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Recent Applications of Enzymes in Food Industry

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Abstract
The application of enzymes in different industries is increasing during the last decades. The enzymes used in food industry have roles in the production of food products. Enzymes used in food industry have been traditionally derived from plant, animal, and microbial sources. Enzymes are used in the food industry for many different applications; production of of dairy, meat, cereal and confectionery products and also in applications of beverage and bakery industry. Enzymes have been used in various food products for improving quality while decreasing processing time and production costs. This review focuses on different types of enzymes recently used during industrial processing for food.

1. Introduction

The majority of currently used industrial enzymes are hydrolytic in action, being used for the degradation of various natural substances. Proteases remain the dominant enzyme type, because of their extensive use in the detergent and dairy industries. Various carbohydrases, primarily amylases and cellulases, used in industries such as the starch, textile, detergent and baking industries, represent the second largest group (Kirk, 2002: 345). Food enzymes can be obtained from different sources, but microbial sources are the most promising and potential one (Srivastava, 2019: 740). On the other hand, the implementation of recombinant DNA technology and process engineering makes these microbial sources more comfortable, flexible, and appropriate for industrial purposes at the commercial level (Srivastava, 2019: 740). Food processing is a transformation practice in the beverage and food industry to make the raw foodstuff of animal and plant origin suitable for consumption. On the basis of their difference from one another, the processed food materials can be distributed into highly processed foods, minimally processed foods, and processed foods. The processing methods are performed through a wide range of biological and chemical agents such as enzymes. The utilization of biological agents in food processing dates back to 6000 BCE and includes bread making, beer brewing, cheese making, and wine making. Currently, these products are mainly recognized in the biotechnology industry (Meshram, 2019: 483).

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There is table below that indicates the enzymes used in food industry (Table 1).

Table 1. Enzymes Used In Food Industry

Enzymes Used In Food Industry	
Industry	**Enzyme class**
Dairy	Protease, Lipase, Lactase, Transglutaminase, Rennin, Catalase
Beverage	Pectinase, Xylanase, β-Glucanase, Laccase, Cellulase
Fats and oils	Lipase, Phospholipase
Other Food	Pectinase, Protease, Lipase, Amylase, Xylanase, β-Glucanase
Sanitation (Antibiofilm enzymes)	Lactonase, DNase, Savinase, α-amylase, Cellulase+Pronase, Glucose oxidase+Lactoperoxidase, Acylase I+ProteinaseK
Bioethanol from Food Crops	Amylases, Cellulase, Xylanase

Enzymes used in food processing have historically been considered non-toxic (Patel, 2016: 67). Some characteristics arising from their chemical nature and source, such as allergenicity, activity-related toxicity, residual microbiological activity and chemical toxicity are of high concern. These attributes of concern must, however, be addressed in light of the growing complexity and sophistication of the methodologies used in the production of food-grade enzymes (Patel, 2016: 67).

2. Enzymes in Dairy Industry

There are many types of microbial enzymes used in the dairy industry, such as catalase, aminopeptidase, proteases, lactoperoxidase, lipases, transglutaminase, etc. They are well known in this field and are different from coagulants as they help improve shelf life. In the dairy industry, microbial enzymes have been utilized to produce diverse products, such as yogurt, cheese, syrup, bread, etc. to enhance their quality (Abada, 2019: 62). Milk coagulants belong to four main categories: animal rennet, plant derived coagulants, microbial coagulants, and geneticaly engineered cymosin. Animal rennet contains the two main enzymes, cymosin and pepsin, in a ratio that depends on the age of the animals when slaughtered (Trani, 2017: 166). Rennet is considered a famous exogenous enzyme used in dairy processing, and has been used since 6000 BCE. Rennin has enzymatic and nonenzymatic action that causes the milk to coagulate. The milk transforms to a gel-like structure during the enzymatic activity due to the temperature and calcium ion effect (Abada, 2019: 64).

