

Prospects and Challenges in Food-Grade Enzymes Industrial Production

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Abstract

Food-grade enzymes are biocatalyst in industrial production of food and they have numerous advantages over conventional chemical processes due to their ability to achieve efficient and sustainable processes and products. It has found extensive application in bakery, beverages, meat, cheese, alcoholic and juice industries. Commercial food-grade enzymes have been preferred to be sourced from microorganisms (bacteria, fungi, yeast, mold) rather than from plants and animals because of their high production yield and controlled conditions. The relevance, applications and challenges of some novel technologies; enzymes screening, enzymes immobilization, solid-state fermentation, recombinant DNA, protein engineering and bioinformatics as techniques for producing food-grade enzymes were discussed. The sources and the industrial applications of some selected food-grade enzymes, such as amylase, proteases, lipase, rennet, cellulase, lactase, invertase, glucose oxidase, glucose isomerase and catalase in the food industry, and food-grade enzymes safety issues, were also discussed.

Keywords

Industrial application · Food-grade enzymes · Food industry · Novel technologies · Safety

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16.1 Introduction

The emergence of the food-grade enzyme for industrial production can be tagged as green revolution approach due to its catalytic biological nature in transforming food into various value-added products in different food industries, such as in beverages and juices, baking, development of functional foods, meat, wine, dairy, fats and oils. The food-grade enzyme has been projected as a potential replacement for chemical additives or harsh physical conditions (temperature, pressure, pH) used in industrial production, therefore guarantees sustainable production for safe and nutritious food supply. Also, with an increase in the awareness on the need to consume a healthier diet which has increased consumers' preference for new and convenient food products manufactured from natural food additives has further increased the use of food-grade enzymes. Presently, food-grade enzymes are being used in the food industry to increase diversity, quality and varieties of food produced. The food-grade enzymes can be generated from the plants, animals and microbial sources. However, out of the entire sources used for enzymes generation, microbial is the most preferred choice in the food industry. Enzymes from microbial sources can be produced in control conditions at a faster rate with minimal harmful by-products and in larger quantities. Large-scale Industrial production has explored the use of *Saccharomyces cerevisiae*, *Aspergillus oryzae*, Fungi *Aspergillus niger* and *Bacillus subtilis* bacteria as sources for microbial enzymes production. Some of the commercially important food-grade enzymes produced from this sources are proteases, rennet, pectinases, invertases, cellulases, lipases, amylases, catalase, lactase, raffinase, pullulanase, glucose oxidase, etc. Most of naturally occurring enzymes from plants, animals or microbial sources are not often suitable for economical production processes and sustainability. Therefore, there is a need to adopt novel technological approaches that can be used to improve the industrial production of food-grade enzymes. Uzuner and Cekmecelioglu (2019) reported that industrial enzymes market is competitive with minimal profit margin and the enzymes are currently manufactured by few companies in the United States, Denmark and Germany with approximately twenty (20) enzymes being commercially produced at present. Hydrolase represents 75% of food-grade enzymes in the market. Carbohydase (i.e. tannase, phytases, amylases, pectinases, cellulases, β -galactosidases), lipase and proteases are family of hydrolase usually employed as food-grade additives or processing aids in the food industry. This chapter provides an overview of the prospect and challenges of a novel application of techniques for improving the industrial production of food-grade enzymes through screening, enzyme immobilization, solid-state fermentation, recombinant DNA technology, protein engineering, and use of bioinformatics tool in enzyme engineering. The potential application of some selected food-grade enzymes used for industrial production and the safety of food-grade enzyme were discussed.

