CHAPTER

2

Microbial Enzyme in Food Biotechnology

Poonam Singh[*], *Sanjay Kumar*[*,†]

[*]Department of Molecular and Cellular Engineering, SHUATS, Allahabad, India [†]Department of Biological Engineering, Indian Institute of Technology Gandhinagar, Gandhinagar, India

2.1 INTRODUCTION

The application of enzymes and microorganism in food processing is traditionally a well-known approach. Enzymes and microorganism have been used in the brewing of beer, bread baking, and the cheese and wine making process for ages (Pandey et al., 1999; Pedro, 2010). Food biotechnology is offering various ways to improve the processing of raw materials to convert them into high nutritional value food products (Underkofler et al., 1957). Enzyme technology is improving food quality in various ways, like enhancing functionality, nutritional value, and the flavor and texture of food products. The sources of enzyme production are animal, plant, and microorganism, with microorganisms (bacteria, fungi, yeasts, and actinomycetes) being the best and most suitable sources of commercial enzyme production. Globally, enzymes viz. α-amylase, glucoamylase, lipase, pectinase, chymosin, and protease are use in food-processing industries. The α-amylase enzyme converts starch into dextrins and produces corn syrup for various applications, such as enhancing the sweetness of various food products. The brewing process uses a solubilization of complex carbohydrates into simple form using barley and other cereals grains. In production of good-quality light beer, glucoamylase (hydrolytic enzymes) convert dextrins into glucose in the form of corn syrup, changing the residual dextrins into fermentable sugars. Lipases are fat-hydrolytic enzymes that enhance flavor, shorten the time for cheese ripening, and produce special fat products with better qualities. Pectinase is a hydrolytic enzyme that is used in extraction, clarification, and filtration of fruit juice. Chymosin enzymes help break down kappa-caseins in the milk curding process. Bacterial and fungal proteases enzymes are applied in the production of fish meals, meat extracts, texturized proteins, and meat extenders. Lactase enzymes break down the lactose present in whey and milk products to produce polyactide. Glucose oxidases are used to change glucose into gluconic acid to stop maillard reactions. Acetolactate decarboxylase converts acetolactate to acetoin to minimize the maturation time in wine making. Cellulase is a nonstarch polysaccharide-solubilizing enzyme that helps in the conversion of

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cellulose to glucose in cell walls in the breakdown of various grains; they facilitate better extraction of cellular products and release nutrients to increases the fiber content in foods (Christopher and Kumbalwar, 2015). Enzyme technologies are positively affecting the food manufacturing industry by providing new and valuable products, lowering production costs, and improving processes.

2.2 SOURCES OF ENZYMES

Initially, enzymes were extracted from the stomach of calves, lambs, and baby goats, but now are produced by microorganisms like bacteria, fungi, yeast, and actinomycetes. Enzymes obtained from microorganisms are better than those of animal and plant origin. Microorganisms can be genetically manipulated to improve the production of commercial scale (Pandey et al., 1999; Sabu, 2003). Enzymes can hydrolyze complex molecules into simple monomer units, like carbohydrates into simple sugars, which are natural substances involved in all types biochemical processes. Every enzyme is substrate, pH, and temperature-specific for catalyzing the reaction to convert a reactant into a product (Afroz et al., 2015; Shuang et al., 2012). The food-processing industry uses more than 55 different microbial enzymes (Table 2.1).

2.3 PRODUCTION PROCESSES OF ENZYMES

There are two main parts of industrial enzyme production: (i) screening of potential microbial strains; (ii) the fermentation process. Production media composition is another important component of commercial enzyme production; suitable media contains carbon sources, nitrogen sources, and micronutrients which support the growth of microorganisms in the fermentation process. Once fermentation is complete to start the downstream process, like recovery of the enzyme, purification and formulation of products can occur within a suitable carrier (Fish and Lilly, 1984). The fermentation process is categorized into three groups: (i) batch process,

