Chapter 10
Microbial Enzymes in Food Industries: Enhancing Quality and Sustainability

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Abstract Even before the discovery of enzymes, they have been used by our ancestors in the production of various food products, and even today they are used routinely in the production of various food products in both households and food industries such as baking, brewing, beverages, juice, dairy, oil refinery, food packaging, etc. Enzymes exhibit various properties such as eco-friendly in nature, work under normal pressure, temperature, and pH, consume less energy, cost-effective, do not produce any greenhouse gases, show high activity and turnover number, high biodegradability, and unsurpassed specificity. Furthermore, enzymes produce specific products with no or minimal byproducts or wastes. Because of these properties they are preferred over inorganic catalysts or chemicals in food and other industries. Enzymes are widely distributed and are present in all living organisms. But in food industries, microbes are given priority for the isolation of enzymes as microbes are easily available, grow at faster speed, and can be manipulated through genetic engineering to produce improved or novel enzymes that can sustain and work optimally at different industrial conditions. Alternatively, enzymes can be extracted from microorganisms that can grow at extreme conditions. Food industries are largest market for enzymes where approx. 55% of all industrial enzymes are used. They are known to enhance the flavor, aroma, palatability, and overall quality of the products produced. In this chapter the authors will discuss the applications of some microbial enzymes used in food industries for various purposes.

Keywords Enzyme · Food industry · Food products · Microbes · Apoenzyme

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10.1 Introduction

Enzymes are biocatalysts that enhance the speed of chemical reactions by lowering down the activation energy. All enzymes are proteinaceous in nature except ribozymes (catalytic RNA). They are macromolecules and their molecular weight varies from 10 to 2000 kDa (Okpara, 2022). Some enzymes require cofactors; some require coenzymes while some require both cofactors and coenzymes for their biological activity. Enzymes with their cofactors or/and coenzymes part are active and are called holoenzymes, whereas enzymes without their cofactors or/and coenzyme part are inactive and are called apoenzymes. Active site is made up of less than ten amino acids and because of its shape and charge properties it enables the enzymes to bind to their substrates specifically, making a particular enzyme unique from other enzymes (Robinson, 2015). Active sites can be present on enzyme's surface, in cleft or pocket or buried deeply within enzyme globular structure (Pravda et al., 2014). On the basis of specificities of enzymes, Enzyme Commission (EC) has classified, named, and numbered over 3000 known enzymes (Patel et al., 2017). Enzymes are broadly classified into six categories based on the reactions catalyzed by them, viz. oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase. In 2018, a new and seventh class of enzymes named translocases has been introduced by enzyme commission. Translocases catalyze the movement of ions and molecules across the membrane, for example, carnitine-acylcarnitine translocase, ADP/ATP translocase, etc. (https://iubmb.qmul.ac.uk/enzyme/). Enzymes of every category have some role in food industries but enzymes of hydrolases category have more frequent role. Functions of enzymes depend upon their tertiary or quaternary structures which are ultimately determined by their primary structures. A small change in the primary structure of enzyme can modify the functions of enzymes. This property can be exploited to produce improved or new biocatalysts with desired features through enzyme or protein engineering (Quin & Schmidt-Dannert, 2011).

Enzymes exhibit the various properties such as eco-friendly in nature, work under normal pressure, temperature, and pH, consume less energy, cost-effective, do not produce any greenhouse gases, show high activity and turnover number, high biodegradability, and unsurpassed specificity (exhibit group specificity, absolute specificity, and stereospecificity). Moreover, enzymes produce specific products with no or minimal byproducts or wastes (biodegradable and non-toxic), consequently, reduce the need for complex downstream processes. This all will be helpful in the development of sustainable food system having positive impact on the environment. Because of these properties they are preferred over inorganic catalysts or chemicals in food and other industries; therefore, industrial enzymes market has grown from 0.31 billion USD in 1960 to 6 billion USD in 2020, which is expected to grow to 9 billion USD by 2027 (Okpara, 2022). Consequently, to fulfill the industrial demand of enzymes, many enzyme producing companies such as DSM, DuPontTM, Novozymes, Advanced Enzyme Technologies, Megazyme, Biocatalysts Ltd., MetGen, and many others came into existence. But among the over 3000 known enzymes only 5% enzymes have been used in industries (Okpara, 2022).

10.2 Sources of Enzymes

Enzymes in food industries can be taken from animals, plants, and microorganisms (fungi and bacteria). In industries, microbes are given priority for the isolation of enzymes due to following reasons: (1) Microbes are easily accessible; (2) They grow at faster speed; (3) They are easy to grow in cheap growth media; (4) Most enzymes (plants, animals, and microbial sources) work optimally at temperature 37 °C, pH 7, and in the absence of inhibitors. But sometimes industrial production conditions are not optimum for the working of enzymes. Temperature, pressure, salinity, pH, or presence of inhibitors during the production process could affect the enzyme activity. In this case microbes can be manipulated through genetic engineering to produce improved or novel enzymes that can sustain and work optimally at different industrial conditions. Alternatively, enzymes can be extracted from microorganisms that can grow at extreme conditions such as extreme temperature, salinity, or pH level; (5) Production of microbial enzymes through fermentation process is cost-effective; (6) Microbial enzymes can be produced in bulk amount in minimal time and space; and (7) Microbial enzymes are free from harmful phenolic compounds as in case of plant derived enzymes and endogenous enzyme inhibitors and protease as in case of animal derived enzymes; and (8) Microbial enzymes are more stable (Patel et al., 2017; Singh et al., 2019; Okpara, 2022; Littlechild, 2015). In industries more than 100 enzymes are used, out of which more than 50% enzymes have been taken from microorganisms. *Aspergillus niger*, *A. oryzae*, *Mucor, Saccharomyces cerevisiae*, *Serratia, Bacillus subtilis*, *B. licheniformis, B. amyloliquefaciens*, *Lactobacillus casei*, *L. acidophilus*, *L. delbrueckii*, *Rhizopus oryzae, Corynebacterium glutamicum* are some common microbial species that are employed in the generation of many commercial enzymes. Enzymes such as lipase, amylase, protease, rennet, pectinase, invertase, cellulase, glucose oxidase, raffinose, catalase, lactase, etc. are obtained from microbial sources and are used in food production and processing industries (Singh et al., 2019).

10.3 Need for Microbial Enzymes in the Food Industries

Biological agents have been used by our ancestors in processing of various food products, viz. tenderization of meat by papaya leaves, making of wine, beer, cheese, curd, soy sauce, vinegar, etc. Traditionally acid hydrolysis method has been used in food industries to hydrolyze starch for the preparation of glucose syrup. Acids such as hydrochloric acid, sulfuric acid, formic acid, nitric acid, and trifluoroacetic acid have been employed for acid hydrolysis of starch. This method is simple and cheap but has few drawbacks such as relatively low yield, high process temperature (120–150 °C), formation of undesirable products, viz. furfural generated from pentose sugar and hydroxymethylfurfural generated from hexose sugar and use of acid can cause serious environmental issues (Azmi et al., 2017). In the late 1990s this

traditional method of starch hydrolysis was replaced by enzymatic method where enzymes, viz. amylase and glucoamylase (amyloglucosidase) have been used (Crabb & Shetty, 1999; Azmi et al., 2017; Haq et al., 2010). Thereafter, demand of enzymes in food industries for production and manufacturing of products has increased rapidly. Furthermore, development of different technologies such as protein engineering, enzyme immobilization, and in 1980s recombinant DNA technology have made a huge impact on the applications and uses of enzymes in food industries (Liu et al., 2013). Of all industries, food industries are largest market for enzymes where approx. 55% of industrial enzymes are used (Guerrand, 2017). In 2020 global food enzymes market was approx. 2.3 billion USD which was valued approx. 40% of all the industrial enzyme market and by 2026 it is expected to grow to 3.3 billion USD (Research and Markets, 2021).