16.2 Food-Grade Enzymes Screening

Screening technique is used for isolating enzymes by judiciously selecting plant, animal and the species and strains of microorganism or cells. Maximum volumetric productivity is used as an objective function for selecting strains or original cultures with the capacity to produce the preferred enzyme of high productivity rate (Ramos et al., 2011). Also, screening is used in finding alternative strains with improved possibility of cloning proposed enzyme. The process of isolating enzymes from extremophile source using screening techniques is useful in stabilizing, showing high activity and turnover numbers, and specificity of enzymes when operated under severe environmental conditions for industrial application (Synowiecki et al., 2006). For instance, there is a rising interest in the sourcing of enzymes from isolations of bacteria from the marine environment, such as proteases from several genera esterase from *Vibrio fischeri*, inulinase from *Cryptococcus aureus*, amylolytic enzymes, glucosidases and invertase/inulinase from *Thermotoga neapolitana* DSM 4359 (Dipasquale et al., 2009; Rastogi & Bhatia, 2019). In the meat and dairy industry, cryophiles are needed potential microbes for screening. Since the industry operates under room-temperature lactase, protease and pectinase can be obtained for the production of lactose-free milk, milk coagulation and cheese maturing, fruit juice or oligosaccharide production, respectively (Nakagawa et al., 2004, 2006; Wang et al., 2008; Mao et al., 2010). In the screening technique, the major challenge is to cultivate the screened and identified microbes in laboratory conditions most especially the extremophile. However, if the extremophiles are expressed in mesophiles using genetic engineering, they can be easily cultured in the laboratory with easier protein extraction and purification (Rastogi & Bhatia, 2019).

16.3 Food-Grade Enzyme Immobilization

Food-grade enzyme immobilization simply means confining enzymes in a given portion of space with the use of microencapsulation or by the use of solid matrix, such as cross-linking, ion exchange, adsorption and covalent binding, so that the enzyme can be physically separated from the reaction medium. The prospect of the industrial application of food-grade enzyme immobilization is that it permits the reuse of the enzymes and also extends its usefulness to be incorporated in the food products or food processing to improve productivity. Among the food-grade supports used of immobilization are alginate, carrageenan, agar, glass beads, polyurethane foam, polyacrylamide, metal surfaces and vermiculite (Ramos et al., 2011). Ramos et al. (2011) reported that for protease enzymes, vermiculite which is less-expensive support using adsorption had been used for immobilization. It is a must to consider food-grade support for any food-grade enzymes immobilization. According to Sumantha et al. (2006), immobilization of microorganism during the production of the enzyme has been reported to produce higher enzyme yields than non-immobilized cells of bacteria or mycelia. Some of the immobilized enzymes that had been applied in the food industry are immobilized glucose isomerase for

manufacturing high-fructose corn syrup, immobilized aminoacylase for manufacturing amino acids, immobilized lactase for producing tagatose, immobilized invertase for producing inverted sugar, immobilized lipases for producing trans-free oils (cocoa butter equivalents, and for modification of triacylglycerols), immobilized β -fructofuranosidase for producing fructooligosaccharides and immobilized isomaltulose synthase for producing isomaltulose, respectively (Rastogi & Bhatia, 2019). From an economic perspective, it is advisable to immobilize expensive enzymes to have a less-expensive process (Fernandes, 2010). The challenges associated with immobilization are that if the procedure of immobilization is not properly done, it might lead to the loss of enzyme activity which might be as a result of mechanical and chemical stress (Rastogi & Bhatia, 2019).

