industry

Many industrial, environmental, and food biotechnology processes employ this enzyme at some stage or another. Besides being used as a major food source, starch is very much harvested and processed into a variety of products such as starch hydrolysates, glucose syrup, fructose, malto-dextrin derivatives, etc. Some important industrial applications of α-amylase are:

##### 48.2.1.2.1 GLUCOSE AND FRUCTOSE PRODUCTION FROM STARCH

One of the major commercial uses of α-amylase is the production of glucose. The chemical conversion of starch into glucose syrup was developed in the early 19th century. For many decades, the diluted acid hydrolysis method was used for commercial conversion of starch into glucose syrup. However, by the late 1990s this was replaced by using a mixture of enzymes (Crabb and Shetty, 1999). The enzymatic process for starch conversion into high glucose syrup starts with liquefaction into short chain dextrins by the action of α-amylase from *B. amyloliquefaciens, B. stearothermophilus*, or *B. licheniformis*. Then saccharification to form a high concentration glucose syrup (>95%) is done by treating starch hydrolysate with fungal *exo*-glucoamylases for which *A. niger* is the primary source (Haq et al., 2010). Another significant industrial use of starch processing is the conversion of high glucose syrup into high fructose syrup. This process is carried out using the enzyme glucose isomerase. Fructose syrup is an important sweetener and additive used extensively in a wide variety of processed foods and beverages ranging from soft and fruit drinks to yogurts and breads (Parker et al., 2010).

##### 48.2.1.2.2 BAKERY INDUSTRIES

In the baking industry, α-amylase plays a major role in the enhancement of quantity, aroma, and taste of the product. Being the main constituent of bread, starch causes hardness and makes it unpleasant to eat with age as the starch crystallizes. The addition of amylase and lipase enzymes in bread making reduces this crystallization and extends the shelf life (Jegannathan and Nielsen, 2013). The enzyme is frequently used during the preparation of

muffins, soft rolls, buns, and breads wherever additional characteristics are desired, such as dough conditioning or improved crust color. α-Amylase also affects antistaling in baking bread and helps to improve the softness (Gupta et al., 2008). All undesirable changes such as a decrease in moisture content and a loss of crispiness after removal from the oven are called staling. The mechanism of bread staling remains a major area of research and extensive study because of the huge economic loss to baking industries. Different supplementations are added during bread baking to reduce staling. The additives may be chemicals, enzymes, or their combinations. The substances that may be added include hydrocolloids such as sodium alginate, carrageenan, and xanthan; emulsifiers such as lecithin and sugar esters; and oxidants such as potassium bromate, ascorbic acid, etc. (Gray and Bemiller, 2003; Guarda et al., 2004; Spendler and Jorgensen, 1997). Enzymes have also been studied as bread improvers and find more acceptance from consumers as being a natural substance. Hemicellulase, lipase, protease, xylanase, and glucose oxidase are some of the examples of bread improvers. However, starch hydrolyzing enzymes, α-amylase, and branching and debranching enzymes were found to be of more use as antistaling agents (Cole, 1982). However, the use of α-amylase still remains out of favor because a small overdose results in sticky bread.

#### 48.2.1.2.3 ALCOHOL INDUSTRY

Raw starch is extensively converted by hydrolysis and fermentation into ethanol, other distilled spirits, and biofuels. Starches such as grains and potatoes are frequently used as the substrate of ethyl alcohol (Juge et al., 2006). However, the conventional method of hydrolysis of starch to liquefy requires the use of strong chemicals (caustic soda, lime, and sulfuric acid) and heating procedures. The energy requirements for temperature control and measures of pH control add to the cost of such procedures (Gray et al., 2006; Robertson et al., 2006). Therefore, many processes have been described to maintain temperature and carry out hydrolysis, then the fermentation of starch, in one step (Reddy et al., 2009; Robertson et al., 2006). Yeast is the most widely utilized organism for industrial bioethanol production (Reddy et al., 2009; Rudolf et al., 2009) due to high productivity, better tolerance, and being in the generally regarded as safe (GRAS) category. A researcher has recently reviewed the possibility and development of starch-degrading amylases by different strains of yeast, recombinant *S. cerevisiae*, and its comparison with the exogenous addition of α-amylase. The production of raw starch-degrading amylases by recombinant *S. cerevisiae* provides opportunities for the direct hydrolysis and fermentation of raw starch to ethanol without cooking or exogenous enzyme addition. Such a consolidated bioprocess (CBP) for raw starch fermentation will substantially reduce costs associated with energy usage. *B. licheniformis*, engineered strains of *E. coli*, and *B. subtilis* are some of the good sources of thermostable α-amylases producing bacterial strains used for the hydrolysis of starch suspensions (Souza, 2010). The combination of α-amylases with gluco-amylase has been described to be the better option for improving raw starch fermentation to ethanol. The most effective mixture found was α-amylase from *Streptococcus bovis* and glucoamylase from *Rhizopus oryzae.*