10.4 Microbial Enzymes: Sources and Applications in Food Industries

Even before the discovery of enzymes, they have been used by our ancestors in the production of food many products, viz. meat tenderization by papaya leaves, preparation of soy sauce, beer, bread, curd, cheese, wine and vinegar, and even today they are used routinely in the preparation of many products in both households and industries such as dairy, baking, brewing, beverages, juice, oil refinery, food packaging, etc. They enhance the flavor, palatability, and improve the quality of the product. In food industries enzymes' main role is the processing of the food products.

10.4.1 α-Amylase (EC 3.2.1.1)

Amylose and amylopectin are the two main components of starch. Amylose is linear and made up of glucose units that attached to each other by α-1,4 glycosidic bond, whereas amylopectin is branched and made up of glucose units that are attached to each other by α-1,4 and α-1,6-glycosidic bonds. α-Amylase (endoamylase) breaks the α-1,4 glycosidic bond of the starch and releases glucose, maltose, maltotriose, and short chain dextrins.

10.4.1.1 Sources

Three types of amylases have been found in nature named as α-amylase, β-amylase, and γ-amylase. α-Amylases can be obtained from archaea, fungi, bacteria, plants, animals, and humans, β-amylases can be obtained from plants and microbes, and

γ-amylases can be obtained from animals and plants (Azzopardi et al., 2016). Among microbial sources *Bacillus* spp. are exploited most for the commercial production of α-amylase. *B. amyloliquefaciens, B. licheniformis*, *B. stearothermophilus*, *Anoxybacillus* sp. AH1, *Geobacillus thermoleovorans*, *Bacillus* sp. BCC 01-50, and *Bacillus subtilis* JS-2004 are used widely for the isolation of α-amylase at industrial scale (Elyasi Far et al., 2020; Okpara, 2022). Thermostable α-amylase is extracted from *Bacillus* spp. such as *B. licheniformis, B. subtilis*, *B. amyloliquefaciens,* and *B. stearothermophilus* (Prakash & Jaiswal, 2010). In the processing of starch some steps such as saccharification, gelatinization, and liquefaction are done at high temperature; therefore, thermostable α-amylases are used in such harsh conditions. Salt tolerant α-amylase can be taken from halophilic bacteria such as *Halobacillus* sp., *Halomonas meridian*, *Bacillus dipsosauri*, *Chromohalobacter* sp., and *Haloarcula hispanica*. Salt tolerant enzyme is used in food processes where salt concentration is high (Singh et al., 2019). *Aspergillus* spp. such as *A. oryzae*, *A. terreus* NCFT4249.10, *A. awamori*, *A. fumigatus* KIBGE-IB_{33}, *A. niger* WLB42 are some examples of α-amylase producing strains (Okpara, 2022). α-Amylases of *Aspergillus* spp. are less thermostable as compared to α-amylases from *Bacillus* spp. (Okpara, 2022). Few *Penicillium* sp. are also used for the production of α-amylase.

10.4.1.2 Applications

In food industries α-amylases are widely used in baking, brewing, starch liquefaction, preparation of livestock feed, fruit juice, starch syrups, and digestive aids. In early nineteenth century acid hydrolysis method was developed for the production of glucose syrup from starch and till 1990s diluted acid hydrolysis method was employed commercially to produce glucose syrup from starch. In late 1990s enzymatic hydrolysis method was developed (Crabb & Shetty, 1999; Haq et al., 2010; Azmi et al., 2017). Commercial production of glucose syrup from starch is a two-step process. First step is the liquefaction where short chain dextrins are produced by α-amylase of *B. stearothermophilus, B. licheniformis, or B. amyloliquefaciens*. Second step is saccharification where starch hydrolysate is converted to high concentration glucose syrup by using exo-glucoamylases which is obtained from *A. niger* (Haq et al., 2010). Glucose syrup can be converted to fructose syrup by using glucose isomerase. Fructose syrup is employed as sweetener and added to many processed products such as soft drink, fruit drink, yogurts, and breads (Parker et al., 2010).

During bread making α-amylase enzyme is used to hydrolyze the starch in flour into fermentable sugars. Later these sugars are used to produce CO_2 by yeast fermentation. Production of CO_2 causes the leavening or rising of the dough. Staling (reduced moisture and crispiness in bread) and starch crystallization cause the huge economic loss to the baking industries. Addition of α-amylase along with other enzymes and chemicals reduces the starch crystallization and staling of the bread.

Moreover, shelf-life, aroma, taste, softness, and quality of bread are improved (Singh et al., 2019; Okpara, 2022). During baking enzymes should be added within the prescribed range as overdoses of enzymes can result in some undesirable changes in the bread. For example, excess use of α-amylase can cause the stickiness in the dough due to production of maltodextrin (Van Der Maarel et al., 2002).

Production of alcohols or biofuels from starch is a demand of today's scenario. Conventional methods for starch liquefaction (acid hydrolysis) require strong chemicals such as caustic soda, lime, sulfuric acid, etc. and 120–150 °C temperature. Maintenance of temperature and pH in conventional methods is a challenge and adds additional cost to the industries (Robertson et al., 2006; Singh et al., 2019). Therefore, in industries enzymatic methods for starch liquefaction are preferred. Simple and fermentable sugars can be obtained from starch by the action of α-amylase. Later these sugars are fermented into alcohols or biofuels by using yeast. Yeast (GRAS category, high productivity, and better tolerance) is commonly used in the industries for the production of alcohol or bioethanol. Fermentation of raw starch into ethanol can be improved by the use of α-amylase in combination with glucoamylase. α-Amylase isolated from *Streptococcus bovis* and glucoamylase isolated from *Rhizopus oryzae* form the most effective combination (Singh et al., 2019). α-Amylase is also used as clarifying agents during the production of beer (Okpara, 2022).

Ingredients present in the feed are not fully digested and absorbed by livestock. Consequently, ingredients of the feed without being utilized fully are passed on into the feces of animals. Therefore, feed industries are adding some enzymes, viz. α-amylase, xylanase, phytase, and protease into the feed of animals. α-Amylase breaks down the starch into glucose units which results in more digestibility of carbohydrates by animals. This results in improvement of body weight of animals, feed conversion ratio, and milk production (Silva et al., 2006; Sidkey et al., 2011; Jegannathan & Nielsen, 2013). In juice industries use of α-amylase is prescribed for improving the juice extraction and its yield. It also acts as clarifying agent in the generation of juice (Vaillant et al., 2001; Sivaramakrishnan et al., 2006).