16.4 Solid-State Fermentation

Fermentation is a technique used for the synthesis of food-grade enzymes. The conventional fermentation method of synthesizing enzymes is through submerged fermentation. The type of substrate used in the submerged fermentation is a determinant in obtaining either high or low enzyme activities. For instance, complex substrate suspended or dissolved in an aqueous medium is expected to give enzymes with high activities as compared with a simple substrate in submerged fermentation. Aside from the type of substrate, the continuous operation has been reported to favour enzyme yield as compared with the batch operation process which is simple to handle and also versatile (Ramos et al., 2011). Optimization of submerged fermentation parameters through the use of advance experimental design, such as response surface methodology, artificial neural network and radial basis function, had been reported as tools that can be used to obtain optimum parameters combination aimed at given maximum volumetric productivity of the enzymes during fermentation (Sumantha et al., 2006). However, solid-state fermentation is gaining greater interest in recent years than the conventional submerged fermentation. The reason is that in solid-state fermentation, cultures are grown on moist solid rather than in aqueous liquid as in submerged fermentation. Also, the culture media are not usually complex; the substrate media for solid-state fermentation can be enriched with nutrient. The enzyme of interest in solid-state fermentation is not diluted, thus paves the way for easy purification after synthesis. There is also low effluent generation, catabolic repression hardly affects the synthesis of enzymes and higher inoculum and relatively low humidity content used in solid-state fermentation reduces extraneous microbial contamination (Ramos et al., 2011). The wide use of agricultural waste as a substrate in solid-state fermentation is another advantage of this technique over submerged fermentation as it aids in reducing the cost associated with enzyme synthesis (Aguilar et al., 2008). Sumantha et al. (2006) reported that the use of wheat bran as a substrate for solid-state fermentation gives a higher yield of proteases than in submerged fermentation using *Aspergillus spp.* and *Mucor pusillus*.

The use of two or more substrates has been reported as a way of increasing the yield of the enzymes in solid-state fermentation (Aguilar et al., 2008).

16.5 Recombinant DNA/Genetic Engineering

According to the Food Drug Administration (FDA) in the USA, *Escherichia coli* K-12 also known as bovine chymosin is the first recombinant enzyme. The recombinant deoxyribonucleic acid (rDNA) techniques or genetic engineering has now been used to produce chymosin by transforming microorganisms with bovine prochymosin gene. At least there are thirty-six (36) enzymes which are genetically modified microorganisms (GMOs) among the one hundred and sixty (160) enzymes produced for use in the food industry based on Association of Manufacturers and Formulators of Enzyme Products (AMFEP) enzymes lists. To achieve the synthesis of efficient enzymes that is free of undesirable enzymes or metabolites of microbes using rDNA, appropriate selection of host microorganisms as recombinant strains with potential DNA sequences that can code for the defined enzyme amino acid sequence is needed. For instance, engineering microbial host strains were applied to some fungi strain to manipulate or eliminate their capacity to release metabolites that are toxic to the formulation of the synthesis enzyme (Olempska-Beer et al., 2006). The application of rDNA has immensely increased the availability of food-grade enzymes with distinct properties to meet the food process and food matrices requirement. Also, it has assisted in synthesizing enzymes that have desirable sensitivity and specificity at a low cost of production.

Rastogi and Bhatia (2019) stated that the application of rDNA can be implemented in batch or continuous operations using cheaper raw material, therefore lessens the criteria for enzyme extraction and purification. The use of food-grade enzymes from genetic engineering has minimized the cost connected with upstream product processing and also increases productivity (Panesar, 2010; Rastogi & Bhatia, 2019). α -amylases, proteases, lipases and pullulanases used in food applications, such as in hydrolysis of starch, are produced by using improved strains (Ramos et al., 2011; Rastogi & Bhatia, 2019). Despite this entire prospect, the main challenge of genetically improved strain is that is not yet certified safe for consumption by the FDA and European Union due to the rise in public consciousness regarding the non-consumption of genetic modified processed foods. Also, the lack of standard in its production and purification procedures has caused a restriction in its application food technology.