#### 48.2.1.2.4 FEED INDUSTRY

A major concern for the industrial production of animal feed is that it is not fully degraded and digested by livestock, which causes underutilization of feed. The protein and minerals are also not fully utilized. The undigested feed excreted by animals leads to environmental

problems as well. To improve this, enzymes are mixed in the animal feed during mass-scale production. α-Amylase, xylanase, phytase, and protease are mixed for the purpose (Jegannathan and Nielsen, 2013). The use of α-amylase in the feed industry is being done to improve the body weight gain and feed conversion ratio. The enzyme hydrolyzes the starch polymers into fructose and glucose, which increase the digestibility of carbohydrates (Sidkey et al., 2011; Silva et al., 2006).

## 48.2.2 Protease

Protease belongs to a group of proteolytic enzymes that is to hydrolyze peptide bonds of proteins. These enzymes are widely distributed in nearly all plants, animals, and microorganisms. The partial digestion by proteases produces peptide sequences that may have biological properties and important functional food ingredients (Udenigwe and Aluko, 2012). Proteases constitute one of the most important groups of industrial enzymes, capturing almost 60% of the total enzyme market (Mala et al., 1998). The vast variety of proteases, with their specificity of action and application, have been exploited widely in the detergent, food, pharmaceutical, and leather tanning industries (Gupta and Khare, 2007; Kalpana et al., 2008). This has been considered as ecofriendly because the appropriate producers of these enzymes for commercial exploitation are nontoxic and nonpathogenic.

### *48.2.2.1 Sources of Proteases*

Proteases from all sources, that is, bacteria, fungi, virus, plants, animals, and humans, have been identified because of their important physiological roles. On the basis of site of action on protein substrates, they are broadly classified as *endo*-peptidases or *exo*-peptidases. Papain, bromelain, keratinases, and ficin are well-known proteases of plant origin (Mala et al., 1998). All these can be categorized into five major classes: cysteine, serine, aspartic, threonine, and metallo-proteases (Zamyatnin Jr., 2015). Papain, extracted from the latex of *Carica papaya* fruits, is the plant protease that has a long history of use. Bromelain, a cysteine protease, is extracted and purified from the fruits of pineapples. Other potential sources of plant erine-proteases are latex of *Wrightiatinctoria, Ipomoea carnea, Fistulosa, Euphorbia milii*, etc. (Guevara et al., 2001; Sanna and Sayed, 2001).

### *48.2.2.2 Proteases From Microbes*

Microorganisms represent a brilliant source of enzymes because of the ease of genetic manipulation and extensive diversity. On an industrial scale, they are always preferred because of various advantages, such as lower manufacturing costs, large-scale production in industrial fermentors, extensive physical and chemical characteristics, rapid culture development, and lack of effect from seasonal variations. Microbial proteases are responsible for approximately 40% of the total worldwide enzyme sales. Most commercial neutral and alkaline proteases are produced by members of the genus *Bacillus*. Neutral proteases of bacteria are active at a narrow pH range (pH 5–8) and show relatively low thermotolerance. But due to their intermediate rate of reaction, these proteases cause less bitterness in hydrolyzed food proteins compared with the animal proteinases; therefore, they are used frequently in the food industry. *Pseudomonas* is a gram-negative bacterium that produces alkaline proteolytic enzymes. A variety of diverse proteases have been isolated from multiple strains of

*Pseudomonas aeruginosa*. Fungi produce a wider variety of proteolytic enzymes than bacteria, for example, *A. oryzae* produces neutral, acid, and alkaline proteases.

