Microbial Enzymes in Food Processing

Praveen Kumar Mehta and Shelly Sehgal

1 Introduction

Enzymes are biological macromolecules (typically proteins), produced by living organisms that accelerate the rate of chemical reactions required for life without permanent alteration to themselves. Enzymes are highly specific, incredibly efficient and operate under mild conditions of temperature and pH. Enzymes are substantial for the sustainability of life in all life forms. They are very efficient catalysts for biochemical reactions and can be obtained from various sources such as animals, plants, and microorganisms.

However, the enzymes derived from the microbial origin are more active and stable than plant and animal enzymes. Moreover, they are considered as potentially interesting candidates for industrial uses because of the ease of culturing substantial quantities in a short span of time by fermentation on incredibly diverse, easy to produce, a wide range of inexpensive and readily available carbon and nitrogen sources.

Microbes represent an important source of the biocatalysts to bring about specific biochemical reactions. Microorganisms are of great importance and proven to be a suitable and leading source of enzymes (Demain and Adrio 2008). History of microbial enzymes reveal that since ancient times, these enzymes have been exploited by humans to production of food products such as cheese making, brewing of beer, bread baking, winemaking and vinegar production etc. (Kirk et al. 2002).

More recently, with the advent of metagenomics and low-cost high-throughput sequencing technologies, it has become possible to characterize the variety of microbial species from nature. In addition, recombinant DNA techniques, have made it possible to develop highly efficient expression systems that can lead to further

P. K. Mehta (✉) · S. Sehgal
Centre for Molecular Biology, Central University of Jammu, Jammu, India
e-mail: mehtapk@cujammu.ac.in

Q. Husain, M. F. Ullah (eds.), *Biocatalysis*,
https://doi.org/10.1007/978-3-030-25023-2_13

enhancement of catalytic properties of enzymes. Microbial enzymes in food applications have not only expanded the food industry but also produced economic assets.

Enzymes tremendously contribute to industrial processes by lowering the energy consumption and maximizing efficiency and yield contributing to its sustainability profile. Microbial sources, enzymes are perceived as natural, nontoxic food components and are preferred by consumers over chemical counterparts used in food-processing.

2 History of Enzyme Technology

Enzymes have been exploited by humans knowingly or unknowingly since prehistoric times. Food processing using biological sources is historically a well-established approach. The ability of the yeast to produce alcoholic beverages from barley was known to the Babylonians and Sumerians before 6000 BC.

The use of chymosin and calve's stomach enzymes for producing cheese has been elucidated in Homer's Greek epic poems as early as 800 BC. Though for centuries enzymes produced by yeast have been used by mankind to produce wine from fermented grape juice, they were technically termed as 'enzymes' only in the eighteenth century.

In the nineteenth century later in 1862, Louis Pasteur studied and discovered the role of yeast in the fermentation of sugar to alcohol. His observation led to the conclusion that this fermentation was catalyzed by '*ferments*', which was functioning within the yeast cells.

The history of modern enzyme technology or industrial production of enzymes for use in food processing started in 1874, when Danish pharmacist, Christian D. A. Hansen developed the first conventional sample of rennet from calve's stomach (Nielsen et al. 1994). Furthermore, the enzyme rennet produced from an animal is a well-set standard even today and is still used in cheese manufacturing. However, at present high-value recombinant chymosin is produced using a microbial source that contains the bovine prochymosin gene introduced through recombinant DNA technology. Apparently this was the first enzyme preparation of relatively high purity which was approved for use in food by the US Food and Drug Administration (FDA) using Bovine chymosin expressed in *Escherichia coli* K-12 (Flamm 1991). In 1878, German physiologist Wilhelm Friedrich Kuhne from University of Heidelberg (1837–1900) introduced the term enzyme from Greek term 'ενζυμον' which literally mean 'in leaven' or 'in yeast'. During the twentieth century, enzyme was recognised as protein, but the emergence of advanced scientific techniques, witnessed the development in industrial production of enzymes. The enzyme urease is noteworthy in the history of enzymology as it was purified and crystallized by James B. Sumner of Cornell University In 1926. Use of enzymes in fruit juice clarification started in 1930, followed by production of invert sugar syrup in the starch industry in the 1960s.

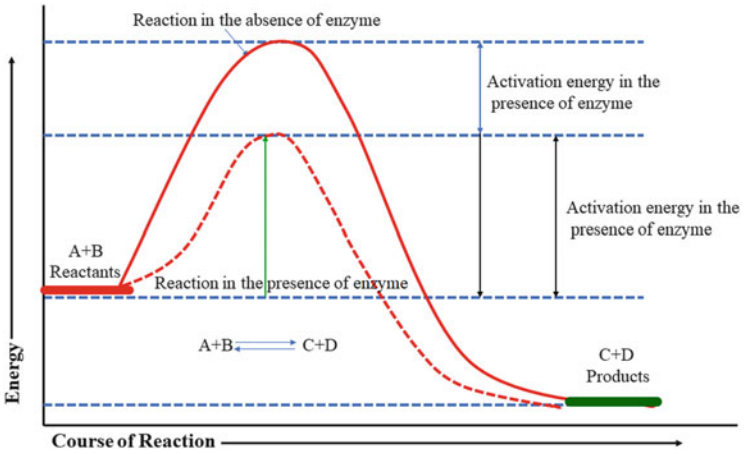

Fig. 1 A schematic diagram showing effect of biocatalyst on free energy changes in a chemical reaction

3 Chemical Nature of Enzymes or Enzyme Classification

Enzymes are natural globular proteins that speed up chemical reactions, and this process is called catalysis. In enzymatic reactions, the molecules upon which enzymes may act are referred to as substrates. Initially, the substrate (S) interacts with the enzyme by binding to the active site of the enzyme (E + S) to form an enzyme-substrate (ES) complex; then the enzyme converts the substrates into different molecules called products.

Enzymes demonstrate extreme specificity for their substrates and therefore catalyze only one or a few types of reactions from among many possibilities. Enzymes facilitate chemical reactions by lowering the activation energy for a reaction (Fig. 1).

Enzymes are remarkably efficient and highly specific biological catalysts produced by a cell that are responsible for carrying out various metabolic processes essential to sustain life (Chapman-Smith and Cronan 1999). Generally, majority of enzymes behave as proteins, although a few important enzymes are catalytic RNA molecules. The latter are known as ribozymes composed of RNA molecules. Enzyme's specificity usually arises from the three-dimensional structure of the enzyme active site. Similar to all other proteins, enzymes are made up of one or more long chain of amino acids linked together by peptide bonds. Each enzyme has a unique sequence of amino acids that causes it to fold into a characteristic shape. Many enzymes require several cofactors (or coenzymes) that is necessary for the enzyme to function properly (Nelson and Cox 2004). These can be metal ions (such as Mn^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}) or organic molecules (such as FAD, NAD, haem, biotin, or coenzyme A). Most of the enzymes are involved in the conversion of substrate

molecule into a product molecule. Enzymes have a high degree of specificity for their substrates, and they accelerate chemical reactions tremendously. Enzymes are classified on the basis of type of chemical reactions they catalyze and the substrate they act upon. According to the enzyme commission (EC) the enzymes are grouped into six main categories. Examples of enzyme types and some of their applications in the food industry are summarized in Table 1.