The proteases of lactate bacteria are necessary for its growth in substrate (milk) and help dramatically in enhancing flavor of fermented milk products. The proteolytic enzyme system constitutes proteinases that first break down the protein of the milk into peptides. Peptidases then breaks down the peptides into amino acids and small peptides, then the transport system takes charge of the uptake of amino acids and small peptides (Abada, 2019: 65). Currently, most effective procedures have been developed by which enzymes are entrapped for controlled release during ripening. Direct encapsulation using food gums or

lyposomes is one of the two entrapping methhods available; the second method is encapsulation of cell free extract from bacteria or whole bacterial cells in milk-fat capsules (Trani, 2017: 167).

Different lipases of animal or microbe origin created clear cheese, low bitterness, improved flavor, and potent malodors, whereas proteinases, in combination with lipases or/and peptidases, developed cheeses with excellent flavor and low bitterness levels. In order to accelerate the ripening of cheese content of peptidases and proteinases we can use attenuate cell-free extract or starter cells to do that (Abada, 2019: 66). Animal lypases that are isolated from the epiglottis (throat) of calves, sheep, or goats, greatly controbute to release short chain fatty acids responsible of sharp and piquant flavors. The result is due to improved fat and moisture retention in cheese caused by the increased emulsifying propert, that is reduction of fat losses in whey and cooking water. A further recent application is enhancing flavor and structure in butter and dairy cream (Trani, 2017: 170). Lipases from *Mucor miehei*, or *Aspergillus niger* are used to give stronger flavors in Italian cheeses from milk before adding the rennet, by a modest lipolysis, increasing the amount of free butyric acid (Patel, 2016: 67). The free fatty acids generated by the action of lipases on milk fat endow many dairy products, particularly soft cheeses with their specific flavour characteristics. The traditional sources of lipases for cheese flavour enhancement are animal tissues, especially pancreatic glands (bovine and porcine) and pre-gastric tissues of young ruminants (kid, lamb and calf) (Aravindan, 2006: 149). They form inter-molecule or intra-molecule cross-linkages in proteins via e-(g-Glu)-Lys bonds which is useful for modifying the physical and functional properties of foods (Zhang, 2018: 31). Transglutaminase (TG) has recently become of great interest to food scientists for its ability in strengthening the structure of protein gels. TG catalyzes the post-translational modification of proteins by transamidation of available glutamine residues by the formation of covalent cross-links between glutamine and lysine residues. Addition of transglutaminase to milk induces of cross-linking of caseins and whey proteins that improves the strength of milk gels (Trani, 2017: 170). Rennet cheese with modified textural and nutritional properties and improved yield could be obtained upon transglutaminase modification but simultaneous addition of rennet and transglutaminase is recommended. Moreover, transglutaminase crosslinking and calcium reduction were investigated as ways to improve the texture and storage stability of high-protein nutrition bars formulated with milk protein concentrate and micellar casein concentrate (Abada, 2019: 66).

Lactase (EC 3.2.1.23) accelerates the breakdown of lactose into galactose and glucose. It is used to improve the sweetness, solubility, as well as digestive agent, for milk products. Lactases are important in reducing and removing lactose in milk products for lactose-intolerant patients in order to protect them from severe diarrhea, fatal consequences, and tissue dehydration. Among various features of milk treated with lactase is the increased sweetness, hence it can avert the need for adding sugars during the production of flavored milk drinks. Lactase is used by the producers of ice cream, yogurt and frozen desserts to enhance spade and creaminess, sweetness, tastiness, and digestibility, and to decrease sandiness because of crystallization that occurs in lactose converged preparations. (Abada,

2019: 68). Commercial enzymes are derived mainly from fungi like *Kluyveromyces sp*. and *Aspergillus sp*. Although many enzymes are commercially available, new efforts are still beingmade using recombinant engineering techniques and new enzymes are being discovered by screening metagenome databases (Spohner, 2015: 128). The enzyme's immobilization, immobilization method, and carrier type can also affect these optima rates (Abada, 2019: 68).