10.4.2 Protease (EC 3.4)

These are proteolytic enzymes which break down the peptide bonds in the proteins resulting in generation of amino acids and smaller peptides. Allergens in the food are mostly proteins. As proteases degrade the proteins, they can be used to reduce the allergenic properties of food products. On the basis of site of cleavage proteases are categorized into endoproteases and exoproteases (aminopeptidases and carboxypeptidases) and on the basis of mechanism of catalysis proteases are categorized into six classes, viz. metalloproteases, glutamic proteases, aspartic proteases, threonine proteases, serine proteases, and cysteine proteases. Glutamic proteases are yet to be discovered in mammals. Aspartic proteases, glutamic proteases, and metalloproteases use water molecules as nucleophile to break peptide bond, while threonine

proteases, serine proteases, and cysteine proteases use amino acids (threonine, serine, and cysteine, respectively) present in their active sites as nucleophile to break peptide bond (López-Otín & Bond, 2008). On the basis of pH optima proteases are categorized into acidic proteases, neutral proteases, and alkaline proteases.

10.4.2.1 Sources

Proteases are found in animals, plants, archea, fungi, and bacteria. In food industries, fungi and bacteria are considered preferred sources of proteases. Of the total global enzymes sales microbial proteases contribute to approx. 40% (Singh et al., 2019). Fungi such as *A. usamii*, *A. niger*, *A. flavus*, *A. fumigatus*, *A. oryzae* DRDFS13MN726447, etc. and bacteria such as *B. subtilis* SMDFS 2B MN715837, *B. licheniformis*, *Chryseobacterium* sp., etc. are exploited for the production of proteases in food industries (Okpara, 2022). *Bacillus* produces the neutral and alkaline proteases, while *Pseudomonas* produces the alkaline protease. Neutral proteases of bacteria are heat sensitive and show activity in the pH range of 5–8. Because of their moderate activities neutral proteases cause the little bitterness in hydrolyzed food proteins when compared to animal proteases; therefore, they are preferred in the food industries. Different strains of *Pseudomonas aeruginosa* are used for the extraction of variety of proteases. Fungi produce more varieties of proteases as compared to bacteria, for example, acid proteases, neutral proteases, and alkaline proteases are produced by *A. oryzae* (Singh et al., 2019). Common proteases produced by plants are papain, bromelain, keratinases, and ficin.

10.4.2.2 Applications

Proteases are used in baking, dairy, food processing, brewing, meat, and animal feed industries. Peptides generated due to partial hydrolysis of proteins by proteases may have some biological functions and can be used in food as functional component. In dairy industries proteases are mainly used for making of cheese. In making of cheese proteases (rennin or rennet) are used to hydrolyze the peptide bond between Phe^{105} and Met^{106} in kappa casein to produce para-k-casein and glycomacropeptide during milk coagulation. Organoleptic and rheological properties of cheese are also improved by proteases. Four types of proteases are used for coagulation/curdling of milk, viz. animal rennet, vegetable rennet, microbial proteases, and genetically engineered chymosin. Chymosin is preferred in dairy industries for making cheese because of its high specificity for casein (Singh et al., 2019; Okpara, 2022). Recently, Mamo et al. (2020) reported that organoleptic properties of cheese were improved by milk clotting protease originated from *A. oryzae* DRDFS13MN726447.

Proteases extracted from *A. oryzae* have been used in the baking industry since 1952 (Lyons, 1982). More the gluten content in the dough more it will be difficult to stretch the dough, consequently, bread will be tough and chewy. Chemical like sodium bisulfite can be used to weaken the gluten structure but it affects the

nutritional properties of dough. Therefore, in bakery industries proteases are used to weaken the structure of gluten. Moreover, use of proteases does not affect the nutritional properties of the dough (Okpara, 2022). In bakery industries, gluten-free products are prepared using proteases for individuals who are intolerant to gluten. Acid proteases extracted from *A. usamii* have been used to improve the rheological properties of wheat gluten used for baking (Deng et al., 2016).

Proteins in the alcoholic beverages (e.g. beer) cause the formation of haze; therefore, microbial proteases are used in brewing industries to prevent haze formation. Moreover, proteases cause the breakdown of proteins of the wort into smaller peptides and/or amino acids. This leads to improvement of fermentation process and the overall quality of beer or whiskey (Singh et al., 2019; Okpara, 2022).

Connective tissues, for example, ligaments, tendons, silver skin, and muscle fibers are present in the raw meat. It causes the meat hard to chew, consequently, meat palatability decreases. Use of papaya leaves (source of protease) for meat tenderization has been a practice of long time. In today's scenario, meat industries are using various proteases extracted from microorganisms for meat tenderization, e.g. aspartic protease from *A. oryzae*, collagenase from *Clostridium histolyticum*, caldolysin from *Thermus* strain, thermophile protease from *Bacillus*. Meat tenderization causes the improvement of rheological properties of meat (Bekhit et al., 2014).

Aspartame or Nutrasweet is a non-saccharide based artificial sweetener that is made up of only two amino acids, viz. L-aspartic acid and L-phenylalanine. As diabetes is growing globally, demand of aspartame and other zero-calorie sweeteners is increasing exponentially. Therefore, 3000–6000 metric tons of aspartame is produced every year by industries. Because aspartame can only be produced by amino acids in L-configuration and maintenance of stereospecificity chemically adds additional cost to the industries. Therefore, synthesis of aspartame by enzymes is recommended as enzymes are stereospecific in nature (Singh et al., 2019). In animal feed industries proteases are added for the breakdown of proteins present in the animal feed. It improves the digestibility and utilization of proteins by animals and reduces the excretion of nitrogen content from the livestock (Okpara, 2022).

10.4.3 Lipase (EC 3.1.1.3)

Lipases causes the breakdown of triglycerides into its components, viz. free fatty acids and glycerol. Lipases aid in proper digestion of fats and lipids in animals including humans.

10.4.3.1 Sources

Lipases are widely distributed enzymes and can be found in all living organisms. In humans and animals, lipases are produced by pancreas and stomach and can be found in blood, pancreatic secretions, intestinal juices, gastric juices, and adipose

tissue. In plants lipases are found in lipid storing tissue such as seeds. High lipase content is found in seeds of plants that belong to Euphorbiaceae, Ranunculaceae and Papaveraceae families. Barley, corn, and cotton seeds are used frequently for extraction of lipase enzyme (Singh et al., 2019) In industries microbes are given priority for the isolation of enzymes as microbes are easily available, microbial enzymes have high stability and are cheap. For industrial applications lipases are extracted from various species of fungi such as *Rhizomucor miehei*, *A. niger* F0215, *A. repens*, *A. oryzae*, *Penicillium camemberti*, *Candida rugosa*, *Mucor javanicus*, *Thermomyces lanuginosus*, and various species of bacteria such as *Bacillus subtilis* LP2, *Staphylococcus aureus*, *S. caprae* NCU S6, *B. cepacia*, *B. megaterium*, *P. aeruginosa* JCM5962(T), *Burkholderia glumae*, *P. alcaligenes*, *P. mendocina*, *S. hyicus* , *S. simulans* PMRS35, *Rhodothermus marinus*, *Serratia marcescens* (Okpara, 2022).

10.4.3.2 Applications

In baking industries lipase is used either along with or in place of traditional emulsifiers to release emulsifying lipids in situ through hydrolysis of wheat lipids. They are employed to enhance the flavor of baked products through production of esters of short chain fatty acids. Along with other enzymes used in the baking industries lipases are used to enhance the loaf volume, shelf-life, texture, and softness of baked products (Guerrand, 2017). Egg white is needed for the production of many baked products. Lipid present (0.02%) in egg white can deteriorate the quality of the dough. Therefore, lipases are needed to decrease the lipid content of the egg white through hydrolysis. This leads to improvement of quality of baked products. In some baked products lipases are added as preservative too (Okpara, 2022).