Table 1 Industrial microbial enzymes, enzyme classes, types of reactions and their role in food processing industries

S. no.	Class	Reactions	Industrial enzymes	Role
1	Oxidoreductases	Oxidation reactions involve the transfer of electrons or hydrogen or oxygen atoms from one molecule to another.	Catalases, Dehydrogenases, Glucose oxidases, Laccases, Lipoxygenases oxygenases, peroxidases	Cheese processing, Dough strengthening, Clarification of juices, flavor enhancer
2	Transferases	Catalyze the transfer of groups of atoms among molecules.	Fructosyltransferases, Glucosyltransferases, Transglutaminase transketolases, acyltransferases, transaminases	Synthesis of fructose oligomers, Laminated dough strength, dough processing, meat processing
3	Hydrolases	Hydrolytic cleavage of bonds	Amylases, Proteases, Peptidases, Pectinases, Phytases, Pullulanase, amylases, Galactosidases, Glucanases, Glucoamylases, Invertases, Lactases, Cellulase, Xylanases, acylases, lipases	Starch liquefaction and saccharification, Lactose hydrolysis, Mash treatment, dough conditioning, cheese ripening, extraction and clarification of fruit juices
4	Lyases	Non-hydrolytic cleavage by addition or elimination reactions	Pectate lyases, acetolactate decarboxylases, hydratases, dehydratases, fumarase, arginosuccinase	Cell wall degradation, fruit softening, Beer maturation
5	Isomerases	Transfer of group from one position to another within the same molecule	Glucose isomerases	High-fructose corn syrup production
6	Ligases	Join molecules together with covalent bonds followed by input of energy in the form of ATP or similar triphosphates	Not used at present	

4 Microbial Enzymes

Microbial enzymes are those enzymes which are derived from microbial sources and are produced through fermentation of the desired microorganisms. Enzymes, particularly of microbial origin, are the worthiest source of commercial enzymes and acquiring much attention with rapid advances in enzyme technology. Microbial enzymes can be obtained from a wide variety of sources such as algae, fungi, yeasts and bacteria, and their properties differ markedly from their source. The microbial source is preferred over plants and animals for the production of enzymes mainly because of the following points:

1. They are comparatively more stable than corresponding enzymes derived from plant or animals.
2. Large-scale production of enzymes can be achieved in short duration of time due to shorter generation times of microbes as compared to other sources.
3. Enzymes from microbial source can be obtained at desired and regular intervals irrespective of seasonal fluctuations.
4. Enzyme contents obtained from the microbial source are more predictable and controllable.
5. Wide range of inexpensive media can be arranged and utilized to acquire microbial enzyme.
6. Ease of process modification and optimization.
7. Microbial sources have better scope for genetic manipulations for achieving a high yield of the end product as compared to rest.
8. Extraction process and purification of microbial enzymes is quite easier and cost effective.
9. Several enzymes can be efficiently produced in spatial and temporal constraints using diverse environmental conditions.
10. Process development is often more environmentally friendly.

Innumerable strains of bacteria, yeasts and molds are commonly used as sources of enzymes for food processing.

5 Strategies to Improve Properties of Microbial Enzymes

The growing demand for the microbial enzyme in food processing industries has led industrialists and researchers to search for new and improved biocatalysts. The majority of currently used microbial enzymes in the food processing industry are derived from recombinant microorganisms. In the 1900s, classic genetics and protein biochemistry were flourishing. However, it got a major boost with the advances in molecular biology and protein engineering which have been leveraged to achieve substantial stabilization and customization of enzymes. There are several different ways by which enzymes can be modified to achieve an improvized end product. Two

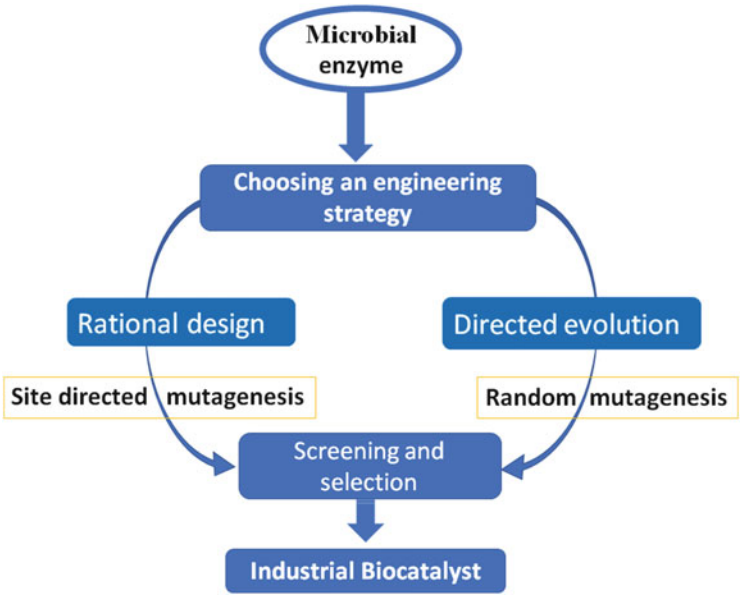

Fig. 2 A schematic diagram illustrating the protein engineering process

strategies which are generally applied in protein engineering are (1) rational protein design and (2) directed evolution. Rational design by site-directed mutagenesis (SDM) is an efficient approach to improve enzyme when the structure, function and catalytic mechanism of the protein of interest are well-known (Fig. 2). In contrast, the directed evolution approach requires only minimal knowledge of its structure and function. It requires only repeated cycles of random mutagenesis and/or gene recombination followed by high throughput screening or selection for positive mutants (Tang and Zhao 2009).

Various recombinant expression systems can be used to produce large scale production of proteins such as *E. coli*, *Bacillus subtilis*, *S. cerevisiae*, *Hansenula polymorpha*, *Pichia pastoris* and species of *Aspergillus* and *Trichoderma*. There are some examples where successful implementation of site-directed mutagenesis method has elevated the overall thermostability of α-amylase after removal of asparagine residues (Declerck et al. 2000). Manipulation of the calcium-binding site of α-amylase such as His-77 to Glu resulted in a four-fold increase in enzyme's half-life (Ghollasi et al. 2013). Likewise, substitution of arginine with proline has resulted in increase in thermostability of the enzyme (Igarashi et al. 1999), or doubly intramolecularly cross-linked lysozyme showed an increase in thermal stability (Ueda et al. 2000). Several enzymes from bacteria and fungi have been engineered to improve their properties to suit their particular applications. Several commercial enzymes for food technology are now derived from particularly selected or genetically modified microorganisms grown in industrial scale fermenters. Table 2 depicts

Table 2 Some examples of microbial technologies and strategies undertaken to improve the performance of microbial enzymes in food processing industries