3. Enzymes in Beverage Industry

Industrial enzymes fall into various groups, of which, the most important are pectinases, cellulases, and tannases which are used in fruit processing. Enzymatic treatment of fruit juice has many advantages over traditional processing. These advantages include an increase in fruit juice yield, enhanced clarification, increased total soluble solids in fruit juice, improved pulp liquefaction, and decreased turbidity and viscosity (Ramadan, 2019: 45). The cloudiness of fruit juice is due to the presence of pectin, cellulose, starch, proteins, tannins, and lignin. The commercial application of enzyme preparations containing pectinases, cellulases, and tannases benefits the fruit juice industry. These enzymes are known as macerating enzymes, which are used in fruit juice extraction and clarification (Ramadan, 2019: 57). Glycoside hydrolases hydrolyze glycosidic bonds and are widely used in syrup, beverage (beer, wine), and dough production. A new low-temperature mashing system containing diverse amylolytic enzymes was applied to produce wort on a commercial scale cost-effectively (Zhang, 2018: 31). Amylases have a broad range of applications in the food and brewing industries (Zhang, 2018: 31). The extensive application of enzymes to brew with high amounts of inexpensive raw materials such as barley focuses on future aspects of enzymes in the brewing industry. In barley, starch has to be broken down into fermentable sugars before the yeast can make alcohol. The majority of enzymes are produced during the germination, for example, α-amylases and proteases, while some enzymes are already present in the barley, for example, β-amylases. In the final malt, entire enzymes essential for the conversion of "grains" into a fermentable liquid are present. However, the malt enzymes do have some boundaries. They can only work at certain pH values, temperatures, etc., and the performance might be too low to do a proper job in a proper time. In comparison, commercial exogenous enzymes can be designed to have more enzymatic power to work at preferred temperatures and pH values. Supplementation of exogenous enzymes can make brewing faster, more consistent, and easier at various steps during the process. Barley malt is the traditional source of enzymes used for the conversion of cereals into beer (Meshram, 2019: 496).

Naringin, limonin, and neohesperidin are bitter compounds presentin citrus juices. The bitterest component is Naringin with a taste threshold in water of approxi-mately 20 ppm. For a long time, the bitterness of grapefruit juices has been a major limitation in the commercial acceptance. The naringin level can be reduced by technologies such as adsorptive debittering, chemical methods, treatment with polystyrene divinyl benzene styrene (DVB) resins and cyclodextrin treatment. Acid hydrolysis, for example, produces not only rhamnose and glucose from naringin, but also naringenin, a very bitter aglycon. The usage of l-Rhamnosidases enabled the specific debittering of juices (Spohner, 2015: 128).

Pectinases have globally attracted great interest as a biological catalyst in many industrial applications. Pectinase catalyzes degradation of pectic substances through de-esterification (esterases) reactions and depolymerization (hydrolases and lyases). Cellulases have gained worldwide interest, as they have valuable potential to process cellulosic biomasses and transform them to useful products. The synergistic effect of cellulases (i.e., exoglucanases, endoglucanases, and β-glucosidases) is needed for cellulose de-polymerization for transformation to useful products using suitable microorganisms. Tannases (tannin acylhydrolases) are important groups of enzymes that are utilized in several industrial applications, including the manufacture of fruit juice, and tea (Ramadan, 2019: 55). Tea and coffee are among the main beverages used worldwide. In coffee industry, enzymes from microbial sources such as cellulases, hemicellulases, galactomannase, pectinases are used from *Leuconostoc mesenteroides*, *Saccharomyces marscianus*, *Flavobacterium spp.*, *Fusarium spp.*. Tea processing requires cellulases, glucanases, pectinases and tannase. Most familiar Asian food products such as soy sauce, koji, moromi, etc. are also made through fermentation of *Aspergillus oryzae* and later inoculated with a bacterium, *Peiococcus soyae*, and yeasts such as *Saccharomyces rouxii* and *Torulopsis spp*., which ferment the mixture for approximately six months (Patel, 2016: 67).