In dairy industries, lipases from various sources are used in the making of varieties of the cheese, viz. Italian cheese, Cheddar, Feta, Manchego, Rumi, Ras, Domiati, blue cheese, etc. Production of free fatty acids through action of lipases on milk fat leads to the generation of cheese and related dairy products. Moreover, production of free fatty acids promotes cheese ripening, increases flavor, taste, texture, and softness of the cheese (Aravindan et al., 2007; Okpara, 2022).

In alcoholic industries lipases are employed for the breakdown of lipids present in barley seeds to produce free fatty acids. Lipid hydrolysis enhances the aroma of alcoholic beverages, for example, sake. For improving the alcoholic content and aroma in apple wine, patented lipase extracted from *R. delemar* or *Candida* sp. have been used for fermentation by Japanese company Tanabe Seiyaku (Singh et al., 2019). In meat industries, lipases are employed to produce lean meat by removal of excess fat. Flavor of meat products can be enhanced by the use of lipases. Lipases of micrococcaceae and lactobacilli are used for the improvement of flavor and ripening of dry sausages (Singh et al., 2019).

In tea industries lipases are employed to improve the flavor and aroma of tea. For example, lipases isolated from *Rhizomucor miehei* have been found to improve the

production of flavored compounds that contributes to the development of flavor and aroma in the tea (Ramarethinam et al., 2002).

In oil industries lipases are employed as degumming agents to eliminate the phosphatides from crude vegetable oils. This process enhances the production and quality of refined oils. Treatment of crude vegetable oils by lipases also enhances the organoleptic properties of refined oils through production of flavor esters (Okpara, 2022). A novel lipase called An-lipase isolated from *A. niger* F0125, and was used to produce butyl butyrate, ethyl lactate and ethyl caprylate flavour esters in crude soybean oil (Cong et al., 2019). Lipase treatment also enhances the content of ω-3 polyunsaturated fatty acids in some oils such as salmon and sardine oil (Okada & Morrissey, 2007; Kahveci & Xu, 2011).

10.4.4 Rennet

Rennet is a mixture of enzymes, viz. lipase, pepsin, and chymosin. It is produced in the stomach of weaning ruminants for the proper digestion of mother's milk. Casein makes up the 80% of total milk protein and is found in milk in the form of micelle. Three types of casein are found in the milk, viz. alpha, beta, and kappa casein. Of total casein alpha casein content is highest and accounts approx. 55%, beta casein accounts approx. 30%, while kappa casein is present in least amount and accounts approx. 15%. But depending upon the size of micelle proportion of each type of casein varies. Rennet or rennin hydrolyzes the peptide bond of kappa casein between Phe^{105} and Met^{106} and releases macropeptide with *N*-acetyl neuraminic acid (glycomacropeptide) and para-k-casein (Morr, 1975; Michael Eskin & Douglas Goff, 2013).

10.4.4.1 Sources

Rennet can be obtained from microorganisms, plants, and mammals. Initially, rennet obtained from mammals was used exclusively for making all kinds of cheese. But with increase in the demand of cheese and need to derive rennet from non-animal sources has pushed industrialist to get rennet from alternative sources. Plants derived proteases can also be used as milk coagulants. Kosher and halal cheeses can be produced using plants derived rennet. However, all kosher cheese are being produced using microbial derived rennet. Globally, currently approx. 30% of the cheese is produced using rennet obtained from microorganisms. Production of rennet from microorganisms is advantageous over rennet derived from animals. Microbial rennet is having low cost, can be produced in bulk, has unlimited availability, and as in case of animal rennet, chances of disease transmission are low. Commercially, rennet can be obtained from microorganisms, viz. *R. miehei*, *R. pusillus*, *A. oryzae*, *Irpex lactis*. Rennet obtained from microorganisms and plants have high proteolytic activity when compared to animal's rennet. High proteolytic activity of rennet,

consequently, decreases the yield as well as taste of cheese and increases the cheese bitterness due to generation of bitter peptides during cheese ripening process. Consequently, people have started producing animal rennet (calf chymosin) using genetic engineering. Calf rennet or chymosin have been produced in genetically modified organisms such as fungi or yeasts (Singh et al., 2019).

10.4.4.2 Applications

Major application of rennet in dairy industries is the production of cheese. Globally, hundreds of varieties of cheese are produced through fermentation. Type of cheese depends upon many factors, viz. type of milk, diet of animal, fat content of milk, type of microorganism used (rennet source), and processing and aging conditions of cheese. It is good source of proteins, minerals, and vitamins.

10.4.5 Catalase (EC 1.11.1.6)

Catalase is one of the three antioxidant enzymes working in human body. The function of catalase is to remove or detoxify free radicals generated by metabolic reactions. It is a tetrameric enzyme and made up of 4 identical subunits of molecular weight 60 kDa each. Turnover number of catalase is highest of all known enzymes. In 1 s, catalase can degrade 2.8 million H_2O_2 molecules to produce H_2O and O_2.

10.4.5.1 Sources

All aerobic organisms like plants, animals, and microbes are equipped with catalase. Mostly microorganisms are exploited for the generation of catalase commercially. *A. niger*, *Pyrobaculum calidifontis*, *Micrococcus luteus, Rhizobium radiobacter* 2-1, *Ureibacillus thermosphaericus* FZSF03, *Bacteroides fragilis, Bacillus maroccanus*, *Enterococcus faecalis* are some examples of microbes exploited for the production of catalase at industrial scale (Okpara, 2022).

10.4.5.2 Applications

Catalases are employed in dairy industries to eliminate peroxides from milk to avoid milk rancidity (Abada, 2018). For production of wine with lower alcohol contents catalase and glucose oxidase can be employed in combination. Combined activity of these enzymes decreases the alcohol content to 2% within 30 h but aroma profile and organoleptic properties of the wine affected significantly. Catalase and glucose oxidase in combination are also used to eliminate O_2 from wine to enhance its shelf-life (Röcker et al., 2016). Packaged food items are susceptible to spoilage

due to oxidation of food. Therefore, catalases are needed for food packaging to avoid oxidation of food products. This treatment increases the shelf-life of packaged food items (Okpara, 2022). Along with other enzymes catalases are also used in small amount during production of cheese and processing of eggs.

10.4.6 Cellulase (EC 3.2.1.4)

Cellulose is linear and made up of glucose units that are attached to each other by β-1,4 glycosidic bond. Cellulase breaks glycosidic bond in cellulose and releases the oligosaccharides, cellobiose, and glucose.

10.4.6.1 Sources

For commercial production of cellulase microorganisms such as fungi and bacteria are preferred. Fungi such as *A. niger* and *Trichoderma reesei* and bacteria such as *B. subtilis* ABDR01, *Paenibacillus* spp., *Streptomyces* sp. strain J2 and *B. licheniformis* are exploited most for the production of cellulase at industrial scale (Okpara, 2022).

10.4.6.2 Applications

During extraction of juice from fruits and vegetables cellulose and hemicellulose present in the fruits and vegetables cause the turbidity or cloudiness in the juice. Ultimately, quality of juices gets reduced. Therefore, cellulases along with other enzymes, viz. pectinases and hemicellulases are used for softening of fruits in juice industries. These enzymes hydrolyze the cellulose, hemicellulose, and pectin in the raw fruit and vegetables. This ultimately increases the extraction, clarification, stabilization, and yield of juices (Bhat, 2000). Cellulase, hemicellulase, and pectinase are also used in the wine industries for improving extraction, skin maceration, clarification, and overall quality of the wine.