Enzyme	Role	Targeted improvement	Strategies applied	Reference
α-Amylase	Starch liquefaction	Specific activity increased by 20 times	Error-prone PCR achieved random mutagenesis Directed evolution	Wong et al. (2004)
		Specific activity and pH-activity profile	Random mutagenesis	Bessler et al. (2003)
		Increased thermostability	Directed evolution	Wang et al. (2006)
	Baking	pH-activity profile	Protein engineering through site-directed mutagenesis	Danielsen and Lundqvist (2008)
α-Glucosidase	Hydrolysis of glycosidic linkages	Thermostability	Random mutagenesis using the standard error-prone PCR	Zhou et al. (2015)
β-Galactosidase	Lactose hydrolysis	Increase in the yields of Galacto-oligosaccharides (GOS)	Site-directed mutagenesis	Wu et al. (2013)
L-arabinose isomerase	Tagatose production	pH-activity profile	Protein engineering through directed evolution	Oh et al. (2006)
Glucoamylase	Starch saccharification	Substrate specificity, thermostability and pH optimum	Protein engineering through site-directed mutagenesis	Allen et al. (2003)
	Starch liquefaction	Thermostable	Directed evolution and site-directed mutagenesis	McDaniel et al. (2008)
Lactase	Lactose hydrolysis	Thermostability	Immobilization	Dwevedi and Kayastha (2009)
Pullulanase	Starch debranching	Activity	Protein engineering through directed evolution	England et al. (2013)
		Thermostability and Catalytic Efficiency	Site-directed mutagenesis based on a structure-guided consensus approach	Duan et al. (2013)
Phytase	Animal feed	pH-activity profile	Protein engineering through site-directed mutagenesis	Tomschy et al. (2002)
		Thermostability	Directed evolution was applied to create thermostable phytases	Kim and Lei (2008)

(continued)

Table 2 (continued)

Enzyme	Role	Targeted improvement	Strategies applied	Reference
Xylose isomerase	Isomerization/ epimerization of tetroses, pentoses and hexoses	pH-activity profile	Protein engineering through directed evolution.	Cha and Batt (1998)
		Substrate specificity; three-fold increase in catalytic efficiency	Site-directed mutagenesis	Karimäki et al. (2004)
	Glucose isomerisation	Thermostability; five-fold higher activity at 60 °C	Protein engineering using directed evolution.	Rubin-Pitel and Zhao (2006), Sriprapundh et al. (2003)

Source: Adapted from (Fernandes 2010)

the representative examples of how genetically modified enzyme could be used in food processing.

These classic examples demonstrate how advances in biotechnology, enzyme engineering, and biocatalyst design have emerged as powerful tools for process improvement and product recovery in the food and beverage industries, apart from their other industrial applications.

6 Sources of Microbial Enzymes

Many enzymes from microbial sources such as bacteria, yeasts, and fungi are globally studied and currently being used in various commercial food processing industries. About 80% of all commercial enzymes are derived from fungi and bacteria, and these enzymes are used in food and pharmaceutical applications (Table 3). Furthermore, a limited number of genera *i.e.*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium*, *Saccharomyces*, *Lactobacilli* and *Bacillus subtilis* are well established, safe, beneficial and best-known microbial sources for enzymes.

7 Global Demand for Microbial Enzymes

Demand for new enzymes is ever increasing owing to the rising awareness of health and nutritional benefits of food enzymes among the consumers all around the world. This has led to the rising acceptance of food enzymes in various food processing

Table 3 Microbial enzyme sources used in food processing industries

S. No	Microbial source	Enzymes
Bacterial enzymes		
	Bacillus amyloliquefaciens	α-Amylase
	Bacillus licheniformis	α-Amylase, pullulanase
	Bacillus subtilis	α-Acetolactate, decarboxylase, α amylase maltogenic amylase, pullulanase, glucanase, maltase
	Bacillus acidopullulyticus	α-Amylase
	Bacillus lentus	Mannanase
	Escherichia coli	Chymosin
Fungal enzymes		
	Aspergillus niger	Phytase, chymosin, lipase, protease, catalase
	Aspergillus oryzae	Esterase, lipase, aspartic proteinase, glucose oxidase, laccase, lipase
	Fusarium venenatum	Xylanase
	Kluyveromyces marxianus var. lactis	Chymosin
	Pseudomonas fluorescens	α-Amylase
	Penicillium notatum	Glucose oxidase
	Trichoderma reesei	Pectin lyase
	Trichoderma longibrachiatum	Protease, xylanase
Yeast		
	Saccharomyces cerevisiae	Invertase
	Saccharomyces fragilis	Lactase
	candida	Lipase

industries. Food enzymes are essential food additives which transform complex molecules into simpler ones in our diet.

The global food and beverage enzymes market is categorized into carbohydrase, lipase, protease, and others. Carbohydrases, which acquire a major share in industries is further categorized into cellulase, amylase, lactase, invertases, inulinases, galactosidases, glucosidases, fructosyltransferases, pectinases, and glucosyltransferases (Husain 2016). Carbohydrases, which have diverse applications in the food and beverage industry, dominate the world food enzyme market and acquire a major share in industries, accounting for almost 70% of this market followed by proteases and lipases (Husain 2017a, b, c).

Food processing industry is currently the leading consumer of industrial enzymes. Furthermore, microbial enzymes are presently among the well-established products in biotechnology and account for 90% of the worldwide market. According to a recent report, the worldwide food and beverage enzymes market is valued at $1200 million in 2011, $1355.8 million in 2012, and is expected to reach $2306.4 million in

2018 (World Enzymes. Freedonia Group: Cleveland, OH, USA, 2011; Food Enzymes Market: Global Trends and Forecasts to 2018). The demand for food enzymes is expected to reach over $2940 million by 2021, at a CAGR of 7.4% from 2016 to 2021 (https://www.marketsandmarkets.com/Market-Reports/food-enzymes-market-800.html). Furthermore, food and beverage applications will remain the largest industrial enzymes market in the near future 2021. There is a significantly large number of companies which are involved in enzyme manufacturing, but major producers are located mainly in North America, Europe, Latin America and the Asia Pacific. Microbial enzymes account for 90% of the global market, and their producers are located mainly in Europe and Asia. In terms of region, North America dominated the global food enzymes market, due to the developed food and beverage industry in the region. It is followed by Europe and Asia-Pacific. The top three dominant global enzyme producers are Novozymes (Denmark), DuPont (USA) and DSM (The Netherlands), which account for more than 70% of the global enzymes business.

Novozymes is the sole company that has focused much on microbes and enzymes for food processing industries. Today, it holds a dominant share of 48% of the enzyme market and turned out to be the world's largest enzyme company (https://report2016.novozymes.com/our-business/trends).

The other leading companies in food processing industries are Associated British Foods plc (U.K.), Chr. Hansen (Denmark), Connell Bros. (U.S.), Dyadic International, Inc. (U.S.), Enmex (India), Advanced Enzymes (India), Puratos Group (Belgium), Kerry Group (Ireland), Amano Enzyme Inc. (Japan), Advanced Enzymes Technologies (India), Amano Enzyme (Japan), Biocatalysts (U.K.), Biocon (India), Fonterra Co-Op Group Ltd. (U.S.), Fytozimus Biotech (India), Genencor International Inc. (India) and Sternenzym (China).