TABLE 2.1 An Overview of Enzymes Used in Food Processing Industry

S.N.	Class	Enzyme	Role
1.	Oxidoreductases	Glucose oxidase	Dough strengthening
		Laccases	Clarification of juices, flavor enhancer (beer)
		Lipoxygenase	Dough strengthening, bread whitening
2.	Transferases	Cyclodextrin	Cyclodextrin production
		Glycosyltransferase	
		Fructosyltransferase	Synthesis of fructose oligomers
		Transglutaminase	Modification of viscoelastic properties, dough processing, meat processing

TABLE 2.1 An Overview of Enzymes Used in Food Processing Industry—cont'd

S.N.	Class	Enzyme	Role
3.	Hydrolases	Amylases	Starch liquefaction and sachcarification, Increasing shelf life and improving quality by retaining moist, elastic and soft nature, Bread softness and volume, flour adjustment, ensuring uniform yeast fermentation, Juice treatment, low calorie beer
		Galactosidase	Viscosity reduction in lupins and grain legumes used in animal feed, enhanced digestibility
		Glucanase	Viscosity reduction in barley and oats used in animal feed, enhanced digestibility
		Glucoamylase	Saccharification
		Invertase	Sucrose hydrolysis, production of invert sugar syrup
		Lactase	Lactose hydrolysis, whey hydrolysis
		Lipase	Cheese flavor, in-situ emulsification for dough conditioning, support for lipid digestion in young animals, synthesis of aromatic molecules
		Proteases	Protein hydrolysis, milk clotting, low-allergenic infant-food formulation, enhanced digestibility and utilization, flavor improvement in milk and cheese, meat tenderizer, prevention of chill haze formation in brewing
		Pectinase	Mash treatment, juice clarification
		Peptidase	Hydrolysis of proteins (namely, soy, gluten) for savoury flavors, cheese ripening
		Phospholipase	In situ emulsification for dough conditioning
		Phytases	Release of phosphate from phytate, enhanced digestibility
		Pullulanase	Saccharification
		Xylanases	Viscosity reduction, enhanced digestibility, dough conditioning
4.	Lyases	Acetolactate decarboxylase	Beer maturation
5.	Isomerases	Xylose (Glucose) Isomerase	Glucose isomerization to fructose

Source: Bloom, J.D., Meyer, M.M., Meinhold, P., Otey, C.R., MacMillan, D., Arnold, F.H., 2005. Evolving strategies for enzyme engineering. Curr. Opin. Struct. Biol. 15, 447–452; Fernandes, P., 2010. Enzymes in food processing: a condensed overview on strategies for better biocatalysts. J. Enzym. Res. 2010, 1–19; Riberiro, D.S., Henrique, S.M.B., Oliveira, L.S., Macedo, G.A., Fleuri, L.F., 2010. Enzymes in juice processing: a review. Int. J. Food Sci. Technol. 45, 224–230.

(ii) fed batch process, and (iii) continuous process. In the batch process, all media components are added at the start of fermentation. Fed batch fermentation is similar to batch fermentation but the production strain is fed with an additional nutrient medium during the fermentation process. Continuous fermentation is a steady state reached by supplying the fresh medium with a simultaneous harvest from the fermenter. The fermentation process can be conducted in

one of two ways—either solid-state fermentation, or submerged fermentation (Rana and Bhat, 2005). Bacterial enzyme production is almost exclusively achieved by submerged fermentation because bacterial cell growth and enzyme secretion are more suitable in a submerged condition than during solid-state fermentation (Aunstrup, 1979). In submerged fermentation, sterilized production media are inoculated by bacterial strains and maintain proper fermentation parameters like aeration, agitation, dissolved oxygen, rotation, temperature, and pH for 48–72 h, depending upon the bacterial strain. However, solid-state fermentation processes are suitable for fungal strains to produce valuable industrial enzymes for various food processing techniques. Fungal filament prefers surface fermentation, or solid-state fermentation, for the production of enzymes, and others use full metabolite. Sterilized solid substrate, like wheat bran, rice bran, and many other grains, support the growth of fungal cell mass at a suitable temperature, humidity, and moisture of the fermentation system. After specific periods of fermentation, the products are harvested and continue downstream processing, like filtration of cell debris, purification, and formulation of enzymatic products. Generally, downstream processing of intracellular enzymes is fairly complex as compared to extracellular enzyme. The purification of enzymes is a very expensive step in downstream processing; techniques like chromatography are mainly used in the purification of enzymatic proteins at the commercial level (Linder et al., 2004). The main issue of the formulation of purified enzymes with a suitable carrier to secure enzymatic activity and stability, to insure enzyme easily release at the site of application. Otherwise the efficiency of the enzymatic product is decreased.