16.6 Food-Grade Enzymes by Protein Engineering

The improvement of the food-grade enzyme in terms of stability and tolerance to severe temperature, pH and organic solvents can be achieved through protein engineering where the catalytic rate is improved and amino acids are modified for enhancing substrate specificity. Rational protein design and direct evolution are two

approaches used by protein engineering either exclusively or alongside each other (Jackel et al., 2008; Tiwari et al., 2012; Rastogi & Bhatia, 2019). According to Singhanian et al. (2010), previous knowledge of the function and structure of the protein to introduce the desirable traits using sited mutagenesis is required for rational protein design approach, while for direct evolution, information of the protein is not compulsory and random mutagenesis is performed by error-prone PCR or chemical mediation. In order to forecast the overall structure of enzyme using rational protein design, multiple sequence alignment technique, homology modelling and protein treading methods are used (Gulati & Poluri, 2016). Rational design is easier to improve properties during screening due to an infinite number of protein variants generated (Singhanian et al., 2010). Quax et al. (1991) used rational design to generate mutant glucose isomerase from *Actinoplanes missouriensis* with enzyme activity that is thermal and pH stable as against the parental enzyme. Double mutant isomerase (G138P, G247D) generated using rational design showed 2.5-fold increases in half-life and 45% specificity increment as compared with the wild type (Zhu et al., 1999). A similar approach was also reported by Lin et al. (2008) for generating amylase mutants from the strain of TS-23 *Bacillus sp.* to enhance the thermal stability of the enzyme. However, some of the biocatalysts generated by direct evolution are glucoamylases, cellulases, α -amylases, lipases and proteinases (Singhanian et al., 2010).

16.7 Application of Bioinformatics Technique in Enzyme Engineering

In order to analyse the information in enzymes data within a limited time either online or off the network, the novel application of bioinformatics technique in enzyme engineering is the recent trend useful in performing sophisticated computations analysis (Alderson et al., 2012). The enormous of structural data and sequencing coming out of nuclear magnetic resonance spectroscopy, X-ray crystallography, mass spectrometry and NGS next-generation sequencing (NGS) for enzyme informatics are now better understood by the application of bioinformatics technique (Rastogi & Bhatia, 2019).

16.8 Application of Food-Grade Enzymes for Industrial Production

The use of food-grade enzymes for industrial production has widened their application in the development of different food products. The application of some selected food-grade enzymes for industrial production of food are as follows.

16.8.1 Application of Food-Grade Amylase Enzymes

α -amylase and β -amylase have been used over years for the industrial production of maltose and glucose by applying the enzyme on starch. The application of α -amylase has paved way for the production of glucose syrup and has replaced the chemical hydrolysis of starch in most starch manufacturing companies (Reddy et al., 2003). The sources used for commercial production of α -amylase are some selected strains of bacteria (*Bacillus* spp.: *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *B. licheniformis*; halophilic bacteria: *Chromohalobacter* sp., *Halobacillus* sp., *Haloarcula hispanica*, *Halomonas meridiana*; fungi: *Aspergillus* species and few species of *Penicillium*: *P. brunneum*, *P. fellutanum*) (Konsoula & Liakopoulou-Kyriakides, 2007; Erdal & Taskin, 2010). α -amylase had been used in the baking industry to play an important role in enhancing the quantity, aroma and taste of baked products. Jegannathan and Nielsen (2013) reported that the addition of amylase and lipase enzymes in bread helps in extending its shelf life and reduces crystallization. Antistaling in bread and improved softness can also be achieved by the addition of α -amylase (Gupta et al., 2008). However, more research still needs to be done on the application of α -amylase in the baking industry as a small overdose of α -amylase can result in sticky bread (Singh et al., 2019). For the fermentation of raw starch into ethanol, a combination of α -amylase produced from *Streptococcus bovis* and glucoamylase from *Rhizopus oryzae* has been reported to improve the ethanol production (Singh et al., 2019).