8 Microbial Enzymes in Food Processing

Food processing industry is one of the leading and most important sectors in the world in terms of production, growth, consumption, and trade. Microbial enzymes are used widely in the food processing industry to improve nutritive value, antioxidant capacity, ripening fruit, developing flavour, appearance, texture strengthening, tenderization and flavor development and nutritive value of processed foods. Every year nearly 30–40% of the food (including fruits and vegetables) is wasted due to various reason in countries like USA, South Africa and India (Sridevi and Ramanujam 2012; Oelofse and Nahman 2013; Gunders 2012). Processing of food using microbial enzyme can assure that our food is safe, prevent spoilage and waste and increase its nutritional value. Consequently, microbial enzymes have found extensive applications in the production and processing of various types of food products. Almost 75% of all industrial enzymes that are widely used in food processing industries are hydrolytic enzymes (e.g., proteases, carbohydrases, lipases, nucleases). Carbohydrases, proteases and lipases dominate the enzyme market, accounting for more than

70% of all enzyme sales. An overview of applications of microbial enzymes in food processing industry are summarised in Table 4.

Enzyme action produces several effects in foods such as clarification, coagulation, color and flavor generation, decolorisation, improved yields, liquefaction, saccharification, tenderization, texture strengthening, maceration and viscosity reduction.

8.1 Microbial Enzymes in Starch Processing

Starch is the most common and main polysaccharide reserve produced by all green plants. It is most abundant biomolecule on earth after cellulose. Also, it is the main component of many agricultural products and forms more than 65% of the dry mass in corn (maize), cereal (wheat, rice) and potato. Starch is primarily composed of two major components *i.e.*, amylose (typically 20–30%) and amylopectin (70–80%) both of which contain α-D-glucopyranosyl units. In amylose 300–3000 glucose units are linked by β-1,4-glycosidic bonds, whereas in amylopectin about one residue in every twenty carbohydrate units are joined by β-1,6-linkages forming branch points (Qureshi and Blaschek 2005; Husain 2017a). It is usually formed by 2000–200,000 glucose units.

Starch is a highly functional natural polymer which possesses many unique properties and is used chemically or enzymatically to process a wide range of derivatives such as starch hydrolysates, glucose syrups, maltodextrin, cyclodextrins, fructose, glucose, and isoglucose.

The enzymatic conversion of the starch comprises of three necessary steps: liquefaction, saccharification, and isomerization. In starch processing industry α-amylase (1,4-α-D-glucan-glucanohydrolase, EC. 3.2.1.1) is one of the main enzymes used in starch liquefaction process during starch hydrolysis, where starch is converted into fructose and glucose syrups.

The second step in the process issaccharification where glucoamylase (Glucan 1,4-α-glucoamylase, EC. 3.2.1.1) hydrolysis starch entirely to glucose along with, maltose and isomaltose. A pullulanase (pullulan 6-glucanohydrolase, EC. 3.2.1.41) is an important de-branching enzyme, which is widely used to hydrolyze the α-1,6 glucosidic linkages in starch and aid in saccharification (Hii et al. 2012). Isomerization is the last step in the process where glucose isomerase, (D-xylose ketol-isomerase EC. 5.3.1.5) catalyzes the reversible isomerization of D-glucose to D-fructose. Amylolytic enzymes have been widely produced by fermentation using a great variety of microbial strains, mainly from bacteria (*Bacillus licheniformis*, *Bacillus flavothermus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*) and fungi (*Aspergillus niger*, *Aspergillus phoenicis*, *Aspergillus awamori*, *Penicillium chrysogenum*, *Penicillium notatum*) (Prakash and Jaiswal 2010; Singh et al. 2016b; Husain 2016).

Currently, large quantities of microbial amylases from bacterial and fungal sources are commercially available for industrial applications due to their economic feasibility, consistency, rapid growth, less space requirement for production, ease of control of process optimization and modification (Ellaiah et al. 2002).

Table 4 Application of microbial enzymes in different food processing Industry

Industry	Enzyme	Function/benefits	Microorganisms
Dairy	Acid proteinase	Milk coagulation	*Aspergillus* sp., *Rhizopus oryzae*, *Pleoticusmuelleri*, *Penicillium citrinum*
	Aminopeptidase	Faster cheese ripening, debittering	*Lactobacillus* sp. *Pseudozymahubeiensis*, *Bacillus licheniformis*
	Catalase	Cheese processing	*Aspergillus niger*, *Lactococcus species*
	Lactase (β-galactosidase)	Lactose-reduced milk and whey products	*Escherichia coli*, *Kluveromyces*sp.
	Lipase	Faster cheese ripening, flavorcustomized cheese	*Aspergillus niger*, *A. oryzae*, *Propionibacterium freudenreichii*
	Neutral proteinase	Faster cheese ripening, de-bittering	*Bacillus subtilis*, *A. oryzae*
	Protease	degrade protein and enables aging of cheese	*Aspergillus niger*
	Transglutaminase	Protein cross-linking	*Streptomyces* sp.
Baking	Amylase	Flour adjustment, bread softness	*Aspergillus* sp., *Bacillus* sp.
	Glucose oxidase	Dough strengthening	*Aspergillus niger*, *Penicillium chrysogenum*
	Lipase	Dough stability and conditioning	*Aspergillus niger*
	Maltogenic α-amylase	Enhance the shelf life of bread	*Bacillus stearothermophilus*
	Oxidoreductases	Giving increased gluten strength	*Bacillus subtilis*, *Zymomonasmobilis*
	Pentosanase	Loaf volume increment of bread	*Tricodermareesei Humicolainsolens*
	Proteases	Dough conditioner, enhance dough extensibility	*Aspergillus niger*
	Transglutaminase	Laminated dough strength	*Streptoverticillium* sp., *streptomyces* sp.
	Xylanase	Dough conditioning	*Aspergillus niger*
Beverages	Aminopeptidases	Protein breakdown during mashing	*Lactobacillus brevis*, *L. plantarum*
	Amyloglucosidases	Increasing glucose content	*Aspergillus niger*
	Cellulase	Fruit liquefaction	*Aspergillus niger*, *Trichoderma atroviride*
	Glucose oxidase	Oxygen removal from beer	*Aspergillus niger*
	Limoninase	Debittering	*Aspergillus niger*, *A. oryzae*
	Naringinase	Debittering	*Aspergillus niger*
	Pectinase	Depectinisation	*Aspergillus oryzae*, *Penicillumfuniculosum*

(continued)

Table 4 (continued)