Cellulase is also employed in the processing purees of vegetables and fruits. Cellulase treatment increases the yield and decreases the viscosity of purees. Animal feed is made up of complex polysaccharides such as lignin, pectin, hemicellulose, cellulose. For the improvement of feed utilization and digestibility and animal performance, cellulase and hemicellulase are used in processing of feed of ruminants. Cellulase treatment of feed of farm animals causes more consumption of diet and 5–25% more production of milk (Murad & Azzaz, 2010).

10.4.7 Lactase (EC 3.2.1.108)

Lactose (milk sugar) is a disaccharide in which galactose and glucose units are attached to each other by β-1,4 glycosidic bond. Lactase (β-galactosidase) breaks the glycosidic bond of the lactose and releases galactose and glucose. Milk is used extensively by dairy industries for the generation of many food products; therefore, lactose hydrolysis is the prime concern for these industries. Microorganisms (yeast, fungi, and bacteria) are exploited most for the generation of lactase because they produce enzyme in more quantity and in cost-effective manner.

10.4.7.1 Sources

Among bacterial sources *Klebsiella oxytoca* ZJUH1705, *B. longum* BCRC 15708, *B. infantis* CCRC 14633, and *E. coli* and among fungal sources *A. oryzae, A. niger*, *Kluyveromyces lactis,* and *K. fragilis* are exploited industrially for the production of lactase (Okpara, 2022). Because of the high cost and toxicity concerns lactases from *E. coli* are not used in the food industries. Lactases of *Kluyveromyces lactis*, *A. oryzae*, and *A. niger* are safe for use in food industries. pH optima for fungal, yeast, and bacteria lactases are 2.5–4.5, 6–7, and 6.5–7.5, respectively. Therefore, yeast and bacteria lactases are ideal for hydrolysis of milk and sweet whey, while fungal lactases are ideal for hydrolysis of acid whey (Singh et al., 2019).

10.4.7.2 Applications

Normally lactase is secreted by the intestinal cells of mammals including humans. Individuals having less amount of intestinal lactase or lactase deficient are not able to digest the lactose present in the milk. Undigested lactose reaches the colon where gut microbiota causes the fermentation of lactose, consequently, short chain fatty acids and gases are generated in the colon. This results in complications such as diarrhea, tissue dehydration, bloating, gas, and pain. Therefore, lactose-intolerant individuals are advised to consume low-lactose or lactose-deficient milk (lactase-treated milk) instead of normal milk. Therefore, in dairy industries, lactases are used in the preparation of lactose-deficient or low-lactose milk or dairy products for lactose-intolerant people. Lactase-treated milk shows improved solubility, sweetness, and digestibility in lactose-intolerant people.

Lactases are also used in making frozen desserts, yogurt, and ice cream to enhance their creaminess, sweetness, and digestibility. Lactose crystallization in dairy products can be avoided by the use of lactases. Moreover, cheese prepared from lactase-treated milk ripens fast when compared to cheese prepared from normal milk. But high cost associated with lactase restricts its use in dairy industries.

10.4.8 Pectinase (EC 3.2.1.15)

Pectinase catalyzes the breakdown of pectin, complex structural polysaccharides found in middle lamella and cell walls of plant cells to produce simpler carbohydrates such as galacturonic acid. A different category of these enzymes utilizes pineapple, tomato, apple, orange, orange peel, lemon pulp, and other citrus fruits as their substrate. On the basis of their mechanism of action they can be categorized as polygalacturonases—hydrolyzes α-1,4 glycosidic bond; pectolyase—cleaves α-1,4-D-galacturonan methyl ester; and pectin esterases—removes acetyl and methoxy group from pectin (Sudeep et al., 2020).

10.4.8.1 Sources

Pectinases can be obtained mostly from fungi for industrial applications. Some bacterial strains (*Bacillus subtilis* ABDR01) produce high amount of pectinases (Yadav et al., 2020). *Penicillium* spp., *Streptomyces* spp., *Moniliella* SB9 (Jaradat et al., 2008), *A. niger* MTCC (Anand et al., 2017), *Aspergillus* spp. Gm, *Fusarium* spp. C, *Aspergillus* spp. T, and *Penicillium* spp. Lco (Sudeep et al., 2020). *Aspergillus kawachii* (Esquivel & Voget, 2004), *Aspergillus fumigatus* (Okonji et al., 2019) are some fungal species that are exploited most for the generation of pectinase at industrial scale.

10.4.8.2 Applications

In fruit juice industries pectinases are used for removal of pectin from cell walls of fruits, consequently, pulp pressability, juice extraction, filterability, flavor, clarification, and yield get improved. In the cell wall of unripe fruit pectin and cellulose microfibrils are attached to each other. This bound pectin is water insoluble and provides hardness or rigidity to the cell wall of fruit. Upon fruit ripening pectin structure gets altered enzymatically, consequently, pectin becomes water soluble and its attachment with the cell wall get weaken and ultimately fruit softens. When fruit is pressed pectin is released into the juice that contributes to increase in the viscosity of fruit juice and pulp particles, while some pectin molecules remain attached to cellulose microfibrils that help in retention of water (Pifferi et al., 1989). Manual pressing of pectin-rich fruits produces viscous and cloudy juice that remains attached to pulp as a jellified mass (lower juice yield). Therefore, in fruit industries the use of pectinase is highly recommended. Pectinases break the jelly like structures in the pulp and increase the juice yield by improving the pulp pressability. Furthermore, pectinases treated juice is found to be less viscous and cloudy, and juice palatability increases (Kashyap et al., 2001). Pectinase treated fruits and vegetables mash provides a high juice extraction and pulp with good pressing characteristics (Soares et al., 2021).

Citrus fruit processing industries release pectinaceous substances into the wastewater. These substances become difficult to get decomposed by microorganisms during activated-sludge treatment. Therefore, pectinolytic enzymes are used to degrade the pectins present in wastewater of food processing industries and make it appropriate for decomposition by sludge treatment (Sharma et al., 2012; Praveen & Suneetha, 2014). Use of pectinolytic organisms during the treatment of activated sludge is an eco-friendly, cheap, and time-saving procedure (Samanta, 2021). *Erwinia carotovora* FERM P-7576 (soft-rot pathogen) is known to produce endo-pectate lyase. Treatment of pectinaceous wastewater with this strain has been found to be effective in removal of pectic substances (Tanabe et al., 1986). Alkalophilic *Bacillus* sp. GIR621 is also known to release endo-pectate lyase at pH 10.0. Removal of pectic substances from wastewater by this strain has been achieved by Tanabe et al. (1987). Due to the safety concerns pectolytic enzymes extracted from bacteria are used to remove pectic substances from the wastewater. Pectinases are also employed in wine industries to enhance the efficiency of process and quality of wine produced.