16.8.2 Application of Food-Grade Protease Enzymes

Protease enzymes can be the source from plant, animal, humans and microorganisms (bacteria, fungi and virus) and are known to hydrolyse peptide bonds of proteins. Protease enzymes produced from microorganisms' source are the most preferred for industrial production due to ease of genetic manipulation and extensive diversity, respectively. They are also known to favour low manufacturing cost, extensive chemical and physical characteristics, lack of seasonal variation, large production in industrial fermentators and quick culture development (Singh et al., 2019). Protease enzymes of a wide variety have been exploited for industrial production in food and pharmaceutical industries because of their specificity in action (Kalpana et al., 2008). Almost 60% of the total food-grade enzymes in the market fall under the category of protease enzymes (Mala et al., 1998). Alkaline and neutral proteases produced for commercial purpose are usually generated from *Bacillus* genus. Powdered bacteria proteases derived from *Bacillus* can be stable and remain 95% active for a year. In the food industry, proteases can be applied in cheese production, baked products making, soy hydrolysates production and meat tenderization. Proteases from *A. oryzae* can improve bread colour and texture when mixed with flour dough. More so it also aids in the reduction of mixing time. Heredia-Sandoval et al. (2016) stated that the gluten content of bakery products can be decreased and degraded

using proteases. In the dairy industry during cheese manufacturing, vegetable rennet, animal rennet, genetically engineered chymosin and microbial milk coagulants are milk coagulating enzymes used. Paracasein and micropeptides are generated from the hydrolysis of peptide bone using protease enzymes in cheese making. However, chymosin is good for generating casein. In beer production, application of protease and amylase enzymes have been used as an efficient alternative way of achieving malting process, thus saves energy and agricultural land that would have been used if the conventional malting that involves drying was to be used (Jegannathan & Nielsen, 2013). Microbial proteases have been reported to hydrolyse proteinaceous in beer that causes haze formation, while production of wort can be achieved by the use of *B. subtilis* proteases to solubilize proteins from barley adjuncts (Singh et al., 2019). For commercial purpose, improved tenderness of meat which is the organoleptic quality of meat as desired by the consumers and for easy marketability can be achieved by using thermophile protease derived from *Bacillus* strain, caldolyisin from *Thermus* strain, collagenase from *Clostridium histolyticum*, and aspartic protease from *A. oryzae* (Bekhit et al., 2014). For industrial production of aspartame which is known as nutrasweet (zero-calorie sweetener), enzymatic synthesis of aspartame has been preferred over chemical method due to their ability to maintain stereospecificity. Proteases have been reported to be used to synthesize aspartame by catalysing the reverse reaction in two amino acids, thus maintain stereospecificity and reduce the cost of production (Singh et al., 2019).

16.8.3 Application of Food-Grade Lipase Enzymes

Lipases are important food-grade enzymes due to their biocatalysts activities in breaking down fats and oils, esterification, hydrolysis, interesterification, acidolysis, alcoholysis and aminolysis. Lipases can be sourced from the plant (barley, corn and cotton), animal (adipose tissues, blood, gastric juices, intestinal juices and pancreatic secretion), yeast (*Candida* and *Torulopsis*), filamentous fungi (*Geotrichum*, *Humicola* and *Rhizopus*), bacterial species (*B. subtilis*, *P. fragi*, *B. megaterium*, *S. aureus*, *Burkholderia cepacia*, *P. aeruginosa*, *P. pseudoalcaligenes*) and fungi species (*Helvinal anuginosa*, *Rhizopus delemar*, *Eurotium herbariorum*, *A. niger*, *M. circinelloides* and *Penicillium citrinum*) (Hasan et al., 2006; Sachan & Singh, 2015; Singh et al., 2019). However, the microbial source is mostly used for commercial production of lipase due to their ability to reduce the cost of production, greater widespread ability in animal and plant lipases, and greater stability (Singh et al., 2019). Hasan et al. (2006) and Treichel et al. (2009) noted that lipase enzymes have found application in food processing, pharmaceuticals, agrochemical, detergents, paper and cosmetic industries. The first commercial lipase from recombinant technique was isolated from *Thermomyces lanuginosus* which is a fungus and since then many other recombinant lipases have been produced for commercial purpose (Singh et al., 2019).