Industry	Enzyme	Function/benefits	Microorganisms
	Protease	Restrict haze formation	*Aspergillus niger*
	Pullulanase	Starch saccharification	*Bacillus* sp., *Klebsiella* sp.
	Tannase	Tea processing	*Aspergillus niger*
	α-Amylase	Starch hydrolysis	*Bacillus*, *Aspergillus*
	β-Amylase	Starch hydrolysis	*Bacillus*, *Streptomyces*, *Rhizopus*
	β-Glucanase	Restrict haze formation, improving wort separation	*Bacillus subtilis*, *Aspergillus* spp.
Juice industry	Amylases	Breaking down starch into glucose	*Aspergillus* spp. *Bacillus* spp. *Microbacterium*
	Cellulases	Pectin hydrolysis	*Aspergillus niger Trichoderma* spp.
	Glucoamylases	Clarifying cloudy juice	*Imperiale*
	Hemicellulose	Lowering viscosity and maintenance of texture	*Aspergillus* spp. *Bacillus subtilis*
	Laccase	Increasing the susceptibility of browning during storage	*Pseudomonas putida*, *Bacillus* sp., *Auerobasidium pullulans*
	Pectinases	Degrading pectins and increasing overall juice production	*Aspergillus* spp., *Penicillium funiculosum*
Starch processing	α-Amylases	Starch hydrolysis	*Aspergillus* spp. *Bacillus* spp. *Microbacterium*
	Amylopullulanases	Starch hydrolysis	*Bacillus* sp., *Staphylothermusmarinus*
	Glucoamylases	Starch hydrolysis	*Aspergillus niger*, *Rhizopus species*
	Glucose isomerases	Catalysing isomerisation of glucose to fructose	*Streptomyces flavogriseus*
	Glycosyltransferases	Increasing the number of branched points to obtain modified starch with improved functional properties such as higher solubility, lower viscosity, and reduced retrogradation	*Streptococcus thermophilus*
	Isoamylases	Starch hydrolysis	*Pseudomonas amyloderamosa*, *Lipmyceskononenkoae*
	Neopullulanases	Starch hydrolysis	*Aspergillus* spp., *Bacillus stearothermophilus*
	Pullulanases	Improvement of saccharification of starch	*Bacillus* spp. *Klebsiella* spp
	β-amylases	Starch hydrolysis	*Bacillus*, *Streptomyces*, *Rhizopus*

(continued)

Table 4 (continued)

Industry	Enzyme	Function/benefits	Microorganisms
Meat and fish processing	Acid proteases	Improve the flavouring, nutritional and functional properties of proteins	*Aspergillus* sp.
	Elastase	Tenderize meat	*Aspergillus fumigatus*, *Pseudomonas aeruginosa*
	Glucose oxidase	Preservation of seafood	*Aspergillus niger*, *Penicillium amagasakiense*
	Glutaminase	Enhance flavor of the meat	*Streptomyces* sp., *Pseudomonas fluorescens*
	Lipase	Hydrolyze triglycerides; Improves flavor in sausages.	*Aspergillus niger*
	Proteases	Removal of scales and skin from fish, production of fish sauce	*Aspergillus niger*, *Bacillus licheniformis*
	Transglutaminase	Improves the structural properties of the processed or cooked meat, meat mince formation	*Streptoverticillium* sp., *streptomyces* sp.
	Tyrosinase	Cross-link meat protein enhances the functional properties of enzymes	*Bacillus megaterium*, *Rhizopus oryzae*, *Trichoderma reesei*
	Urease	Removal of off-odor and fishy taste	*Streptococcus thermophilus*, *Aspergillus niger*

Source: Adapted from (Singh et al. 2016a)

After completion of the process in the starch-processing industries, the syrups and starch derivatives, which comprise of diverse compositions and physical properties, can be used in a wide variety of food and beverage industries. On the basis of type, the market is sectioned into glucose syrup, maltodextrin, cyclodextrin, hydrolysates, and spray-dried starch. Among the types of starch derivatives, glucose syrup acquires the largest market. However, the maltrodextrin sector is growing at the highest compound annual growth rate CAGR of 7.0%.

The global starch derivatives market is one of the burgeoning industries and is projected to grow at a rate of 6.2% from 2014 to 2019 to reach $58.2 billion by 2019. (http://www.marketsandmarkets.com/Market-Reports/starch-derivatives-market-116279237.html).

Globally, the United States dominates the starch industry with 51% of world production. Starch market at industrial level is expected to reach USD 106.73 Billion by 2023, witnessing growth at a CAGR of over 6%, in value terms owing to the rising popularity of ready-to-use convenience foods among the working population who do not have time to cook their meals.

8.2 Enzymes in the Bakery Industry

Microbial enzymes can effectively amend gluten network and are hence useful for modulating flour and dough rheology in addition to flavor enhancement of the baked products. Enzymes lipoxygenases carry out the oxidation of fatty acids and are utilized for bleaching of fat-soluble flour pigments and provide elasticity in the dough (Miguel et al. 2013). They have been obtained from fungus *Ascomycota incertaesedis* such as *Gaeumannomyces*, *Pyricularia*, *Geotrichumcandidum*. The fungal lipoxygenase may be from *Gaeummanomyces graminis*, *Fusarium oxysporum* or *F. proliferatum*, or *Penicillium* sp. (Borch et al. 2006). Microbial proteases (*Bacillus* sp.), α-amylases (*Aspergillus* sp.), xylanases (*Bacillus*, *Aeromonas*, *Cephalosporoim*, *Aspergillus niger*) and lipases (*Acinetobacter*, *Aermonas*, *Bacillus*, *Candida*, *Penicillium*, *Pseudomonas* sp.) are widely used to enhance the homogeneity, softness, colour, longer freshness and crunch to the bakery products (Singh et al. 2016c; Ahmed et al. 2014; Goswami and Rawat 2015; Andualema and Gessesse 2012). The enzymes glucose oxidase and asparaginase often inhibit off flavors in end products due to chemical reactions (e.g. Maillard reaction). Microbial phytases (*Thermomyces lanuginosus*, *Talaromyces thermophilus* and *Sporotrichum thermophile*) cause oxidation of phenol groups and reduce the phytate content and stickiness, thereby increasing dough strength, stability and volume of bread (Dahiya 2016). Transglutaminases (*Streptoverticillium* sp. and *Physarumpolycephalum*) along with laccases (*S. lavendulae*, *S. cyaneus*, *Marinomonas mediterranea*, *Phanerochaete chrysosporium*, *Theiophora terrestris*, *Lenzites, betulina*) are used to obtain gluten-free bread and increase the shelf life of the products (Kieliszek and Misiewicz 2014).

8.3 Enzymes in Fish and Sea Food Processing

Both endogenous and added enzymes play a fundamental role in the development of products and processes encompassing seafood and meat processing industries. The paradigm of research has progressed from traditional screening methods to innovative/improved molecular and metagenomics approaches in conjunction with regulatory formulations. Though plenty of plants and mammalian sources of enzymes exist, still microbes remain the preferred source due to ease of cultivation and manipulation (Anbu et al. 2015). Proteases like papain, pepsin, trypsin, subtilisin, bacillolysin, ficin have been widely used for descaling/deskinning, peeling, tenderization, ripening etc. to minimize mechanical damage and to increase shelf-life and yield of the products (Ghaly et al. 2013; Faisal et al. 2015; Fernandes 2016; Husain 2018). Fungi belonging to genus *Penicillin*, *Mucor*, *Fusurium*, *Thermomyces*, *Aspergillus*, *Rhizopus*, *Humicola*, *Trichoderma* and *Thermoascus* remain a powerful source to deliver proteases (Singh et al. 2016c). On the other hand, meat tenderizing enzymes like subtilisin and neutral proteases are produced by bacteria *Bacillus subtilis*, *B. licheniformis*, *B. alcalophilus*, *B. lentus*, etc. Both crude enzymatic