2.4 APPLICATION OF ENZYMES IN FOOD PROCESSING

Enzymes are secreted by nearly all living cells for catalysis of their own specific biochemical reactions in the metabolic process. Enzymes are playing an important role in food processing techniques for improving nutritive value and flavor of processed food. The food-processing industry—the making of cheese, leavened bread, wine and beer, yogurt, and syrup—is successfully using enzymes at the commercial level (Dewdney, 1973).

2.4.1 Glucose Oxidase

The glucose oxidase enzyme is commercially produced from *Aspergillus niger* and *Penicillium glaucum* through a solid-state fermentation method. Muller was first to report the catalyzation of glucose oxidase and the breakdown of glucose into gluconic acid in the presence of dissolved oxygen (Muller, 1928). Fungal strains *Aspergillus niger* are able to produce notable amounts of glucose oxidase. Glucose oxidase enzymes are used to remove small amounts of oxygen from food products or glucose from diabetic drinks. Glucose oxidase is playing an important role in color development, flavor, texture, and increasing the shelf life of food products (Khurshid et al., 2011).

2.4.2 Laccase

Laccase enzymes were first obtained from the cell sap of the Japanese lacquer tree. Laccase enzymes are isolated from plants, bacteria, fungi, and insects (Saqib et al., 2012). Laccase is

responsible for discoloration, haze, wine stabilization, baking, and flavoring in food processing (Rosana et al., 2002). Laccase improves the baking process through an oxidizing effect, and provides an additional development in the strength of dough and baked products, including enhancing crumb structure and increasing softness and volume. Another diverse application of laccase is in environmental sectors, which degrade various ranges of xenobiotic compounds.

2.4.3 Cyclodextrin Glycosyl Transferase

Cyclodextrin glycosyl transferase (CGTase) enzymes catalyze the change of starch into nonreducing cyclic sugars (cyclodextrin) (Coelho et al., 2016). Cyclodextrins (CD) are cyclic homogeneous oligosaccharides of glucose residues, which are composed of 6–8 D-glucose units linked by a -1,4 glycosidic bond. Cyclodextrins are being used in the food-processing industry for preparation of reduced-cholesterol products and rising bioavailability of desired molecules, because cyclodextrins facilitate hydrophobic-hydrophilic interactions within protein-protein and other molecules. Production of Cyclodextrin glycosyl transferase (CGTase) is reported in different bacterial groups; major CGTase producers belong to the genus Bacillus. However, *Klebsiella pneumonia*, *Micrococcus luteus*, *Thermococcus*, *Brevibacterium* sp., and hyperthermophilic archaea are reported as major CGTase-producing strains (Mori, 1999; Szerman et al., 2007; Tachibana, 1999).

2.4.4 Transglutaminase

Transglutaminase enzymes catalyze reactions to alter proteins by merging amine, cross-linking, and deamination. Transglutaminase is responsible for acyl transfer, deamidation, and the inter- and intra-molecular crosslink between amino acid residues of glutamine and lysine (Chanyongvorakul et al., 1995; Christensen et al., 1996; Kuraishi et al., 1997). The commercial application of transglutaminase enzymes in the food-processing industry is improving the protein-emulsifying capacity, gelatation, viscosity, and production of various types of protein ingredients to enhance the quality of food products. Transglutaminase is enhancing the water-holding capacity, softness, foam formation, and stability of food products. Extracellular transglutaminase is isolated from cultural filtrate of *Strepto verticillium* spp., *Strepto verticillium mobarens*, *Strepto verticillium ladakanum*, and *Strepto verticillium lydicus* (Ando et al., 1989; Dickinson, 1997; Jiang et al., 2000; Motoki et al., 1984; Tsai et al., 1996; Zhu, 1995). Intracellular transglutaminase is secreted by common microbial species *Bacillus subtilis* and *spherules* (Tsai et al., 1996).