#### *48.2.2.3 Applications of Proteases in Food Industry*

Proteases are of great importance in the food industry because of their biochemical characteristics such as temperature, pH, metal requirement, activity, and stability. The cost of production, ease of development, markets, and the economy of applications are added advantages. The proteases have been regularly used for purposes such as cheese making, baking, making of soy hydrolysates, and meat tenderization.

##### 48.2.2.3.1 DAIRY INDUSTRY

The important application of proteases in the dairy industry is in the manufacturing of cheese. The milk-coagulating enzymes are divided into four main categories: animal rennets, microbial milk coagulants, vegetable rennet, and genetically engineered chymosin. Both animal and microbial milk coagulating proteases belong to a class of acid aspartate proteases. In cheese making, the basic role of proteases is to hydrolyze the specific peptide bond (the Phe105-Met106 bond) to generate para-casein and macro-peptides. Chymosin is preferred because of its high specificity for casein, a major reason for its excellent performance in cheese making.

##### 48.2.2.3.2 BAKING INDUSTRY

Proteinase from *A. oryzae* has been permitted in baking since 1952 (Lyons, 1982). When proteases are mixed in flour dough, they reduce mixing time and generate a bread loaf with better texture and crust color. Bacterial proteinases are derived primarily from *B. subtilis*. These powdered enzymes are very stable and remain 95% active for up to 1 year. Proteases have been used to degrade and decrease gluten content in bakery products (Heredia-Sandoval et al., 2016). Sourdough bread, a staple food of Central and Eastern Europe, is prepared by a long fermentation process that mainly uses lactic acid bacteria such as *Lactobacillus alimentarius, Lactobacillus brevis*, etc.

##### 48.2.2.3.3 SOY SAUCE PRODUCTION

A salty seasoning agent, soy sauce is one of the most widely used Asian fermented products. It is prepared by microbial fermentation of soybeans and wheat in salt water. On an industrial scale, proteolytic and amylase enzymes are used in the digestion of deflated soybeans in salt brine. This significantly reduces the time for brine fermentation. Large amounts of protease and amylase enzymes are produced by the mold *A. oryzae* after a 48-h culture during soybean koji fermentation (Chancharoonpong et al., 2012). Further immobilized cells of *Pediococcus halophilus* bacterium, *Zygosacchromyces rouxii*, and *Candida versatilis* are used in bioreactors for lactic acid formation during soy sauce production (Luh, 1995).

##### 48.2.2.3.4 BREWING INDUSTRY

In beer production, sugar fermentation is converted into alcohol. During mashing, enzymes from malt, which is germinated barley, act on the starch of different grains to form sugars. Jegannathan and Nielsen (2013) have described an alternative malting process that uses industrially produced amylase and protease enzymes for fermentation of starch. Because malting requires grains and heat for drying, bypassing this process will save energy as well

as agricultural land. *B. subtilis* proteases are used to solubilize proteins from barley adjuncts for production of wort. Haze formation due to proteinanceous substances in beer is also hydrolyzed by microbial proteases.

#### 48.2.2.3.5 MEAT TENDERIZATION

The organoleptic quality of meat is of paramount importance for its marketability. Numerous publications have stated the importance of tender meat and consumers' willingness to pay more for such meat. Exogenous proteases such as collagenase from *Clostridium histolyticum*, aspartic protease from *A. oryzae*, thermophile protease from *Bacillus* strain, and caldolysin from *Thermus* strain are being used commercially to improve the tenderness of meat (Bekhit et al., 2014).

#### 48.2.2.3.6 INDUSTRIAL PRODUCTION OF ASPARTAME

Aspartame, also known as Nutrasweet or Equal, is an artificial noncarbohydrate, zero-calorie sweetener that is the methyl ester of dipeptide L-aspartic acid and L-phenylalanine. Aspartame is an important ingredient in >5000 consumer goods and beverages worldwide. Therefore, it has an industrial production of 3000–6000 metric tons every year. Because the L-forms only synthesize sweet aspartame, the maintenance of the stereospecificity always adds to the cost of production by chemical methods. Thus, enzymatic synthesis of aspartame is carried out by proteases, which catalyze the reverse reaction to maintain stereospecificity of the two amino acids.

### 48.2.3 Lipase

Lipases are the biocatalysts that carry out esterification, interesterification, hydrolysis, alcoholysis, acidolysis and aminolysis as well as break down fats and oils. Due to their wide applicability, lipases become one of the most important industrial enzymes. Several sources of lipases having different catalytic properties have been identified.