4. Enzymes in Other Food Industry

Industrial applications of enzymes include food (baking, dairy products, starch conversion) and beverage processing (beer, wine, fruit and vegetable juices), animal feed, textiles, pulp and paper, detergents, biosensors, cosmetics, health care and nutrition, wastewater treatment, pharmaceuticals and chemical manufacture and, more recently, biofuels such as biodiesel and bio-ethanol (Homaei, 2016: 145). Quality parameters like meat tenderness is influenced by a number of factors, which may be preslaughter or post slaughter in nature. This toughness may be resolved by the enzymes present in the muscle cell or from exogenous sources to obtain a tender meat. The meat industry's use of enzymes improves the manufacturing process and upgrades poor quality meat. Meat has been found to be a source of several bioactive peptides, and researchers have reported different health-promoting applications of these peptides. They are obtained after strategic use of enzymes on the meat as substrate. Bioactive peptides exhibiting antihypertensive, antioxidant, and antimicrobial effects have been observed in the meat protein hydrolysates. In addition, the enzymes can be used for binding small meat pieces to improve their market value. This also enhances the efficiency of carcass utilization. The protein cross-linking enzymes, like transglutaminases (TGase), have been used extensively in several food products to improve the texture (Singh, 2019: 112). Much of the early interest in enzymology was developed by scientists like Pasteur, Payen and Persoz, who were associated with food, wine and beer industries (Aravindan, 2007: 141). Although not in isolated form, enzymes have been used traditionally for dairy, baking, brewing, and winemaking for centuries. Enzymes are needed for cheese production and a wide varietyof other dairy goods. For example, their application keeps breadsoft and fresh longer, leads to crispy crusts, increases dough vol-ume and can compensate for variations in flour and malt quality. Additionally, enzymes are used to lower

alcohol concentrationand calories in beer. In winemaking, the sulphur content can be reduced, clarity and wine colour can be maintained, flavours can be enhanced and the filterability can be improved with enzymes (Spohner, 2015: 119).

In the production of baked goods, enzymes can be added individually or in complex mixtures at a very low level that may act in a synergistic way. Baking comprises the use of enzymes from three different sources:

- The endogenous enzymes in flour.
- Enzymes associated with the metabolic activity of the dominant microorganisms.
- Exogenous enzymes added in the dough.

In the baking industry, there is a rising focus on lipolytic enzymes. Because the enzymes degrade polar wheat lipids to produce emulsifying lipids, recent findings suggest that phospholipases can be used to supplement or substitute traditional emulsifiers (Meshram, 2019: 496).

The main constituent of bread is starch. As the starch crystallizes, bread becomes hard and unpleasant to eat with age. To avoid wasting bread and to extend shelf life, the addition of lipase and amylase enzymes in bread making reduces the crystallization of starch in the bread. The savings were chiefly determined by avoided grain production and bread transportation (Meshram, 2019: 497). The use of cathepsins in meat tenderization is doubtful, as there is dearth of evidence that they are released from lysosomes during the postmortem storage of meat. Moreover, cathepsins have been found to have an effect on myofibrillar proteins like myosin, actin, and α-actinin, and during normal aging of muscle, only a small amount of these proteins is affected. Many types of cathepsins have been identified, and out of them cathepsins B (EC 3.4.22.1) and L (EC 3.4.22.15) are said to be the key factors for deterioration of muscle proteins. Effective degradation patterns of myofibrillar proteins were observed in salmon muscle tissue which was almost reproduced upon treating myofibrils with purified cathepsin L. Studies reveal that the myofibrillar proteins are least affected during postmortem storage and the softening of muscle is due to proteolytic digestion of minor cell components that link the major structural units (Singh, 2019: 115).

Glutaminase (l-glutamine aminohydrolase, EC 3.5.1.2), an enzyme produced by starter cultures to impart flavor, is important in products like meat sausage. Incorporating the enzyme to fermented seasoning agents leads to an increase in the glutamic acid content, and enhancing the “umami” taste in foods. The enzyme is responsible for hydrolytic deamidation of l-glutamine to produce l-glutamic acid, the element indicated as a flavor enhancer, and ammonia, another component which acts as an acid neutralizer. It is a ubiquitous enzyme in bacteria and eukaryote, but its presence in archaea, thermophiles, and plants is doubtful (Singh, 2019: 122).