preparations (*e.g.* pepsin, trypsin, chymotrypsin, thermolysin) from *Bacillus subtilis*, *Geobacillus stereothermophilus*, *B. thermoproteolyticus*, *T. thalophilus* and commercial formulations (*e.g.,* Alcalase derived from submerged fermentation of *B. licheniformis*, Flavorzyme—a peptidase preparation from *Aspergillus oryzae*, Nutrase from *Bacillus amyloliquefaciens*, etc.) are used for manufacturing the products involving protein hydrolysis *e.g.* fish protein hydrolysate (FPH) and fish sauce. Transglutaminases are another important group of enzymes isolated from *Streptoverticillium* sp., *Streptomyces* sp. and are used for formulations of fish meat mince, texture modification, the formation of collagen and gelatin bonds and minimization of drip after thawing (Zheng et al. 2001; Suresh et al. 2015). Lipases find a great application in this industry for the preparation of ω-3-poly-unsaturated fatty acids (ω-PUFAs) and for isolation of fats and oils from seafood by-products (Chaurasia et al. 2016; Guerrand 2018). Most of the industrial microbial lipases are derived from fungi (*Aspergillus*, *Candida*, *Geotrichum*) and bacteria (*Bacillus*, *Pseudomonas*, *Chromobacterium*, etc.) (Aravindan et al. 2007).

8.4 Enzymes in Meat Industry

Microbial enzymes are routinely used in meat industries in numerous production processes. Faced with the new market trends, production of nutritionally enhanced meat foods is being focused upon by reformulation of raw materials and products and advanced technological processes. For the breakdown of proteins in muscle and hydrolyze myofibrillar and connective tissue proteins of meat and meat products several proteolytic enzymes are utilized. Proteases from both fungal (e.g., *Aspergillus oryzae*) and bacterial (e.g., *Bacillus subtilis*) sources are used for meat tenderization (Arihara 2006). Bacterial proteases like subtilisin and neutral proteases find major applications in the meat industry because of their relative specific activity and low inactivation temperature. Enzymes like alkaline elastase are obtained from the alkalophilic *Bacillus* sp., are routinely used for collagen, elastin, and myofibrillar hydrolysis (Avendano et al. 2016). Transglutaminase is another enzyme of microbial origin produced by *Streptoverticilliummobarence* which in addition to processing also enhances the nutritional value by adding essential amino acids (Maróstica and Pastore 2010). It also aids in binding, texture maintenance, and improvement, emulsification, homogenization of meat and meat products. Together proteases and thermolysin (EC 3.4.24.27) are used to for the ripening of dry sausages (Avendano et al. 2016).

8.5 Enzymes in the Dairy Industry

Microbial enzymes are extensively used for three major applications in dairy industries like cheese production, product ripening, shelf-life extension and function

alteration. Flavor development and ripening in cheese are met by controlled hydrolysis of triglycerides. Microbial community structure and hence enzyme secreted by them play an important role for developing different cheese types. *Rhizomucorpusilus*, *R. meihi*, *Endothiaparasitica*, *Aspergillus oryzae*, *Irpexlactis* are the sources of rennet-like proteinases used for cheese manufacture. Milk clotting protease has been isolated from *R. pusilus* (Neelakantan et al. 1999). *Mucor miehei* rennet, and *Cryphonectriaparasitica* rennet is also being widely used in dairy industries. For flavor development in dairy products, lipolysis (enzymatic hydrolysis of triglycerides to fatty acids) of the milk fat is essential. Lipases isolated from *Propionibacterium* sp. contribute to the development of Swiss-type cheese. Esterases produced by *L. helveticus*, *L. delbrueckiisubsp. Bulgaricus*, *L. delbrueckiisubsp. Lactis*, *L. casei*, *L. paracasei*, and *L. plantarum* are lipolytic in nature are widely used in dairy industries for enhancing the cheese flavor (Neelakantan et al. 1999; McSweeny and Sousa 2000; Raveendran and Parameswaran 2018). Enzymes isolated from *Micrococcus* and *Pediococcus* also show weak lipolytic activity. Psychrotrophic bacteria (e.g., *Pseudomonas florescens*) also contribute to lipolysis in cheese made from milk. Studies reveal that K- and β-caseins were most susceptible to proteolysis while the α-casein was less affected enzymes secreted by psychrotrophs (Shammet et al. 1992). *E. faecium*, *E. durans* produce biogenic amines like tyramine used for cheddar cheese production and development (Rea et al. 2004). Lipases are also used for the production of different types of cheese like Camembert cheese using lipase from *Penicillium camemberti* and cheddar cheese using *Aspergillus niger* or *A. oryzae* (Aravindan et al. 2007). Lipase catalysis also prolongs the shelf life of dairy products. Antimicrobial enzymes like bacteriolysins are used to control microbial contamination, improving safety and shelf life of dairy products. Another class of enzymes, *i.e.,* catalases (apparently from the genus *Bacillus*) are also used for the treatment of milk to remove H_2O_2 before cheese production (Tarhan 1995). Galactosidase produced from yeast *Kluyveromyces lactis*, *K. fragilis* is generally used for the hydrolysis of lactose in the milk of whey (Hussein et al. 1989).

8.6 Enzymes in the Vegetable and Fruit Juice Industry

Fruits and vegetables contain approximately 50–70% water creating a turgor pressure and juices are prepared from them by mechanically squeezing or mashing them with little or no application of heat or solvents. Enzymes are used in these industries for (1) maceration (2) treatment of extracted fruit/vegetable juice (3) obtaining better clarification by reducing viscosity (4) increasing product stability (5) colour and flavor enhancement. The cell walls of plant tissues comprise of a methoxylated galacturonic acid polymer called pectin which is present as a complex macromolecular structure and provides integrity and rigidity. Pectinolytic enzymes like protopectinases, lyases and pectin esterases are used for hydrolysis of pectin or dissolution of protopectin (Kumar and Suneetha 2016). Most of these enzymes have been isolated from fungi like *Aspergillus niger*, *Penicillium oxalium*, etc. For

clarification of orange, papaya, pear, peach, plum, apricot, apple and blueberry juices pectinase enzyme from *Rhizopus oryzae*, pectin methylesterase from *Aspergillus tubigensis*, pectinase from *Aspergillus niger*, purified polygalacturonase from *Aspergillus awamori* and some strains of *Bacillus* Ar1.2, Ega16, Ega22 and VIT sun-2 are utilized for reducing viscosity and increasing stabilization (Kumar 2015; Garg et al. 2016; Kumar and Suneetha 2016).

9 Conclusion

Role of enzymes is indispensable in our daily lives being the most proficient catalysts with high specificity and stability. Globally these micro-machines, primarily made up of proteins; have been the major research focus and increasing efforts are made for their discovery, engineering and production for industrial applications. Their role is tremendous in competitive and cost-effective processes in food and beverage industries. They have been greatly exploited in the development of innovative bioprocesses and for speeding-up production processes. A vast diversity in the types and actions of enzymes enable them to serve as efficient biological catalysts for innumerable chemical reactions ensuring highest market profitability, yet least logistic load on user and environment.