#### *48.2.3.1 Sources of Lipases*

In eukaryotes, lipases are involved in various stages of lipid metabolism including absorption, lipoprotein metabolism, fat digestion, and reconstitution. If lipase is produced in sufficient quantity, it can use fat stores to be burnt as fuel. In animals, lipases are found in a wide diversity of sources such as blood, gastric juices, adipose tissues, intestinal juices, and pancreatic secretion.

In the plant kingdom, lipases appear to have wide distribution, where lipases are present in energy reserve tissues. Lipases of plants origin were isolated frequently from barley, corn, and cotton (Hasan et al., 2006). The seeds belonging to the families Euphorbiaceae, Ranunculaceae, and Papaveraceae have a high lipase content. Microbial lipases may originate from fungi, molds, or bacteria and most of them are formed extracellular, therefore they are easy to recover or isolate. Yeast such as *Candida* and *Torulopsis* and filamentous fungi such as *Rhizopus, Geotrichum,* and *Humicola* are some of the sources of extracellular lipases of microbial origin. Some of the lipolytic bacterial species are *B. subtilis, P. fragi, B. megaterium, S. aureus, Burkholderiacepacia, P. aeruginosa* (Sachan and Singh, 2015), and *P. pseudo alcaligenes*. Some other common lipase-producing fungal species are *Helvinal anuginosa, Rhizopus delemar,*

*Eurotrium herbanorium, A. niger, M. circinelloi,* and *Penicillium citrinum*. Microbial lipases are commercially important because of their low production cost, greater stability, and greater widespread availability than plant and animal lipases. Therefore, they are most useful for industries. Maximum production of microbial lipases on an industrial scale is being done by submerged culture as well as solid state fermentation.

#### *48.2.3.2 Applications of Lipases in the Food Industry*

Lipases form an integral part of the industries that include cosmetics, leather, textile, paper, pharmaceuticals, agrochemical, detergents, tea, and bioremediation processes along with almost every field of the food processing industry (Hasan et al., 2006; Treichel et al., 2009). The first recombinant and commercial lipase was isolated from the fungus *Thermomyces lanuginosus*, expressed in *A. oryzae*. Since then, many other recombinant lipases have been made for commercial purposes.

##### 48.2.3.2.1 DAIRY INDUSTRY

In dairy industries, lipases are extensively used for the hydrolysis of milk fat. They are also used to enhance the flavor of cheeses in acceleration of cheese ripening, in the manufacturing of cheese products, and the lipolysis of cream. When acting on milk fat, lipases generate free fatty acids that lead to the production of many dairy products, mainly cheese (Aravindan et al., 2007). All blue veined cheese relies on *Penicillium roqueforti* and varieties of it for flavor enhancement. The major bacterial flora in a rennet cheese is the lactic streptococci.

##### 48.2.3.2.2 BAKERY FOOD

In the baking industry, there is an increasing interest in lipolytic enzymes. They can be used to replace traditional emulsifiers as the enzymes degrade wheat lipids to produce emulsifying lipids in situ. It is basically used to improve the flavor of bakery products by releasing short-chain fatty acids through esterification along with flavor enhancement.

##### 48.2.3.2.3 BEVERAGE PROCESSING

Barley is the most important grain in use for making beer all over the world. The total lipid content in barley ranges from 3% to 5% of a grain's dry matter. Lipase is used to hydrolyze the lipid of barley and also improve the aroma in alcoholic beverages such as sake. The Japanese company Tanabe Seiyaku has been using a patented lipase isolated from *R. delemar* or a *Candida* species during fermentation in the preparation of apple wine to improve the aroma and increased alcohol content.

##### 48.2.3.2.4 MEAT OR FISH PROCESSING AND FOOD DRESSING

In the processing of meat and fish products, lipases are used for fat removal and flavor development. Lipases are also found in the fat of meat, fish, eggs, milk, and cereals. Micrococcaceae and Lactobacilli lipase are being used for flavor development and ripening of dry sausages. Some Micrococcus species were found to be lipolytic to pork fat while various volatile and nonvolatile fatty acids were identified in ripened sausages (Caserio and Gervasini, 1969). Lipases are being used extensively in mayonnaise, dressing, and whipping to improve the quality and texture.