Traditional methods for isolation of oils from *Canola*, coconut germ, sunflower seeds, palm, kernel, olive, etc. commonly use hexane (potential carcinogen) as organic solvents. Therefore, in oil industries pectinases and other cell wall degrading enzymes are employed to disintegrate the cell wall of oil crops to extract the oil. Pectinase treatment improves oil extraction from different sources such as olives, dates, flaxseeds, and many more (Anand et al., 2020). An enzyme preparation (Olivex) taken from *A. aculeatus* has enzymes for the degradation of cellulose (cellulase), hemicellulose (hemicellulase), and pectin (pectinase). Oil extracted from this enzyme preparation has higher yield, improved stability and rich in vitamin E and polyphenols (Kashyap et al., 2001).

Alkaline pectinases have a very important role in pectin degradation and removing mucilaginous coats from the coffee bean. Pectinolytic microbes are employed to degrade the mucilaginous coat from coffee during fermentation, thereby improving the coffee quality (Sharma et al., 2012; Praveen & Suneetha, 2014; Bhardwaj et al., 2017). Enzyme preparation having cellulolytic, hemicellulolytic, and pectinolytic activities is employed to degrade the mucilage coat and pulpy layers of the coffee beans, thereby enhancing the coffee quality. Pretreatment of coffee beans with commercial enzyme preparations reduces the fermentation time of coffee production. In tea industries pectinases of fungi are employed for quicker fermentation of tea leaves. Foam-forming properties of properties of instant tea powders can be reduced by treatment with pectinase. This treatment improves the quality, aroma, color, and market price of tea (Kashyap et al., 2001; Praveen & Suneetha, 2014; Hassan & Ali, 2016). Along with other enzymes pectinases are employed in feed industries to process the feed rich in pectin to enhance digestibility of nutrients and performance of animals.

10.4.9 Xylanases (EC 3.2.1.8)

Xylan is a structural heteropolysaccharide found in the cell wall of plants. It makes up the significant part of dry weight (approx. 25–35%) of woody tissues and lignified tissues in dicots and monocots, respectively. In some grasses and cereal grain tissues it makes up approx. 50% of dry weight. Xylan is made up of linear chain of xylose units which are attached to each other by β-1,4 glycosidic bond. Arabinose, methyl glucuronic acid, glucuronic acid, and *O*-acetyl groups are also found attached to main chain of xylan as branches (Ebringerová & Heinze, 2000; de Vries & Visser, 2001; Heinze et al., 2004; Mussatto et al., 2008). Xylanases degrade polysaccharide xylan into monosaccharide xylose through breakdown of β-1,4 glycosidic bond. Majorly three enzymes are needed for the complete catalysis of xylan into xylose. (1) Endoxylanases make the cut in between the chain of xylan to xylooligosaccharides (XOS). (2) Exoxylanases remove the xylose from the non-reducing end of xylan. (3) β-xylosidases cleave XOS and xylobiose and produce xylose. Depolymerization of xylan for industrial applications requires a few more xylanolytic enzymes such as *p*-coumaric esterase, α-1-arabinofuranosidase, ferulic acid esterase, and acetylxylan esterase and α-glucuronidase (Collins et al., 2005; Walia et al., 2017; Bhardwaj et al., 2019).

10.4.9.1 Sources

Xylanases can be obtained from actinomycetes, bacteria, and fungi. Commercially bacteria are used frequently for the isolation of xylanases. Xylanases work effectively at pH in between 5 and 9 and temperature in between 35 and 60 °C. *Clostridium acetobutylicum* (Walia et al., 2017), *Streptomyces* sp., *Pediococcus acidilactici* GC25 (Adiguzel et al., 2019), *B. subtilis* ABDR01 (Yadav et al., 2020), *B. licheniformis* DM5 (Ghosh et al., 2019), and *B. pumilus* (Chakdar et al., 2016) are some bacterial strains that are exploited most for the generation of xylanases at industrial scale. Fungi produce extracellular xylanases with a higher yield. Also fungal xylanases show higher activity as compared to bacterial and yeast xylanases. *Aspergillus japonicus*, *Penicillium occitanis* Pol6 (Driss et al., 2012), *Fusarium* sp., *Pichia pastoris* (de Queiroz Brito Cunha et al., 2018), etc. are some fungal sources of xylanases.

10.4.9.2 Applications

In food industries xylanases find many applications such as fruit juice clarification, enhancing the overall quality cookies and breads, synthesis of prebiotics (xylooligosaccharides and arabinoxylooligosaccharides), etc.

Along with different enzymes such as α-amylase, arabinofuranosidase, laccase, and glucanase, xylanases are used to enhance the rheological properties of the bakery products. During making of bread xylanases are used to transform the water

insoluble hemicellulose (arabinoxylan) into water soluble hemicellulose (arabinoxylan). Formation of water soluble arabinoxylan in the dough causes the increase in its volume. Moreover, more uniform and finer crumbs are formed and hardness of dough reduces. Also, xylanases treated dough does not stick to machine parts while making dough (Butt et al., 2008). Degradation of xylan by xylanases causes redistribution of water in the wheat flour. It makes the kneading easier and dough softer. Also it delays the formation of crumb and causes the dough to rise during baking of bread (Polizeli et al., 2005). Xylanases work as anti-staling agent and increases the shelf-life and quality of bread (Harris & Ramalingam, 2010). In 2018, xylanases were produced from genetically modified *Pichia pastoris* having xynBS27 gene of *Streptomyces* sp. It was observed that xylanases decreased the sugar content, hardness of bread, and increased the volume of bread (de Queiroz Brito Cunha et al., 2018). Xylanases are also used to enhance texture, taste, aroma, and quality of biscuits.

Naturally extracted juices are hazy or turbid due to existence of cellulose, hemicellulose, protein, pectin, lipid, and other components. Therefore, in juice industries various enzymes that degrade cell wall such as pectinase, cellulase, hemicellulose are employed. Treatment with cell wall degrading enzymes improves the clarity, extraction, yield, and palatability of fruit juices. Xylanases are used to degrade hemicelluloses present in raw juice. consequently, extraction, clarification, quality, and yield of juices get improved (Bhardwaj et al., 2019). Xylanases produced from *Bacillus stearothermophilus* were used for the clarification of citrus fruit juices (Dhiman et al., 2011). *Pediococcus acidilactici* GC25 derived endoxylanase was employed for clarification of different fruit juices (Adiguzel et al., 2019).

In the making of papad from black gram xylanases are used. In black gram content of arabinoxylan remains high that causes hardness in the papad dough, consequently, process of papad making becomes difficult. Addition of xylanases to black gram flour causes the breakdown of arabinoxylan that leads to reduction of papad hardness and requirement of water. Also oil requirement for frying of papad decreases after xylanases treatment while other characteristics (color, taste, or texture) of papad remains unaffected (Awalgaonkar et al., 2015).

Degradation of xylan by xylanases produces XOS. XOS are considered as prebiotics and added to many food products as food supplement. Prebiotics are known to confer many beneficial effects to the host. Prebiotics also known to increase the growth of probiotics (good bacteria) in the gut of humans. Therefore, prebiotics are recommended as functional food supplements to sustain healthy lifestyle.

Xylanases are also used in the processing of ruminants and non-ruminant animal feed. Due to high fiber content (cellulose and hemicellulose) it is difficult for ruminant and non-ruminant animals to digest plant-based feed. Therefore, xylanases are used to degrade hemicellulose present in the feed to enhance feed digestibility by these animals (Bhat, 2000).

During the production of beer xylanases are employed for the degradation of cell wall of barley. Xylanases treatment degrades the xylan present in the cell wall of barley into arabinoxylans and oligosaccharides. This treatment causes beer clarification.