References

Ahmad Z, Butt MS, Ahmed A, Riaz M, Sabir SM, Farooq U, Rehman FU (2014) Effect of *Aspergillus niger* xylanase on dough characteristics and bread quality attributes. J Food Sci Technol 51(10):2445–2453

Allen MJ, Fang TY, Li Y, Liu HL, Chen HM, Coutinho P, Honzatko R, Ford C (2003) Protein engineering of glucoamylase to increase pH optimum, substrate specificity and thermostability. U.S. Patent No. 6,537,792. Washington, DC: U.S. Patent and Trademark Office

Anbu P, Gopinath SC, Chaulagain BP, Tang TH, Citartan M (2015) Microbial enzymes and their applications in industries and medicine 2014. Biomed Res Int 2015:816419

Andualema B, Gessesse A (2012) Microbial lipases and their industrial applications. Biotechnology 11(3):100–118

Aravindan R, Anbumathi P, Viruthagiri T (2007) Lipase applications in food industry. Indian J Biotechnol 6:141–158

Arihara K (2006) Strategies for designing novel functional meat products. Meat Sci 74(1):219–229

Avendano KA, Anguiano M, Lopez CE, Montanez LE, Sifuentes L, Balagurusamy N (2016) Microbial enzymes applications in food processing. Agro Food Ind Hi Tech 27:63–67

Bessler C, Schmitt J, Maurer KH, Schmid RD (2003) Directed evolution of a bacterial α-amylase: toward enhanced pH-performance and higher specific activity. Protein Sci 12(10):2141–2149

Borch K, Erlandsen L, Jensen M (2006) Treatment of dough with a lipoxygenase and a lipolytic enzyme. U.S. Patent Application No. 10/528,330

Cha JAEHO, Batt CA (1998) Lowering the pH optimum of D-xylose isomerase: the effect of mutations of the negatively charged residues. Mol Cells 8(4):374–382

Chapman-Smith A, Cronan JE Jr (1999) The enzymatic biotinylation of proteins: a post-translational modification of exceptional specificity. Trends Biochem Sci 24(9):359–363
Chaurasia SP, Bhandaria K, Sharmaa A, Dalai AK (2016) A review on lipase catalysed synthesis of DHA rich glyceride from fish oils. Int J Res Sci Innov 3:9–19
Dahiya S (2016) Industrial application of phytases. Int J Appl Res 2:95–98
Danielsen S, Lundqvist H (2008) Bacterial alpha-amylase variants. WO Patent 825, 2008
Declerck N, Machius M, Wiegand G, Huber R, Gaillardin C (2000) Probing structural determinants specifying high thermostability in *Bacillus licheniformis* α-amylase1. J Mol Biol 301(4):1041–1057
Demain AL, Adrio JL (2008) Contributions of microorganisms to industrial biology. Mol Biotechnol 38(1):41
Duan X, Chen J, Wu J (2013) Improving the thermostability and catalytic efficiency of *Bacillus deramificans* pullulanase by site-directed mutagenesis. Appl Environ Microbiol 79(13):4072–4077
Dwevedi A, Kayastha AM (2009) Stabilization of β-galactosidase (from peas) by immobilization onto Amberlite MB-150 beads and its application in lactose hydrolysis. J Agric Food Chem 57 (2):682–688
Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P, Srinivasulu B (2002) Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated Aspergillus species. Process Biochem 38(4):615–620
England G, Kolkman M, Miller B, Vroemen C (2013) Pullulanase variants with increased productivity. U.S. Patent No. 8,354,101. Washington, DC: U.S. Patent and Trademark Office
Faisal M, Islami SNE, Islam MN, Kamal M, Khan MNA (2015) Study on microbial and physical changes in fish sauce during fermentation. Res Agric Livestock Fish 2(2):375–383
Fernandes P (2010) Enzymes in food processing: a condensed overview on strategies for better biocatalysts. Enzyme Res 2010:1–19. https://doi.org/10.4061/2010/862537
Fernandes P (2016) Enzymes in fish and seafood processing. Front Bioeng Biotechnol 4:59
Flamm EL (1991) How FDA approved chymosin: a case history. Nat Biotechnol 9(4):349
Food Enzymes Market by Type (Carbohydrase, Protease, Lipase), Application (Beverages, Bakery Products, Dairy Products, Confectionery Products, Processed Foods), Formulation (Lyophilized Powder and Liquid), Source, and Region – Global Forecast to 2023. https://www.marketsandmarkets.com/Market-Reports/food-enzymes-market-800.html
Food Enzymes Market: Global Trends and Forecasts to 2018. http://www.researchandmarkets.com/research/3xgvqh/food_enzymes
Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R (2016) Microbial pectinases: an ecofriendly tool of nature for industries. 3 Biotech 6(1):47
Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D (2013) Fish processing wastes as a potential source of proteins. Amino acids and oils: a critical review. J Microb Biochem Technol 5(4):107–129
Ghollasi M, Ghanbari-Safari M, Khajeh K (2013) Improvement of thermal stability of a mutagenised α-amylase by manipulation of the calcium-binding site. Enzym Microb Technol 53(6–7):406–413
Goswami GK, Rawat S (2015) Microbial Xylanase and their applications – a review. Int J Curr Res Acad Rev 3(6):436–450
Guerrand D (2018) Economics of food and feed enzymes: status and prospectives. In: Enzymes in human and animal nutrition, pp 487–514
Gunders D (2012) Wasted: how America is losing up to 40 percent of its food from farm to fork to landfill. Natural Resources Defense Council, 26
Hii SL, Tan JS, Ling TC, Ariff AB (2012) Pullulanase: role in starch hydrolysis and potential industrial applications. Enzyme Res 2012:921362
Husain Q (2016) Magnetic nanoparticles as a tool for the immobilization/stabilization of hydrolases and their applications: an overview. Biointerfaces Res Appl Chem 6(6):1585–1606
Husain Q (2017a) Nanomaterials as novel support for the immobilization of amylolytic enzymes and their applications: a review. DeGruyter Biocatal 3(1):37–53