Difructose anhydride (DFA) III is a non-cariogenic sweetener and non-digestible disaccharide that promotes the absorption of calcium magnesium, and other minerals in the intestine. DFA III is produced from inulin by the exo-acting inulin fructotrnasferase (EC 4.2.2.18) from *Arthrobacter ureafaciencs*. Since then, inulin

fructotrnasferase from *Arthrobacter sp*. and other bacteria has been identified. However, the industrial use of DFA III was limited by a low thermal stability and expensive inulin. A great deal of effort has been made to isolate heat-stable inulin fructotrnasferase from various microorganisms and to develop a novel process using a cheap substrate. Recent reports have shown a novel inulin fructotrnasferase *Arthrobacter pascens* T13-2, *Arthrobacter sp.* L68- 1, and other *Nonomuraea species*, stable up to 70–80 °C after 1 h of heat treatment. Fructooligosaccharides (FOS) as a prebiotic can be synthesized fromsucrose using fructosyltransferase (EC 2.4.1.19). Inulin has a similar fructofuranosidic linkage to FOS, which is the smallest substrate for inulin fructotransferase. The utilization of sucrose as a substrate to produce DFA III, resulting in about a 10% (w/w) yield, was attempted through a coupled enzyme reaction as a novel approach (Choi, 2015: 1448).

Papain is a highly efficient enzyme causing significant degradation of myofibrillar as well as collagen proteins. Grzonka et al. (2007) found that papain had an optimum activity at a wide range of pH levels (5.8–7.0) and temperatures (50–57°C) specially when the substrate used was casein (Singh, 2019: 117). Ficin (EC3.4.22.3, MW = 26 kDa) is obtained from the latex of *Ficus glabrata*, *Ficus anthelmintica* etc., which contains about 10 proteases with the half-life of 1.5 h at 60°C. In fact, the ficin is most commonly obtained from the fig fruit and reported to have a meat tenderizing effect (Singh, 2019: 117). Actinidin, or actinidain (EC 3.4.22.14), is obtained from the kiwi fruit (*Actinidai deliciosa*). The enzyme varies greatly among different cultivars of fruit and it ranges from non-detectable to 10.7 mg/mL juice. The actinidin was stable at a pH range of 7–10, however, it had an optimal activity at 58–62°C. Actinidin hydrolyzes both myofibrillar proteins and connective tissue proteins but appears to have higher proteolytic activity toward collagen (Singh, 2019: 119). Transglutaminase (TGase) is used for the improvement of textural characteristics of food products. The enzyme is calcium-dependent and catalyzes acyl transfer reactions with the ε-amino group lysine, where γ-carboxamide groups of glutamine act as acyl donors and lysyl residues act as acyl acceptors. The reaction involves the intermolecular linking between the glutamine and lysine component of proteins by creating a complex network. Hence, in raw meat products, it provides an enzymatic way of producing uniform shapes and sizes. The enzyme results in an effect on soluble proteins in such a manner that a gel network is produced and combines pieces of meat together (Singh, 2019: 120). A large number of fat clearing enzymatic lipases are produced on an industrial scale. Most of the commercial lipases produced are utilized for flavour development in dairy products and processing of other foods, such as meat, vegetables, fruit, baked foods, milk product and beer. The function of phospholipase in egg yolk treatment is to hydrolyze egg lecithin, iso-lecithin, which improves the emulsifying capacity and heat stability. The egg yolk thus produced can be useful in the processing of custard, mayonnaise, baby foods, dressings and in dough preparation (Aravindan, 2006: 150).