#### 48.2.3.2.5 LIPASES IN TEA PROCESSING

The quality of black tea is dependent mainly on the dehydration, enzymatic fermentation, and mechanical breaking. While processing black tea, the enzymatic breakdown of membrane lipids initiates the formation of volatile products with characteristic flavor properties (Verma et al., 2012). The exogenous addition of lipase secreted by *Rhizomucor miehei* has resulted in an increase in flavor volatiles and the aroma of tea (Ramarethinam et al., 2002).

#### 48.2.3.2.6 LIPASES IN FAT AND OIL PROCESSING

Modification of fats and oils is one of the prime areas in the food processing industry. Chemical modification of fats and oils is not only nonspecific but also energy consuming whereas lipase-mediated modifications are highly specific and can be carried out under mild conditions. Microbial lipases, which can be used for converting cheap oils into nutritionally rich oils, low calorie tri-acylgylcerols, PUFA, and oleic acid-rich oils, have been extensively reviewed (Gupta et al., 2003). Unilever Ltd. has a patent for a mixed hydrolysis and synthesis reaction to produce a cocoa butter substitute using an immobilized lipase (Jaeger and Reetz, 1998). The removal of phospholipids in vegetable oils is also a recently developed environmentally friendly process.

## 48.2.4 Rennet

Rennet is a complex of enzymes containing chymosin, pepsin, and lipase. The enzyme is synthesized in a weaning ruminants' stomach to digest mother's milk. Industrially, rennet is the main enzyme in cheese making, where it is used to separate milk into solid curds for cheese making and liquid whey. All the manufacturing of cheese with a variety of flavors and textures is dependent on the rennet enzyme. In milk, casein accounts for 80% of total milk proteins and exists as a large organized soluble structure, termed the micelle. There are three major caseins (α, β and κ-casein) distributed all over the micelle. The percent of each type of casein varies according to the micelle size, but approximately it is α—55%, β—30%, and κ—15%. The smaller the micelle size, the higher the κ-casein content in the micelle. The action of rennet is on κ-casein, in which it splits the peptide bond between phenylalanine ($Phe^{105}$) and methionine ($Met^{106}$) to form glycomacropeptide with *N*-acetyl neuraminic acid (Morr, 1975). Rennet action is, however, inhibited if the milk is heated. The reduced availability of the peptide bond in the κ-casein for rennet may be the reason.

### *48.2.4.1 Sources of Rennet*

The source of the animal rennet enzyme is the fourth stomach of young ruminants. It may contain 50%–95% chymosin, depending on the age of the animal (Addis et al., 2008). Plant origin proteolytic enzymes are also milk coagulants but the high proteolytic action decreases the cheese yield and increases the bitter taste in cheese (Lo Piero et al., 2002). Plant origin rennet may be used in the production of kosher and halal cheeses, but nearly all kosher cheeses are produced with microbial rennet. For commercial purposes, rennet is generally obtained from the mold *R. miehei*. Spain and Portugal have a great variety of cheeses that use Cynara sp. as a plant coagulant (Tejada et al., 2008). Initially, the mammalian rennet was exclusively used in the preparation of all types of cheese. But the demand for cheese production and the

need for a nonanimal derivative have led to substitute sources, such as microbial rennets. Presently, almost one-third of the cheese produced worldwide is through microbial rennets. Marketed since the 1970s, microbial rennets from various microorganisms have proved suitable for the production of different kinds of cheeses. These rennets are more proteolytic in nature in comparison to those isolated from animal sources and may result in the production of some bitter peptides during the cheese ripening (Fox and McSweeney, 1997). Therefore, efforts have been made to clone the gene for calf chymosin, and to express it in selected bacteria, yeasts, and molds. Microbial rennets have advantages over animal rennets such as bulk production, unlimited availability, less cost, and no risk of disease transmission, as may be in case of animal rennets. Many microorganisms have been identified as good sources of rennet. Microorganisms such as *R. pusillus*, *R. miehei*, *A. oryzae*, and *Irpex lactis* are widely used for the production of rennet in cheese making (Bailey and Siika-Aho, 1988; Escobar and Barnett, 1993; Neelakantan et al., 1999). Also the calf rennet or chymosin has been expressed in genetically modified microorganisms (GMOs) such as yeast or fungi (Dutta and Banerjee, 2006). The rennets by GMOs have been commercially produced since the 1980s in India; only the microbial rennet is being used as there is a ban on calf rennet (Pai, 2003).