Lipases have been applied extensively in the dairy industries for hydrolysis of milk fat, lipolysis of cream, producing cheese products and in enhancing the flavour

of cheeses in order to accelerate cheese ripening (Aravindan et al., 2007). In the bakery industry, lipolytic enzymes have been used to produce emulsifying liquids in situ by degrading wheat lipids instead of using traditional emulsifiers. Also, the application of lipolytic enzymes helped in the release of short-chain fatty acids through esterification, thereby aids in improving the flavour of baked products flavour. Apple wine has been produced by the Japanese company Tanabe Seiyaku with improved alcoholic content and aroma by isolating lipase enzyme from *R. delemar* or a *Candida* species during fermentation (Singh et al., 2019). Lipases have also been used to improve the quality and texture of mayonnaise, whipping and dressing products. In the meat and fish processing, lipases are used to enhance and remove fat. Ramarethinam et al. (2002) reported that volatile flavours and the aroma of black tea were increased using lipase secreted by *Rhizomucor miehei*. Lastly, in the fat and oil industry, cheap oils have been converted into nutritional-rich oils, oleic acids oils, low calorie triacylglycerols and polyunsaturated fatty acid using microbial lipases (Gupta et al., 2003). Besides commercial production in fats and oils modification, low energy consumption and high level of specificity can be achieved using lipase enzyme modification when compared with chemical modification.

16.8.4 Application of Food-Grade Rennet Enzymes

Rennet is enzyme made up of chymosin, lipase and pepsin and it is usually referred to as complex enzymes. Rennet can be generated from the plant, animal and microbial sources. Rennet has been extensively applied in the commercial production of different types of cheese. However, rennet from the microbial source is mostly used for cheese production worldwide. For instance, *R. miehei*, *Irpex lactis*, *A. oryzae* and *R. pusillus* are the good source of microorganism that can be used to generate rennet for cheese production (Neelakantan et al., 1999; Singh et al., 2019). Also, microbial rennet has served as a replacement for mammalian rennet used in cheese production due to consumers' demand of non-animal derivative. Unlimited availability, low cost of production, bulk production and no risk of disease transmission are some of the advantages of microbial rennet over animal rennet (Singh et al., 2019). The application of rennet enzyme is one of the most important uses of enzymes in the food industry.

16.8.5 Application of Food-Grade Catalase Enzymes

Catalase enzymes also known as hydroperoxidases and is popularly known commercially for converting hydrogen peroxidase to water and oxygen. According to Singh et al. (2019) one molecule of catalase can transform millions of hydrogen peroxide to water and oxygen per second. Catalase can be found in all living organism but mostly source from microorganisms and bovine liver (Chelikani et al., 2004; Tukul & Alptekin, 2004). Plant leaves with high antioxidant properties are *M. sylvestris* L., black gram (*Vigna mungo*) seeds, cotton, sunflower and

pumpkin (Eising et al., 1990; Kandukuri et al., 2012). Catalase found industrial application in the removal of hydrogen peroxide used in sterilizing, oxidizing and bleaching agents (Ertaş et al., 2000). The combination of catalase and glucose oxidase has been used for the preservation of some selected foods. The enzyme can also be used in egg processing but has limited usage in cheese production.