5. Extremozymes

In addition to enzymes from conventional sources, the bioprocessing of extremozymes (enzymes produced by organisms living under extreme conditions)

has the potential to expand the application scope of enzymes and therefore further increase the market value of enzymes. Extremozymes have potential application in food for a number of reasons: (a) extremozymes are hardy enzymes that can survive other-than-usual food processing conditions, (b) extremozymes are more suitable for substrates whose solubility is enhanced only under extreme conditions, (c) extremozymes may allow in situ catalysis during food processing, for example, breaking down acrylamide during the baking of food, (d) extremozymes are more suitable for use in foods that require aging under extreme conditions (high salt, low temperature, etc.), and (e) the use of extremozymes helps control microbial contamination by microorganisms that grow under normal conditions (Akanbi, 2019: 796). Extremozymes have great economic potential in many industrial processes (e.g. agriculture, food, feed and drinks, detergents, textile, leather, pulp and paper) (Gomes, 2004: 232). Extremozymes are enzymes that can function under extreme conditions of temperature, pressure, pH, and alkalinity. They are derived from extremophilic microorganisms (extremophiles) that are metabolically active and grow under extreme conditions. Extremophiles thrive in ecological niches such as deep-sea hydrothermal vents, hot springs, solfataric fields, shallow marine boiling water, heated sea floor volcanoes, hot lakes, coal and copper mines, and some environments contaminated with nuclear waste (Akanbi, 2019: 796). With an average pressure of 38 MPa, the world's oceans are home to piezophilic (formerly called barophilic) microorganisms, including a variety of thermophilic and hyperthermophilic archaeal strains. Given the use of high-pressure conditions in various industries (e.g. the food industry), the availability of extremozymes capable of catalysis at high pressures would offer a novel biotechnological alternative to currently employed processes (Eichler, 2001: 271). Extremozymes may therefore be useful for (a) the production of glucose from cellulose in wood chips; (b) the release of bioactive peptides from keratinous materials such as horns, hoofs, and feathers; and (c) the in situ degradation of polyacrylamide in foods during baking, etc (Akanbi, 2019: 809).

6. Antibiofilm Enzymes

The persistence and expansion of foodborne pathogens and their associated diseases are a prime concern for public health. It has been reported that gastrointestinal, neurological, gynecological, and immunological symptoms are some of the effects frequently caused by foodborne illnesses, often resulting in disability and mortality (Nahar, 2018: 1). If we take a look at the cleaning and disinfection aspects, enzymes have a duty here. Diverse microorganisms are able to grow on food matrixes and along food industry infrastructures. This growth may give rise to biofilms (Galie, 2018: 1). Biofilms are polymeric mixtures (polysaccharides, lipids, and nucleic acids) produced by microorganisms, which are supported on biotic and abiotic surfaces. There are several favorable conditions for the formation of biofilms, such as the presence of moisture, nutrients, and inocula of microorganisms from the raw material. These conditions provide a persistent source of contamination, causing the deterioration of food as well as creating an important route for cross contamination during production processes. In some scenarios, biofilm formation can play an important and beneficial role, for example, plant growth promotion, the biodegradation of

environmental pollutants, and controlling the microbial balance in living organisms. However, in others, such as clinical settings and some industrial processes, it can cause significant problems (Gutierrez, 2019: 321). Biofilms can become resistant to the chemical and physical treatments applied during cleaning and sanitizing procedures in the food industry. Therefore, the use of enzyme treatments to break down EPS in biofilms is a possible alternative when standard cleaning agents do not give satisfactory results in removing biofilms (Lequette, 2010: 421). The extracellular matrix is mainly composed of polysaccharides, such as cellulose, proteins or exogenous DNA. This matrix can be fixed to hard surfaces (food industry equipment, transport, dispensing and storage surfaces, soil, etc) or to biological structures (vegetables, meat, bones, fruits, etc.). The extracellular matrix has a structural role, which is responsible for the strong persistence of these biofilms in the food industry. It generates complex gradients with respect to nutrients and oxygen diffusion, contains extracellular enzymes used for nutritional purposes, allows for the transfer of cell communication molecules, and protects the embedded cells against toxic compounds. In summary, biofilm formation confers many advantages to the microbial cells in a food industry environment, such as physical resistance (against desiccation), mechanical resistance (against liquid streams in pipelines) and chemical protection (against chemicals, antimicrobials and disinfectants used in the industry) (Galie, 2018: 2). One strategy that has proved effective for the removal of biofilms from industrial systems has been the use of enzymes, for example the treatment of biofilms formed in food areas. Enzymes and detergents have also been used as synergists to improve disinfectant efficacy. The combination of surfactants/proteolytic enzymes increased the wettability of biofilms formed by a thermophilic *Bacillus* species, thus enhancing cleaning efficiency also reported the synergistic action of phenolic antimicrobials and surfactants in combination with enzymes. The specificity of the mode of action of each enzyme makes this technique complex. It also means that it is extremely difficult to identify enzymes that are effective against all the different types of biofilms (Gutierrez, 2019: 323).