#### *48.2.4.2 Applications of Rennet in the Food Industry*

Cheese is an extensively consumed fermented dairy product with a growing consumer demand. More than a hundred varieties of cheese are produced in the world. It is an excellent dietary source of protein, vitamins, and minerals such as calcium. The variety of cheese depends on the type of milk, the animal's diet, the butter fat content, bacteria and mold, and the processing and aging conditions of cheese (Fox et al., 1996; Miller et al., 2007). The use of the rennet enzyme in cheese production is one of the major applications of enzymes in food processing. Rennin acts on the milk protein in two stages, by enzymatic and by nonenzymatic action that results in coagulation of milk. Recently, Kethireddipalli and Hill (2015) have critically reviewed in detail the role of heat processed milk on rennet coagulation during cheese making as well as the addition of whey proteins to cheese milk. Mild heating conditions with some other process adaptations such as ultrafiltration and pH adjustments will lead to the production of cheeses with the desired organoleptic qualities and a higher protein content.

### 48.2.5 Catalase

Catalases (EC 1.11.1.6), one of the most studied enzymes, is also named hydroperoxidases. It is a tetrametric enzyme of four identified subunits of 60 kDa each. Catalase is one of the major enzymes which are important in revenue generation. One catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen per second.

#### *48.2.5.1 Source of Catalase*

Catalase is a common enzyme found in nearly all living organisms (Chelikani et al., 2004). Catalase enzymes are usually obtained from the bovine liver and microorganisms ((Tukel and Alptekin, 2004; Costa et al., 2001). Plants such as *M. sylvestris* L. leaves with high antioxidant properties, including radical-scavenging activity, are good sources of catalase (Barros et al., 2010). The enzyme has been studied, purified, and characterized from many plants such as black gram (*Vignamungo*) seeds (Kandukuri et al., 2012), cotton, sunflower (Eising et al., 1990), and pumpkin.

#### *48.2.5.2 Industrial Applications of Catalase*

Catalase has great industrial importance for its applications in the removal of hydrogen peroxide used as an oxidizing, bleaching, or sterilizing agent (Arıca et al., 1999; Ertaş et al., 2000). The enzyme can be used in a limited amount in cheese production. It is used in the food industry and also in egg processing, along with other enzymes. Catalase together with glucose oxidase is used in some food preservation, where superoxide dismutase, an antioxidant used in foods, produces $H_2O_2$, which is removed by catalase. Glucose oxidase and catalase are often used together in selected foods for preservation.

### 48.2.6 Lactases

Lactose, the sugar found in milk and whey, and its corresponding hydrolase, lactase, or β-galactosidase, have been widely researched in the past decade (Mehaia and Cheryan, 1987). This is because of the enzyme immobilization technique, which has given new and exciting prospects for the utilization of this sugar. Due to an insufficient intestinal enzyme, some individuals and/or populations show lactose intolerance and difficulty in consuming milk and dairy products. Therefore, a low-lactose or lactose-free food aid program is essential for lactose-intolerant people to prevent severe tissue dehydration and even diarrhea. Another advantage of lactase-treated milk is the improved sweetness of the milk, thus reducing the addition of sugars in the production of flavored milk drinks. Producers of ice cream, yogurt, and frozen desserts use lactase to increase scoop and creaminess, sweetness, and digestibility. Cheese manufactured from hydrolyzed milk ripens more rapidly than the cheese produced from normal milk.