2.4.5 Lactase

Lactase enzymes catalyze the breakdown of the milk sugar lactose into simple sugar monomer units like glucose and galactose. Lactases are obtained from plants, animal, bacteria, fungus, yeasts, and molds. Commercial production of lactase enzymes are developed from *A. niger*, *A. oryzae*, and *Kluyveromyces lactis* (Mehaia, 1987). Fungal origin lactases have optimum activity at acidic pH ranges, and yeast and bacterial-originated lactases have optimum pH ranges near to neutral (Gekas and Lopez-Levia, 1985). The lactase enzyme is predominantly

rich in infancy and is called a brush border enzyme. Some people do not produce enough of the lactase enzyme so they do not properly digest milk. This is called lactose intolerant, and people who are lactose intolerant need to supplement the lactase enzyme to aid in the digestion of milk sugar. Another useful application of the lactase enzyme is it increases the sweetness of lactase-treated milk, and assists in the manufacturing of ice cream and yogurt preparation.

2.4.6 Catalase

Catalase enzymes break down hydrogen peroxide (H_2O_2) to water and oxygen molecules, which protects cells from oxidative damage by reactive oxygen species. Commercial catalases are produced from *Aspergillus niger* through a solid-state fermentation process (Fiedurek and Gromada, 2000). The major applications of catalase in the food-processing industry include working with other enzymes like glucose oxidase, which is useful in food preservation and egg processing, and sulphydryl oxidase, which under aseptic conditions, can eliminate the effect of volatile sulphydryl groups, that is, they generate from thermal induction and are responsible for the cooked/off-flavor in ultra-pasteurized milk (Maur, 1996).

2.4.7 Lipase

Lipases catalyze the hydrolysis of ester bonds in lipid substrates and play a vital role in digestion and the transport and processing of dietary lipids substrate (Svendsen, 2000). Lipases catalyze the biochemical reaction like esterification, interesterification, and transesterification in nonaqueous media which frequently hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. Microorganism like *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcoccus aureus*, and *Bacillus subtilis* are the best sources of lipase enzymes. Lipases are widely used in pharmacological, chemical, and food industries. The commercial applications of lipases in the food industry are the hydrolysis of milk fats, pronounced cheese flavor, low bitterness, and prevention of rancidity. Lipases may combine with many other enzymes like protease or peptidases to create good cheese flavor with low levels of bitterness (Wilkinson, 1995).

2.4.8 Protease

Proteolytic enzymes are also termed as peptidases, proteases, and proteinases, which are able to hydrolyze peptide bonds in protein molecules. Proteases are generally classified as endopeptidases and exopeptidases. Exopeptidases cut the peptide bond proximal to the amino or carboxy termini of the protein substrate, and endopeptidases cut peptide bonds distant from the termini of the protein substrate. Proteases are obtained from diverse groups of organisms such as plants, animals, and microorganisms, but commercially viable proteases are obtained from microorganisms, especially bacterial and fungal species. Microorganisms secrete the extracellular and intracellular proteases in both the submerged and solid-state fermentation process. Bacillus species of bacteria, like *Bacillus licheniformis, Bacillus subtilis,* and *Aspergillus* species of fungus like *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. oryzae*, are the best sources of protease enzyme. Broad working range of temperature (10–80°C) and pH (4–12) of protease enzymes increases their application in the food-processing industry, the

major role in cheese and dairy product manufacturing. Aminopeptidases are significantly improving the flavor in fermented milk products. Other basic applications of proteases in the food-processing industry are to increase the nutritive value of bread, baked goods, and crackers (Law and Haandrikman, 1997).