Food industry biofilms constitute a serious economic and health issue. On the one hand, the existence of biofilms along food manufacturing surfaces can lead to financial losses as a result of corrosion on the metal surfaces by some bacteria, requiring the replacement of these parts. Furthermore, some bacterial species, such as *Pseudomonas spp*. and *Bacillus spp*., secrete many different proteolytic and lipolytic enzymes that can generate unpleasant odors (rancid) and tastes (bitter). In these instances, the affected manufacturing batches must be removed and destroyed. These disruptions may not only represent a significant financial loss for the company, but may also potentially damage their brand with respect to their competitors (Galie, 2018: 12).

7. Other Industrial Applications of Enzymes

The by-products of the vanilla extract were not utilized earlier, but in 2010, the enzymatic treatment and reutilization of the exhausted vanilla pods were reported. Exhausted vanilla pods contain only small amounts of aroma and flavor, but after treating them with various enzymes, the aroma and flavor can be extracted and potentially be used as food additives (Patent, 2010, US 7803412 B1: Enzymatic

treatment of spent vanilla beans, United States) (Rastogi, 2019: 853). The use of enzymes as feed additives is also well established. For example, xylanases and β-glucanases have been used throughout the past decade in cereal-based feed for monogastric animals which, contrary to ruminants, are unable to fully degrade and utilize plant-based feeds containing high amounts of cellulose and hemicellulose. During recent years focus has been on the utilization of natural phosphorus bound in phytic acid in cereal-based feed for monogastrics (Kirk, 2002: 348). Mushrooms are known to be rich in proteins while containing significant amounts of free glutamic and aspartic acid, which are found to elicit umami flavor. In order to improve the taste-contributing free amino acid contents, these proteins need to be hydrolyzed. Flavourzyme contains both endo- and exo-peptidase activities that are suitable to yield greater amounts of free amino acids while also producing flavor-active amino acids and peptides from proteins. Consequently, the treatment of mushrooms with the cell-wall degrading enzyme β–glucanase and the protease Flavourzyme significantly enhanced the umami and other taste-contributing free amino acids (Rastogi, 2019: 853). From the enzymatic pretreatments to the fermentation of sugars by yeasts, enzymes are the core part of every biotechnological process, including those comprised within biorefineries. The most important enzymes for bioethanol biorefineries are amylases, cellulases, and xylanases, yet expansin-like proteins (loosenins), ligninases, and pectinases are also of potential interest. The main enzymes for carbohydrate hydrolysis in bioethanol biorefineries are amylases and cellulases. Whereas amylases are the main enzymes in first-generation bioethanol, cellulases are for second-generation bioethanol. Moreover, it is a concerted prediction that through heterologous expression of hydrolases, enzyme recycling and immobilization, and complementation of enzyme cocktails with hemicellulases and accessory enzymes, second-generation bioethanol production will come closer to a full industrial scale reality (Ríos-Fránquez, 2019: 262). Conventional techniques of food analysis may be extremely tedious and time consuming. It is therefore essential to replace such conventional methods with rapid and easy-to-use techniques. Biosensors are showing great promise in this area, mainly due to their simplicity and quick results. They allow the testing of multiple samples for multiple characteristics with minimum error. Research groups developing biosensors have paid a lot of attention to achieving a low response time, a low detection limit, a wide linear detection range, and long-term stability, desirable characteristics for any biosensor to be applicable for commercial usage. With increasing simplicity and portability as well as improving sensitivity and reproducibility, biosensors are rapidly emerging as an alternative to conventional methods of food quality assessment (Vaidya, 2019: 672).