#### *48.2.6.1 Industrial Applications of Lactases*

Lactose crystallizes easily, which restricts its use in the dairy industry. The use of lactase to overcome this problem cannot be exploited completely due to its associated high costs. Also, the discharge of large quantities of cheese whey pollutes the environment. However, the discharged whey could be utilized as a substitute source of lactose for the production of lactic acid by fermentation. The whey permeate, which is a by-product in the manufacture of whey protein concentrates, by ultrafiltration could be fermented efficiently by *L. bulgaricus* (Mehaia and Cheryan, 1987). Lactose can be isolated from several sources such as plants, animal organs, bacteria, yeasts (intracellular enzyme), or molds. Some of these sources are utilized for the preparation of the enzyme. Lactase production from *A. niger*, *A. oryzae*, and *Kluyveromyces lactis* is considered safe because it has been subjected to numerous safety tests and has a history of safe use. The most explored *E. coli* lactase is not used in food processing due to its cost and toxicity issues. The properties of the enzyme depend on its source. Temperature and pH depends on the type of source and the method of preparation. Immobilization of the enzymes, the procedure of immobilization, and the type of carrier can also affect these optima values. Universally, fungal lactase has a pH optima in the acidic range 2.5–4.5, and yeast and bacterial lactases in the neutral region 6–7 and 6.5–7.5, respectively. The difference in the pH optima of lactases marks them as appropriate for specific applications. For example, yeast and bacterial lactases are suitable for milk (pH 6.6) and sweet whey (pH 6.1) hydrolysis whereas fungal lactases are used for acid whey hydrolysis. Lactases from the *Bacillus* species are superior with respect to thermostability, pH operation range, product inhibition, and sensitivity against

high-substrate concentration. Its high activity for skim milk and less inhibition by galactose has made it suitable for use as a production organism for lactase (Gekas and Lopez-Levia, 1985). The enzymatic hydrolysis of lactose can be attained either by free enzymes (in the batch fermentation process) or by immobilized enzymes. Though various hydrolysis methods have been studied, only a few of them have been successfully scaled up and very few have been applied at an industrial level. Large-scale methods that use the free enzyme process have been established for the processing of UHT-milk and the treating of whey, using *K. lactis* lactase (Maxilact, Lactozyme). Beta-galactosidase is a very significant enzyme used in the dairy industry. It is involved in the hydrolysis of lactose into glucose and galactose with improved solubility and digestibility of milk and dairy products. Food thus obtained with a low lactose content is perfect for lactose-intolerant consumers (Mahoney, 1997; Pivarnik et al., 1995). It is also a good choice for consumers who have less tolerance to dairy products.

### 48.2.7 Cellulases

Cellulases enzymes break the glucosidic bonds of cellulose microfibrils and release oligosaccharides, cellobiose, and glucose (Dillon et al., 2004). Apart from the use of these hydrolytic enzymes in the food, drug, cosmetics, detergent, and textile industries, they are also used in wood pulp and paper industry, in waste management, and in the medical and pharmaceutical fields (Bhat and Bhat, 1997). In the food industry, cellulases are used in the extraction of constituents from green tea, soy protein, essential oils, aromatic products, and sweet potato starch. Together with hemicellulases and pectinases, these are used in the extraction and clarification of fruit juices. After the crushing of fruit, the enzymes are used to enhance liquefaction through the degradation of the solid phase. These enzymes are also used in the making of orange vinegar and in the extraction and clarification of citrus fruit juices. Cellulases complement pectinases in the juice and wine industries as extraction, clarification, and filtration services, with an increase in yield and flavor (Pretel, 1997). Cellulase is manufactured by a variety of fungal populations, such as *Trichoderma, Chaetomium, Penicillium, Fusarium, Aspergillu,s* and *Phoma*; aerobic bacteria, such as *Acidothermus, Bacillus, Celvibrio, Pseudonoma, Streptomyces, Staphylococcus,* and *Xanthomonas*; and anaerobic bacteria, such as *Bacteroides, Butyrivibrio, Caldocellum, Acetovibrio, Clostridium, Erwinia, Eubacterium, Ruminococcus, Pseudonocardia,* and *Thermo anaerobacter* (Soares et al., 2012). Filamentous fungi, *Aspergillus* stands out as key producers of cellulolytic enzymes. *A. niger*, a fermenting microorganism, produces cellulolytic enzymes, organic acids, and other products with great value by solid-state fermentation processes (Castro and Pereira Jr., 2010; Chandra et al., 2007; Couto and Sanroman, 2006).

### 48.2.8 Glucose Oxidase

Glucose oxidase (GOx) is an enzyme that catalyzes the oxidation of beta-D-glucose with the formation of D-gluconolactone. This enzyme consists of the prosthetic group flavin adenine dinucleotide (FAD), which allows the protein to catalyze oxidation-reduction reactions. Guimarães et al. (2006) accomplished a screening of filamentous fungi that could majorly yield glucose-oxidase. The result of the study showed high levels of GOx in *Rhizopus stolonifer* and *Aspergillus versicolor*. The literature states that the genus *Aspergillus* is a chief producer. This enzyme is used in the food industry for the elimination of harmful oxygen. Packaging materials

and storage environments are primarily responsible for the quality of products containing probiotic microorganisms because the metabolism of the microbial group is basically anaerobic or microaerophilic (Mattila-Sandholm et al., 2002). Therefore, the oxygen level during storage should be minimal to circumvent toxicity, the death of the organism, and the consequent loss of the product's functionality. Glucose oxidase may be a great strength to biotechnology to improve the stability of probiotic bacteria in yogurt without chemical additives.