10.4.10 Glucose Oxidase (EC 1.1.3.4)

Glucose oxidase (GOX) is the one of the members of oxidoreductase family of enzymes. GOX is made up of 2 identical subunits of molecular weight 80 kDa each. It is a flavoprotein having FAD at its active site. It uses O_2 as an electron acceptor and catalyzes the oxidation of D-glucose into D-glucono-δ-lactone and H_2O_2. D-Glucono-δ-lactone is hydrolyzed to D-gluconic acid by enzyme lactonase, whereas catalase breaks down H_2O_2 into O_2 and H_2O.

10.4.10.1 Sources

Among the microbial sources fungi are exploited commonly for the generation of glucose oxidase commercially. GOX was firstly isolated from *A. niger* in 1928 (Wong et al., 2008). Different species of *Aspergillus* such as *A. carbonarius*, *A. niger*, *A. nidulans*, *A. tubingensis*, *A. oryzae,* and *A. terreus* are known for the generation of glucose oxidase (Kornecki et al., 2020). Many *Penicillium* species like *P. amagasakiense*, *P. purpurogenum*, *P. glaucum*, *P. notatum*, and *P. adametzil* are known for the generation of glucose oxidase (Khatami et al., 2021). Some other glucose oxidase producing fungal sources include *Mucor circinelloides* (Kornecki et al., 2020) and *Cladosporium neopsychrotolerans* (Ge et al., 2020). Many bacterial species are also exploited for production of glucose oxidase.

10.4.10.2 Applications

Glucose oxidase is employed for various purposes in food industries. GOX is used to remove glucose and oxygen from the food products, thereby improve their shelf-life. In food processing industries GOX is used to produce D-glucono-δ-lactone from glucose molecules available in the food products. After production D-glucono-δ-lactone acts as preservative; therefore, shelf-life, stability, quality, and flavor of food products get improved. GOX is also used to decrease the content of glucose from the drinks, consequently, GOX treated drinks become appropriate for diabetic patients.

For the generation of wine with lower alcohol contents GOX and catalase can be used in combination. Combined activity of these enzymes decreases the alcohol content to 2% within 30 h but aroma profile and organoleptic properties of the wine affected significantly. GOX activity reduces the glucose content, thereby availability of glucose for the production of alcohol through anaerobic fermentation decreases. GOX and catalase in combination are also used to eliminate molecular oxygen from wine to enhance its shelf-life (Röcker et al., 2016).

In baking industries, GOX are used for making of strong dough and to enhance the bread volume. A novel GOX produced by researchers was also effective in

enhancing the bread volume (Ge et al., 2020). Glucose oxidase is also used in food packaging industries as it has property to remove oxygen.

10.4.11 Laccase (EC 1.10.3.2)

Laccases are copper metalloenzymes and have four copper atoms per molecule. They belong to oxidoreductase class of enzymes and to blue oxidase subgroup. They cause the oxidation of phenolic compounds, ascorbic acids, and aromatic amines. They are produced by bacteria, fungi, plants, soil algae, and some insects. Laccase of microbial sources degrades the lignin of wood to produce cellulose and hemicellulose, while laccase of plants synthesizes the lignin in plants.

10.4.11.1 Sources

Fungi are most exploited for production of laccase at industrial scale. *Funalia trogii*, *Trametes versicolor*, *Pleurotus eryngii*, *P. flabellatus*, *P. lampas* (Struch et al., 2016), *Abortiporus biennis* (Yin et al., 2017), *Pleurotus ostreatus* (Lettera et al., 2016) are some fungal sources of laccase. Some strains of bacteria, viz. *Bacillus licheniformis* are employed for the generation of recombinant laccase for use in industries.

10.4.11.2 Applications

Wastewater from olive-oil industries has high concentration of phenols (1.5–8.0 g/L). Therefore, laccase taken from fungal sources is utilized to oxidize the different phenolic compounds released into wastewater from olive-oil industries (Osma et al., 2010). In wine industries laccase is employed to eliminate the O_2 from the wine to improve its shelf-life (Okpara, 2022). Laccases are also used for stabilization of wine through control of phenolic compounds (Osma et al., 2010). During production of beer laccase is used for the oxidation of polyphenols, consequently, formation of haze is minimized, and beer clarification and stabilization get improved. Moreover, laccase acts as preservative through eliminating the molecular oxygen during the process of beer production. Laccases are also used in fruit juice industries either alone or in combination with cellulase and pectinase to enhance the juice yield and clarification. In 2016, a group of researchers have reported the reduction of phenol content in fruit juice by 45% through the use of immobilized laccase taken from fungal sources (Lettera et al., 2016). In 2017, another group of researchers have reported the efficient clarification of litchi juice through the use of thermostable laccase taken from *Abortiporus biennis* strain J2 (Yin et al., 2017). In baking industry, laccase is utilized to crosslink biological polymers, consequently, dough strength, stability, and rheological properties get improved (Manhivi et al., 2018). In

dairy industries, laccase is utilized to crosslink milk proteins in skim milk to enhance the quality of yogurt (Mokoonlall et al., 2016; Struch et al., 2016).

10.4.12 Naringinases (EC 3.2.1.40)

Naringin, limonin, and neohesperidin are most bitter substances found in the citrus fruits. In grapes naringin is the major component, and most bitter compound. Naringinases, an enzyme complex, have α-L-rhamnosidase and β-D-glucosidase activities. Naringinases catalyze the breakdown of naringin to simpler compounds like prunin, rhamnose, naringenin, and glucose due to its activities. Naringin is hydrolyzed first by α-rhamnosidase to produce prunin and rhamnose. Thereafter, prunin is hydrolyzed by β-glucosidase to produce naringenin and glucose. Therefore, naringinases are used in industries as debittering enzymes during the generation of citrus fruit juices and to enhance the taste, flavor, and aroma of fruit juices.

10.4.12.1 Sources

Microorganisms mainly fungi and bacteria are extensively exploited for the isolation of naringinases commercially. Fungal sources are utilized largely for the production of high yield of enzyme. *Thermomicrobium roseum*, *Bacillus amyloliquefaciens*-D1 (Pegu et al., 2021), *Burkholderia cenocepacia* (Patil et al., 2019), *B. amyloliquefaciens* strain 11568 (Zhu et al., 2017), *Thermotoga maritima* MSB8, *Thermoclostridium stercorarium* DSM 8532, *Thermotoga neapolitana* Z2706-MC24, *Caldicellulosiruptor bescii* DSM 6725 (Baudrexl et al., 2019), *Cryptococcus albidus* (Borzova et al., 2018) are some examples of bacterial strains producing naringinase. *A. usamii*, *A. niger*, *A. oryzae*, *A. flavus*, *Penicillium decumbens*, *Rhizoctonia solani*, *Cochliobolus miyabeanus*, *Lasiodiplodia theobromae, Rhizopus nigricans*, *Coniothyrium diplodiella* (Patil et al., 2019) are some examples of fungal species producing naringinase.