The cost of the immobilized enzyme process may decrease with the advances in microbial biodiversity, molecular biology, and genomics; this also may increase the use of immobilized enzymes in food processing sectors. Immobilized enzymes have to be very heat stable and must be able to work in a reliable optimized process system so the products will be cheap. But if in the market any cheap source of soluble enzyme is available and if other processes are well established, then the newly developed immobilized enzyme process cannot survive (Adhikari, 2019: 719).

Laccases, also known as polyphenol oxidase, are part of the family of blue multicopper oxidases that catalyze single electron oxidation of food-reducing substrate molecules with four electrons of molecular oxygen into water. In addition, these enzymes induce the oxidation of a wide range of substrates, including mono-, di-, and polyphenols, amino phenols, methoxy phenols, aromatic amines, and ascorbate with the concomitant four electron reduction of oxygen to water. Laccases exhibit a wide range of substrate while oxidizing compounds in the presence of mediators with high redox potential. However, the application of laccases in the food industries requires a large amount of laccase enzyme; for this, many strategies can be applied along with media and process optimization. Applications of ligninolytic enzymes (Laccase, Manganese peroxidase, Lignin peroxidase) to some processes that improve or modify the color of food or beverages for the elimination of unwanted phenolics accounts for the browning, haze formation, and turbidity in clear fruit juice, beer, and wine (Chowdhary, 2019: 186).

Lipases are the enzymes that alter the lipid properties by changing the position of fatty acid chains and exchanging one or more fatty acid chains with new chains in the glyceride. This precise property modifies lipids to a higher fat value from a relatively inexpensive and less-desirable lipid. Lipases can cause esterification and interesterification while catalyzing the hydrolysis of fats and oils. This esterification and interesterification process are used to attain value-added products by the lipolytic alteration of oils and fats. This fatty acid and positional lipase specifically show higher industrial potential than the bulk production of fatty acids through hydrolysis. Lipase is a flexible enzyme having a potential role in the food, pharmaceutical, leather, detergent, cosmetic, textile, and paper industries (Meshram, 2019: 491). Lipases occur widely in nature, but microbial lipases are commercially significant because of low production cost, greater stability and wider availability than plant and animal lipases. They may originate from fungi, molds or bacteria and most of them are formed extracellularly. This ready availability has created an enormous spin-off with respect to the enantioselective hydrolysis and formation of carboxyl esters (Aravindan, 2006: 142).

Lipoxygenases are enzymes classified in the class oxidoreductases (linoleate means oxygen, LOXs) present in plants, fungi, and animals. Lipoxygenases produced by plants have a noteworthy importance in the food industry. Lipoxygenases generate approximately 9832 aromas and flavor in several plant products (Meshram, 2019: 492).

Tyrosinase is a multifunctional metalloenzyme that catalyzes the o-hydroxylation of monophenols to o-diphenols (cresolase or monophenolase activity) and the subsequent oxidation of the latter to reactive o-quinones (catecholoxidase or diphenolase activity) using oxygen. Further, these o-quinones polymerize into brown-black pigments through a series of enzymatic and nonenzymatic reactions. These two activities are the basis for the widespread biotechnological and industrial applications of tyrosinase, for instance biosensors for the monitoring of the phenolic content in wastewater and food products; in environmental technology for the bioremediation of phenol-containing wastewater and contaminated soils; in pharmaceutical industries for the biotransformation of l-

tyrosine to l-DOPA, which is the preferred drug for Parkinson's disease; and in the cosmetic and food industries due to either undesirable or beneficial oxidative browning reactions (Agarwal, 2019: 4).

8. Results

Enzymes are used in several different food products. Many processes and application are being added by modern biotechnology. Recent researces are mostly on enzymes working at the extreme conditions. Also, researches on metagenomics are helped exploring of these new trend enzymes. Novel biotechnological applications have been established successfully. The industrial enzyme market has a large potential not only for food industry but also detergent, textile and paper industries.

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