16.8.6 Application of Food-Grade Lactase Enzymes

This enzyme is very important in the dairy industry, most especially in the production of low-lactose or lactose-free food, such as flavoured milk drinks, ice cream, yoghurt and frozen dessert. Lactase can be the source and isolate from animal organs, plants, yeasts, bacteria or molds. For industrial production of lactase, it is usually source from *A. niger*, *A. oryzae* and *Kluyveromyces lactis* due to their safety of use history and the numerous safety tests they undergo before use. The source used for generating the lactase and the method of preparation determines its properties, such as the temperature and pH. For optimal performance of immobilized lactases, the procedure of immobilization and the type of carrier are important. Acidic pH of 2.5 to 4.5 is favourable for fungi lactase production, while neutral region pH is favourable for bacterial and yeast lactase with ranges of 6.5–7.5 and 6–7, respectively. The prospects in the utilization of lactase, hydrolase and β -galactosidase have been extensive research due to the discovery of enzymes immobilization techniques (Mehaia & Cheryan, 1987). The application of lactase in milk has assisted in preventing diarrhea and severe tissue dehydration which affect lactose intolerance people. It has also been reported to increase digestibility, sweetness and scoop and creaminess of ice cream, yoghurt and frozen dessert. Increase in sweetness and sugar addition level reduction has also been ascribed as another advantage of lactase-treated milk in the production of flavoured milk drinks.

16.8.7 Application of Food-Grade Cellulase Enzymes

Cellulases are enzymes used for extracting constituents from soy protein, essential oils, aromatic products, green tea and sweet potato in the food industry. Dillon et al. (2004) described cellulase enzymes as enzymes that break the glucosidic bond of cellulose microfibrils and release oligosaccharides, cellobiose and glucose. Cellulases can be produced from filamentous fungi (*Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium*, *Phoma* and *Trichoderma*), anaerobic bacteria (*Acetovibrio*, *Bacteroides*, *Butyrivibrio*, *Caldocellum*, *Clostridium*, *Eubacterium*, *Erwinia*, *Pseudonocardia*, *Ruminococcus* and *Thermoanaerobacter*) and aerobic bacteria (*Acidothermus*, *Bacillus*, *Cellvibrio*, *Pseudomonas*, *Streptomyces*, *Staphylococcus*, and *Xanthomonas*) (Soares et al., 2012; Singh et al., 2019). Among all the sources of producing cellulolytic enzymes, *Aspergillus* has been reported to be the most outstanding (Singh et al., 2019). Combination of cellulases, hemicellulase and

pectinase is currently being used in the food industry to extract and clarify fruits juice. Cellulase can also be used to degrade solid phase, thus enhances liquefaction.

16.8.8 Application of Food-Grade Invertase Enzymes

This enzyme has a great commercial significance in the production of inverted sugar and is known for its sweetness effect. Invertase can catalyse sucrose to glucose and fructose through hydrolysis. Temperature and pH within the range of 40–60 °C and 3–5, respectively, are more favourable for more active invertase. At the industrial level, invertase is mostly isolated from yeast, such as *S. cerevisiae*, and there is ongoing research for the use of high yielding filamentous fungi for production of invertase. Examples of the filamentous fungi are *Rhizopus* sp. And *Aspergillus casingii*. The filamentous fungi *Rhizopus* sp. was cultivated in the medium of wheat bran to obtain invertase (Soares et al., 2012); *Aspergillus casingii* was inoculated in a medium of soybean meal and the invertase was isolated after 72 h, while in yeast, invertase (S-b D-fructofuranosidase) is isolated from *S. cerevisiae* (Singh et al., 2019).

16.8.9 Application of Food-Grade Glucose Isomerase Enzymes

In the food industry glucose isomerase has found extensive application in the production of fructose-rich corn syrup. The enzyme is also known as d-xylose ketol isomerase which aids in catalysing reversible isomerase from d-glucose and d-xylose into d-xylulose and d-fructose, respectively. Protein engineering techniques are being explored to generate thermostable glucose isomerase enzymes (Hartley et al., 2000). Glucose isomerase has also been used for bioconversion of hemicellulose into ethanol (Singh et al., 2019). For commercialization of glucose isomerase, the application of biotechnology in isolating mutants of promising prospect is important.

16.8.10 Application of Food-Grade Glucose Oxidase Enzymes

Glucose oxidase is majorly used in the food industry to eliminate harmful oxygen in food product containing probiotic microorganisms. The enzyme allows catalysing oxidation–reduction reaction through the prosthetic group flavin adenine dinucleotide (FAD). High level of glucose oxidase can be isolated from filamentous fungi, such as *Aspergillus versicolor* and *Rhizopus stolonifera* (Guimarães et al., 2006). Singh et al. (2019) suggested that the use of glucose oxidase through biotechnology can be used to improve the stability of probiotic bacteria in yoghurt without adding chemical additives.