### 48.2.9 Glucose Isomerase

Glucose isomerase (GI) (D-xylose ketolisomerase) catalyzes the reversible isomerase from D-glucose and D-xylose into D-fructose and D-xylulose, respectively. The enzyme is very significant in the food industry because of its uses in the manufacturing of fructose-rich corn syrup. Saprophytic bacteria has nutritional requirement for interconversion of xylose into xylulose by GI, which has a major application in the bioconversion of hemicellulose into ethanol. The hunt for GI thermostable enzymes has been the main goal of protein engineering (Hartley et al., 2000). Biotechnology plays a significant role in isolating mutants of promising prospects for the commercialization of the GI enzyme.

### 48.2.10 Invertase

Invertase is S-bD-fructo-furanosidase isolated from *S. cerevisiae* and other microorganisms. Hydrolysis from sucrose to fructose and glucose is catalyzed by this enzyme. The production of inverted sugar is one of invertase's numerous applications. Due to its sweetening effects, which are more than sucrose, it has great industrial significance and has good prospects for its use in biotechnology. Invertase is more active at temperatures between 40 and 60°C and pH ranging from 3 to 5. Microorganisms such as filamentous fungi are good producers of invertase with potential use in various industries. Soares et al. (2012) cultivated the filamentous fungus *Rhizopus* sp. in a wheat bran medium and obtained invertase. Another potential invertase-producing fungus is *Aspergillus casiellus*. It was inoculated in a soybean meal medium and after 72 h its crude extract was isolated. As most invertases used in the industry are produced by yeasts, the search for high yielding fungi is a requisite.

## 48.3 FRIENDS OR FOES

Very few enzymes show hazards, due to their catalytic activity, to those handling them in normal conditions but the major areas of risk are their source and chemical nature. These are allergenicity, activity-related toxicity, residual microbiological activity, and chemical toxicity.

All enzymes, being proteins, are possible allergens and have particularly strong effects if inhaled as a dust. Once an individual has developed an immune response as a result of inhalation or skin contact with the enzyme, reexposure produces increasingly severe responses that can become dangerous or even fatal. Because of this, dry enzyme preparations have been replaced to a large extent by liquid preparations. Enzyme producers and users recognize that allergenicity will always be a potential problem and provide safety information concerning

enzyme preparations. Liquid preparations are inherently safer but it is important that any spilt enzyme is not allowed to dry as dust formation can then occur. The formation of aerosols (e.g., by poor operating procedures in centrifugation) must be avoided as these are at least as harmful as powders. Activity-related toxicity is very rare but it must be remembered that proteases are possibly dangerous, particularly in concentrated forms, if inhaled. No enzyme has been found to be toxic, mutagenic, or carcinogenic by itself as might be expected from its proteinaceous structure. Enzyme preparations cannot be regarded as completely safe as such dangerous materials may be present as contaminants, derived from the enzyme source, or produced during its processing or storage. The organisms used in the production of enzymes may themselves be sources of hazardous materials and have been the chief focus of attention by the regulatory authorities.

## 48.4 FUTURE PROSPECTS

Being natural products, enzymes are always preferred by the consumers. The industrial demand for replacing chemical additives with enzymes and other such substances is increasing, especially as regards to preservatives and additives in food products. The use of enzymes has been successfully done for the production of gluten-free food, fiber-rich bread (Damen et al., 2012), glucose syrup, and natural sweetener. Microorganisms are being explored for the production of industrial enzymes because of the ease for bulk production and the reduction in cost. Various strategies are being employed for the purpose ranging from isolation of novel enzymes from microorganisms obtained from unique environment to genetically modifying organisms, rational protein design and protein engineering for recombinant proteins directed evolution to modify enzyme properties and high throughput screening to select proteins (Miguel et al., 2013).

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