10.4.12.2 Applications

Citrus fruit juice industries use the naringinase as debittering enzyme and to enhance the taste, flavor, and aroma of fruit juice. In 2017, a group of researchers isolated the naringinases from *B. amyloliquefaciens* strain 11568 and used it to decrease the bitterness of citrus fruit juice through degradation of naringin present in citrus fruits (Zhu et al., 2017). Naringinases are also used by various food processing industries to synthesize different food additives such as sweeteners to enhance the taste and flavor of food. Kinnow peel is the major waste product of citrus fruit processing industries. Naringin is the major component in the peel of kinnow. Therefore, α-L-rhamnosidase activity of naringinase can be utilized to produce the L-rhamnose

(Puri et al., 2011). L-Rhamnose has applications in pharmaceutical industries and can be used as a plant protective agent. Naringinase is used in wine industries along with arabinosidase for improving the aroma of wine.

10.4.13 Esterase (EC 3.1)

Esterase is one of the members of hydrolase family of enzymes. They degrade the esters into alcohols and acids in aqueous solution. Esterases degrade the short chain acylglycerols into their components like glycerol and fatty acids rather than long chain acylglycerol, which makes them different from lipases. Feruloyl esterase is the one of the members of esterases family of enzymes. This enzyme produces the ferulic acid through the degradation of ester bond between ferulic acid (hydroxycinnamic acid) and different polysaccharides present in the plant cell wall. These enzymes are also useful in the management of waste as they can degrade the lignocellulosic biomass produced from the plants.

10.4.13.1 Sources

Bacteria are exploited most for production of esterases at industrial scale. *Lactobacillus acidophilus*, *L. farciminis*, *L. amylovorus*, *L. fermentum* (Xu et al., 2017), *Bacillus licheniformis* (Alvarez-Macarie & Baratti, 2000) are exploited to produce feruloyl esterase.

10.4.13.2 Applications

Esterases are mainly used in beverage industries for the making of beer, wine, alcohol, and fruit juices. Esterases and lipases are used to convert the low-value fat or oil into high value fat or oil through transesterification reactions. To improve the flavor and aroma in cheese and its related products esterase and lipase isolated from *L. casei* CL96 are used to hydrolyze the milk fat (Choi & Lee, 2001). Ferulic acid produced by the action of feruloyl esterase has found many applications in food industries. Ferulic acid is utilized to synthesize vanillin, an aroma compound. Vanillin being a major ingredient of vanilla is used to enhance the flavor of beverages (Gallage et al., 2014). Moreover, ferulic acid is employed in food industries as an additive in functional foods. In juice industries esterases are utilized to enhance the flavor and aroma of various fruit juices through modification of oil and fat in juices (Panda & Gowrishankar, 2005). A protease resistant feruloyl esterase was isolated from the microbes of cow rumen and was utilized to produce ferulic acid through hydrolysis of wheat straw. This enzyme is of great industrial significance as it has high pH resistance, thermal resistance, and protease resistance (Cheng et al., 2012).

10.4.14 Glucoamylase (EC 3.2.1.3)

Glucoamylase is a type of exoamylase that releases the D-glucose from the non-reducing end of starch. It is also known as saccharifying enzyme and is found in all living organisms. D-Glucose generated by the action of glucoamylase can be used as substrate for various fermentation processes in food and beverage industries.

10.4.14.1 Sources

Rhizopus oryzae F-923 (Fadel et al., 2020), *A. niger* (Bagheri et al., 2014), and *Aspergillus awamori* (Coutinho & Reilly, 1997; Blanco et al., 2014) are common fungal sources for production of glucoamylase at industrial scale.

10.4.14.2 Applications

In confectionary industries glucoamylases are employed for making of glucose and/or fructose syrup for the making of candies. In baking industries glucoamylases are used to generate simple sugars from starch. Fermentation of these sugars by yeast produces CO_2 that causes the dough to rise. They also acts as anti-staling agent and enhance the quality of fluor, bread crust colour and high fibre baked products. In brewing industries glucoamylases are used for the production of simple sugars that are ultimately fermented by *S. cerevisiae* to produce ethanol.

10.4.15 Phospholipase (EC 3.1.1.4)

Phospholipase causes the breakdown of phospholipids to produce fatty acids and other lipophilic compounds. Phospholipases are divided into two categories, acyl hydrolases and phosphodiesterases. Phospholipase A1, A2, and B are types of acyl hydrolase, while phospholipase C and D are types of phosphodiesterase.

10.4.15.1 Sources

Fungi are exploited most for the isolation of phospholipase commercially. Some fungal sources of production of phospholipase are *Fusarium oxysporum* (Su et al., 2017), *B. cereus* (Elena et al., 2017), *Streptomyces chromofuscus* (Cerminati et al., 2019), and *A. oryzae* (Wang et al., 2021).

10.4.15.2 Applications

Phospholipases are mostly used in oil, dairy, and bakery industries. In dairy industries phospholipases are employed for the generation of cheese. They decrease the loss of milk fat in whey, consequently, cheese texture, flavor, aroma, and yield get improved (Lilbaek et al., 2006). Alike lipases, phospholipases are employed as degumming agent during the production of refined oil to improve its yield and quality. A chimeric enzyme named Lecitase ultra was prepared by fusing phospholipase A1 gene and lipase gene for degumming of vegetable oils. Phospholipase A1 was taken from *Fusarium oxysporum* while lipase gene was taken from *Thermomyces lanuginosus* (Virgen-Ortíz et al., 2019). Degumming of crude soybean oil by using alkaline cold active phospholipase C of *Aspergillus oryzae* has been reported elsewhere (Wang et al., 2021). A mutant version of phospholipase C (F66Y) isolated from *Bacillus cereus* has been used for degumming of soybean oil. This enzyme was able to remove almost 90% of phosphatidylethanolamine (Elena et al., 2017). Phospholipases are also employed to enhance the quality and shelf-life of sauces, mayonnaise, and baked products. Phospholipases are also employed to enhance the nutritive value of soya lecithin (fat containing animal feed).

10.4.16 Phytase (EC 3.1.3.8)

Phytase is a member of phosphatase class of enzymes and removes the phosphorus from phytic acid present in grains and oilseeds.

10.4.16.1 Sources

Phytase is widely distributed enzyme and found in all living organisms. However, microbes are exploited most for the generation of phytase at industrial scale. *A. ficuum*, *A. fumigatus*, *A. niger*, *K. oxytoca, K. terrigena*, *E. coli*, *B. amyloliquefaciens*, *B. subtilis*, *Schizosaccharomyces pombe* are some microbial species that are exploited for the isolation of phytase (Pandey et al., 2001; Ciofalo et al., 2003; Selle & Ravindran, 2008).

10.4.16.2 Applications

In grains and oilseeds, minerals such as calcium, zinc, iron sometimes remain bound to phytic acid, consequently, bioavailability of these minerals to monogastric animals gets reduced. Therefore, to improve the bioavailability of minerals bound to phytic acid, phytases are mixed with the feed of monogastric animals. This leads to increase in nutritive value of animal feed (Ciofalo et al., 2003; Selle & Ravindran, 2008).

10.5 Conclusion and Future Perspectives

Enzymes are used routinely in food industries for the generation of wine, bread, juices, ice cream, cheese, beer, refined oil, gluten-free food, etc. Sometimes maintenance of optimum conditions for the working of enzymes during industrial production of products seems difficult and tedious. Therefore, innovative methods are being employed for the design of improved/new biocatalysts with desired features such as less sensitive to change in temperature and pH, less susceptible to presence of inhibitory agents, less or no requirement of cofactor and/or coenzyme, without affecting the activity of novel enzymes. Development of different technologies such as protein engineering and recombinant DNA technology has made a huge impact on the development of improved or novel biocatalyst.

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