16.9 Safety of Food-Grade Enzyme

Industrial food-grade enzymes are generally considered safe and are known as food additives, while some considered them as food processing aids. The safety regulation on the use of food-grade industrial enzymes differs among countries, premarket approval of the enzymes and the information provided by the manufacturer. The prominent occupational food safety risks that have been associated with the use of industrial food enzymes are mainly allergic reaction (in the respiratory tract), irritation risks, residual microbiological activity, chemical toxicity and oral toxicity among consumers (Spök, 2006; Ramos et al., 2011; Singh et al., 2019). Ramos et al. (2011) stated that enzymes are sensitizers and can cause allergic reaction in the respiratory tract (asthma) upon inhalation and also irritation upon contact with the skin. However, aside from the occupational risk, the final consumers of food products containing food-grade enzymes rarely report allergic reaction because low levels of the enzymes are usually used in food formulation which would have eventually be deactivated upon processing and before reaching the consumers. The future challenges that might be associated with the safety concern of food-grade enzymes are toxic substance arising from the by-product of contaminants present in the preparation of the enzyme. Adequate attention must be paid towards fermentation broth contaminate or environmental conditions of some phylogenetical strains used in enzyme production. Although with the advent of genetic engineering technique, toxicity problems can easily be eliminated. Food industry and national or European regulatory bodies must develop strategies to harmonize and enforce legislation on the available food enzymes that are categorized as a novel or those with unusual properties and their food safety assessment practice to eradicate occupational risks that may arise from their use. One of the current issues related to food enzymes legislation is that in the USA, Japan and Canada food-grade enzymes are considered as food additives, while Australia regards food enzymes as food processing aids. WHO/FAO, joint committee of food additives, does not differentiate food-grade enzymes either as a food processing aids or food additives (Ramos et al., 2011). However, EU food legislation considered more than 160 food-grade enzymes as food processing aids, while invertase and lysozyme enzymes are the only two food-grade enzymes considered as food additives. National legislation of France, Hungary, Denmark and Poland do subject food-grade enzymes that are referred to as food processing aids to authorization before use, while in the UK the authorization is voluntary (Ramos et al., 2011). Therefore, it is imperative for legislation of each country worldwide to define the ranges of the permitted enzymes and their applications.

16.10 Conclusions

For the future purpose, there is need to explore the benefits of novel approaches, such as recombinant DNA technology, screening, protein engineering, enzyme immobilization, solid-state fermentation and use of bioinformatics tool in enzyme

engineering in generating food-grade enzymes, and to adapt the enzymes for sustainable and economical industrial production processes. Understanding the novel approaches can aid the industrial production of food-grade enzymes which can be useful in improving food product quality and consistency, preventing potential harmful by-products in the food, reducing raw material dependence during processing, replacing chemical additives, reducing process time, increasing shelf life and creating more opportunities for enhancing the aromatic, structural and textural properties of food. In addition, application of food-grade enzymes for industrial processes should be able to lead to the development of new or improve healthy food products and food properties within the food processing industry; bakery, dairy, beverages, juice, alcoholic, meat, fats and oils, vegetables and other industries. Proper selection of enzymes source, enzymes isolating method and purification technique that is safe and non-toxic must be carefully selected as they influence the cost of production of food-grade enzymes and yield. Solid-state fermentation produces higher enzymes than submerged fermentation at a lower cost. National legislation of countries should make provision for permitting and regulating products from food-grade enzymes and their applications. More research should be channel towards the application of enzymes technologies in food processing to broaden our knowledge and future use because consumers are now demanding for food products or additives that are free of chemicals.

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