Husain Q (2017b) Nanomaterials immobilized cellulolytic enzymes and their industrial applications: a literature review. JSM Biochem Mol Biol 4(3):1029
Husain Q (2017c) Nanosupport bound lipases their stability and applications. Biointerfaces Res Appl Chem 7(6):2194–2216
Husain Q (2018) Nanocarriers immobilized proteases and their industrial applications: an overview. J Nanosci Nanotechnol 18(1):486–499
Hussein L, Elsayed S, Foda S (1989) Reduction of lactose in milk by purified lactase produced by *Kluyveromyces lactis*. J Food Prot 52(1):30–34
Igarashi K, Ozawa T, Ikawa-Kitayama K, Hayashi Y, Araki H, Endo K, Hagihara H, Ozaki K, Kawai S, Ito S (1999) Thermostabilization by proline substitution in an alkaline, liquefying α-amylase from *Bacillus* sp. strain KSM-1378. Biosci Biotechnol Biochem 63(9):1535–1540
Karimäki J, Parkkinen T, Santa H, Pastinen O, Leisola M, Rouvinen J, Turunen O (2004) Engineering the substrate specificity of xylose isomerase. Protein Eng Des Sel 17(12):861–869
Kieliszek M, Misiewicz A (2014) Microbial transglutaminase and its application in the food industry. A review. Folia Microbiol 59(3):241–250
Kim MS, Lei XG (2008) Enhancing thermostability of *Escherichia coli* phytase AppA2 by error-prone PCR. Appl Microbiol Biotechnol 79(1):69–75
Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13 (4):345–351
Kumar S (2015) Role of enzymes in fruit juice processing and its quality enhancement. Health 6 (6):114–124
Kumar P, Suneetha V (2016) Microbial pectinases: wonderful enzymes in fruit juice clarification. Int J MediPharm Res 2:119–127
Maróstica MR, Pastore GM (2010) Some nutritional, technological and environmental advances in the use of enzymes in meat products. Enzyme Res 2010:480923
McDaniel A, Fuchs E, Liu Y, Ford C (2008) Directed evolution of *Aspergillus niger* glucoamylase to increase thermostability. Microb Biotechnol 1(6):523–531
McSweeney PL, Sousa MJ (2000) Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. Lait 80(3):293–324
Miguel ÂSM, Martins-Meyer TS, da Costa Figueiredo ÉV, Lobo BWP, Dellamora-Ortiz GM (2013) Enzymes in bakery: current and future trends. In: Muzzalupo I (ed) Food industry. InTech, UK
Neelakantan S, Mohanty AK, Kaushik JK (1999) Production and use of microbial enzymes for dairy processing. Curr Sci 77(1):143–148
Nelson DL, Cox MM (2004) Lehninger principles of biochemistry, 4th edn. WH Freeman, New York
Nielsen PH, Malmos H, Damhus T, Diderichsen B, Nielsen HK, Simonsen M, SchiV HE, Oestergaard A, Olsen HS, Eigtved P, Nielsen TK (1994) Enzyme applications, industrial. In: Kirk-Othmere Encyclopedia of chemical technology. Wiley, New York
Novozymes (https://report2016.novozymes.com/our-business/trends)
Oelofse SH, Nahman A (2013) Estimating the magnitude of food waste generated in South Africa. Waste Manag Res 31(1):80–86
Oh DK, Oh HJ, Kim HJ, Cheon J, Kim P (2006) Modification of optimal pH in L-arabinose isomerase from *Geobacillus stearothermophilus* for D-galactose isomerization. J Mol Catal B Enzym 43(1–4):108–112
Prakash O, Jaiswal N (2010) Alpha-Amylase: an ideal representative of thermostable enzymes. Appl Biochem Biotechnol 160(8):2401–2414
Qureshi N, Blaschek HP (2005) Butanol production from agricultural biomass. In: Food biotechnology. CRC, Boca Raton, FL, pp 541–565
Raveendran S, Parameswaran B (2018) Applications of microbial enzymes in food industry. Food Technol Biotechnol 56(1):16–30

Rea MC, Franz CMAP, Holzapfel WH, Cogan TM (2004) Development of enterococci and production of tyramine during the manufacture and ripening of Cheddar cheese. Ir J Agric Food Res 43(2):247–258

Rubin-Pitel SB, Zhao H (2006) Recent advances in biocatalysis by directed enzyme evolution. Comb Chem High Throughput Screen 9(4):247–257

Shammet KM, Brown RJ, McMahon DJ (1992) Proteolytic activity of some milk-clotting enzymes on κ-Casein1. J Dairy Sci 75(6):1373–1379

Singh R, Kumar M, Mittal A, Mehta PK (2016a) Microbial enzymes: industrial progress in 21st century. 3 Biotech 6:174

Singh R, Mittal A, Kumar M, Mehta P (2016b) Amylases: a note on current applications. Int Res J Biol Sci 5(11):27–32

Singh R, Mittal A, Kumar M, Mehta PK (2016c) Microbial proteases in commercial applications. J Pharm Chem Biol Sci 4:365–374

Sridevi DV, Ramanujam AR (2012) Performance of mixture of vegetable wastes with high cabohydrate content in anaerobic digestion process. Int J Environ Sci 3(1):181

Sriprapundh D, Vieille C, Zeikus JG (2003) Directed evolution of *Thermotoga neapolitana* xylose isomerase: high activity on glucose at low temperature and low pH. Protein Eng 16(9):683–690

Starch Derivatives Market by Type (Maltodextrin, Cyclodextrin, Glucose Syrup, Spray Dried Starch, Hydrolysates), Application (F & B, Feed, Paper, Cosmetics, Pharmaceutical), Function (Emulsifying, Binding, Stabilizing, Thickening), & by Region – Global Trends & Forecast to 2020. http://www.marketsandmarkets.com/Market-Reports/starch-derivatives-market-116279237.html

Suresh PV, Nidheesh T, Pal GV (2015) Enzymes in seafood processing. In: Chandrasekaran M (ed) Enzymes in food and beverage processing. CRC, Boca Raton, FL, pp 354–377

Tang WL, Zhao H (2009) Industrial biotechnology: tools and applications. Biotechnol J Healthc Nutr Technol 4(12):1725–1739

Tarhan L (1995) Use of immobilised catalase to remove H_2O_2 used in the sterilisation of milk. Process Biochem 30(7):623–628

Tomschy A, Brugger R, Lehmann M, Svendsen A, Vogel K, Kostrewa D, Lassen SF, Burger D, Kronenberger A, van Loon AP, Pasamontes L (2002) Engineering of phytase for improved activity at low pH. Appl Environ Microbiol 68(4):1907–1913

Ueda T, Masumoto K, Ishibashi R, So T, Imoto T (2000) Remarkable thermal stability of doubly intramolecularly cross-linked hen lysozyme. Protein Eng 13(3):193–196

Wang Y, Fuchs E, da Silva R, McDaniel A, Seibel J, Ford C (2006) Improvement of *Aspergillus niger* glucoamylase thermostability by directed evolution. Starch-Stärke 58(10):501–508

Wong DW, Batt SB, Lee CC, Robertson GH (2004) High-activity barley α-amylase by directed evolution. Protein J 23(7):453–460

World Enzymes (2011) World food & beverage enzyme demand by market (million dollars). Freedonia Group, Cleveland, OH. https://www.freedoniagroup.com/industry-study/world-enzymes-2824.htm

Wu Y, Yuan S, Chen S, Wu D, Chen J, Wu J (2013) Enhancing the production of galacto-oligosaccharides by mutagenesis of *Sulfolobus solfataricus* β-galactosidase. Food Chem 138 (2–3):1588–1595

Zheng M, Du G, Guo W, Chen J (2001) A temperature-shift strategy in batch microbial transglutaminase fermentation. Process Biochem 36(6):525–530

Zhou C, Xue Y, Ma Y (2015) Evaluation and directed evolution for thermostability improvement of a GH 13 thermostable α-glucosidase from *Thermus thermophilus* TC11. BMC Biotechnol 15 (1):97