2.4.9 α-Amylase

Amylase enzymes hydrolyze complex starch molecules into simple monomer units of glucose. Sources of α-amylase are plants, animals, and microorganisms, but commercially viable amylases are produced from microorganisms, especially bacterial and fungal species. Thermostable α amylase is produced by some potential bacterial species like *Bacillus licheniformis* and *Bacillus stearothermophillus*, *Pseudomonas*, and the *Clostridium* family. Starch-converting properties of α-amylases are playing an important role in the food, beverage, and sugar industries. α-Amylase is improving the quality of breads that have reduced size and poor crust color, and compensates for the nutritional deficiencies of the grain. α-Amylase also degrades the starch in wheat flour into small dextrins, thus allowing yeast to work continuously during dough fermentation, proofing, and the early stages of the baking process. α-Amylases are also employed in many other aspects of the food industry like clarification of beer, fruit juices, and pretreatment of animal feed to improve the digestibility of fiber (Ziegler, 1999).

2.4.10 Pectinase

Pectinase breaks down pectin components, which are found in the middle lamella of plant cell walls. Pectin is made up of complex colloidal acid polysaccharides with a back bone of galacturonic acid residue with a α-1-4 linkage. Pectinase therefore helps to break down plant cell walls to extract cell sap. Potential microbial strains like *Moniliella SB9*, *Penicillium* spp. and *Aspergillus* spp. are good sources of commercial pectinase (Priya and Sashi, 2014). Pectinases are now an essential part of the fruit juice industry, as well as having various biotechnological applications in the fermentation of coffee and tea, the oil extraction processes, and the treatment of pectic waste water from the fruit juice industry. Pectinase is lowering down the viscosity of fruit juice during the clarification process through the degradation of pectin substance in fruit juice and getting better pressing ability of pulp, simultaneously jelly structure are breaking down and increases the yields of fruit juice (Dupaign, 1974). Another significant application of pectinase enzymes in industrial processes is the refinement of vegetable fibers during the starch manufacturing process, such as the curing of coffee, cocoa and tobacco, canning of orange segments, and extracting sugar from date fruits.

2.4.11 Acetolactate Decarboxylase

Acetolactate decarboxylase catalyzes the conversion of acetolactate into acitoine and release carbon dioxide, a type of decarboxylation reaction. α-Acetolactate decarboxylase is commercially produced by the submerged fermentation of *Bacillus subtilis*, genetically improved *Bacillus brevis* and *Enterobacter aerogenes* strain 1033. In conventional brewing procedures, α-diacetyl is produced from α-acetolactate and this further reduces to acetoin over

a 2–4 week maturation period, but α-acetolactate decarboxylase causes direct decarboxylation of α-acetolactate to acetoin and avoiding maturation period.

2.4.12 Xylose (Glucose) Isomerase

Xylose isomerase (D-xylose ketol-isomerase) catalyzes the isomerisation reaction of D-xylose into xylulose. This is initial step of xylose metabolism in microbial cell physiologies (Wovcha et al., 1983). Xylose isomerases are also referred to as glucose isomerases because of their capability to exchange D-glucose into D-fructose. Microorganisms are most suitable sources of xylose isomerase; some potential microbial species are *Streptomyces olivochromogenes*, *Bacillus stearothermophilus*, *Actinoplanes missouriensis*, *Thermotoga maritime* and *Thermotoga neapolitana*, known xylose isomerase procurers. Xylose isomerase loses its catalytic activities up to 50% under acidic conditions (Oshima, 1978). The greatest application for glucose isomerase is in the food-processing industry; it mainly catalyzes two significant reactions such as reversible isomerization of D-glucose to D-fructose, and D-xylose to D-xylulose.

2.5 FUTURE ASPECTS OF ENZYMES IN FOOD PROCESSING

Food and feed processing areas are having great success using biological agents like enzymes and microorganisms for the manufacturing of valuable food products (Pedro, 2010). In the future, recombinant strains will be playing a significant role in the production of industrial enzymes, but currently there is some hesitation applying them to genetically modified food products. There is no doubt that the genetically engineered production culture is superior to the wild strain. The sophisticated fermentation techniques and downstream processing will also provide support to the manufacturing of pure and large-scale food processing enzymes. There is a need to develop a novel food processing technology to reduce the cost and time of manufacturing processed food items. Therefore, more advanced research is needed in the area of recombinant DNA technology for improvement of production strains, commercially viable enzyme production, and the development of new food-processing techniques for maximum cost-effective product formulation.

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