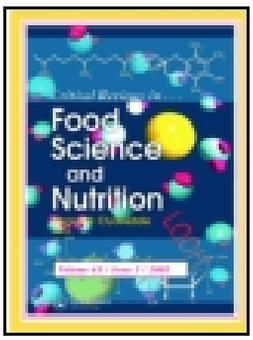

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Muhammad Bilal & Hafiz M. N. Iqbal (2019): State-of-the-art strategies and applied perspectives of enzyme biocatalysis in food sector — current status and future trends, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2019.1627284

To link to this article: https://doi.org/10.1080/10408398.2019.1627284

Published online: 18 Jun 2019.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2019.1627284

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REVIEW

State-of-the-art strategies and applied perspectives of enzyme biocatalysis in food sector — current status and future trends

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ABSTRACT

With the recent progress in biotechnology, a wide variety of novel enzymes with unique physicochemical properties and diverse applications has been introduced, and new application list continues to extend in the future. Enzymes obtained from microorganisms, including bacteria, fungi, yeast are widely applied in numerous food formulations for intensifying their texture and taste. Owing to several desirable characteristics such as easy, cost-efficient and stable production, microbial-derived enzymes are preferred source in contrast to animals or plants. Enzymatic processes have a considerable impact in controlling the characteristics such as (1) physiochemical properties, (2) rheological functionalities, (3) facile process as compared to the chemical-based processing, (4) no or minimal consumption of harsh chemicals, (5) overall cost-effective ratio, (6) sensory and flavor qualities, and (7) intensifying the stability, shelf life and overall quality of the product, etc. in the food industry. Also, enzyme-catalyzed processing has also been designed for new food applications such as extraction of bioactive compounds, nutrient-rich and texture improved foods production, and eliminating food safety hazards. Herein, we reviewed recent applications of food-processing enzymes and highlighted promising technologies to diversify their application range in food industries. Immobilization technology enabled biocatalysts to be used cost-effectively due to reusability with negligible or no activity loss. Integrated progress in novel enzyme discovery, and recombinant DNA technology, as well as protein engineering and bioprocess engineering strategies, are believed to rapidly propagate biocatalysis at industrial-scale food processing or green and sustainable chemical manufacturing.

KEYWORDS

Enzyme catalysis; food industry; brewing; baking; juice clarification; genetic engineering; protein engineering; computational design

Introduction–problem statement and opportunities

Enzymes, as biological catalysts, offers a unique set of physiochemical, biological, and biocatalytic functionalities that enable biocatalysts with unsurpassed selectivity and specificity in transforming raw materials. Additionally, biocatalysts also sustain a superior quality of the final products by maintaining industrially requisite physiochemical properties, biochemical characteristics, and bioactive functional attributes of the product (Bilal and Iqbal 2019a; Singh et al. 2019). Enzymes developed from different microbial sources find a significant position to design efficient bioprocesses in numerous industries, including food and beverages, animal feed, pharmaceuticals, cosmeceutical, textile, leather, detergent, pulp and paper, personal care products, and energy, along with many others. Fascinatedly, enzymes are natural, safe, and process friendlier as they carry out catalytic reactions under mild reaction conditions (at optimal, pH, temperature, and pressure) with a high turnover rate. To date, more than 500 products have been developed by enzyme-based processes in different industries (Kumar and Singh 2013). Approximately around 150 industrial bioprocesses from the industries mentioned above exploit enzymes as green catalysts. Among the various industrial segments, food, and beverage enzymes encompass the leading segment of industrial enzymes (Patel, Singhania, and Pandey 2016). In 2011, they shared a high revenue nearly $1.2 billion which was predicted to realize up to $1.8 billion by 2016 (World Enzymes to 2015 2011). In addition to having a great impact on controlling the quality of final food products, enzymes are also employed in the food industry as direct food additives to impart or modify the physicochemical and rheological properties of the foods (Aguilar 2008). This review article summarizes current applications of food-processing enzymes. Additional emphasis is given to state-of-the-art genetic engineering, protein engineering, and immobilization approaches to tailor the catalytic properties of enzymes or design enzymes with new and improved functionalities to diversify their application range in food industries. The biocatalyst engineering approach might offer economically viable and environmentally acceptable biotransformation following the optimization of all the variables mentioned above.

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Applied perspectives of enzymes in the food sector

Applied perspectives of enzymes in the food industry are illustrated in Fig. 1. The enzymes employed in baking, beverage, brewing, dairy, oils, fats, fruit juice, and wine industries are terms as food enzymes. Most of them share their contribution at different stages of the industrial process such as (1) preprocessing (i.e. initial foodstuff preparation and pretreatment), (2) production and processing, (3) refining, (4) packaging, and (5) storage (Ghorai et al. 2009; Porta, Pandey, and Rosell 2010). Among them, the first two stages are carried out to eliminate excessive amounts of certain components to ensure the physicochemical stability and clarity of the resulting products. The clarification and fining of fermented beverages are often costly, labor-intensive, and generates a massive amount of disposal resulting in a substantial loss of product. This also confines the aroma (odor) and flavor (taste) compounds from the residual product. Aiming to overcome these significant drawbacks and induce the product quality, several enzymes subject to their functionality and purpose are added to the fermentation media to stimulate the overall production process. Examples of such enzymes include but not limited to the amylases, lipases, xylanases, pectinases, proteases, lactases, aminases, glucanases, reductases, cellulases, arabinofuranosidases, laccases, glucose oxidases, peroxidases, and esterases, etc. The deployment of these enzymes is highly suitable and superior to the traditional thermal and mechanical processing of several food products (fruits/vegetables). Moreover, the use of enzymes has been reported as process friendlier and eco-friendly with no or minimal waste generation. Additionally, these enzymes accelerate the overall extraction process efficiency and produce a high-quality product. Following green agenda, noticeable attention has also been paid on the both, production and exploitation of aroma liberating enzymes, for example, pectinases, glucanases, glycosidases, and arabinofuranosidases, etc. Engineered yeast can be utilized to produce better-flavored alcoholic beverages (Hasan, Shah, and Hameed 2006). The industrial potentialities of extensively studied enzymes in the food industry are displayed in Fig. 2.

Figure 1. Applied perspectives of enzymes in food industry.

Food enzymes—starch industry

Starch, consists of amylose and amylopectin, is a leading renewable industrial raw feedstock that can be processed, either chemically or enzymatically, into an array of valuable compounds with diverse industrial significance, such as high-fructose sirups (Zobel 1992; Park et al. 2018). Due to its complicated architecture, the depolymerization of starch into oligosaccharides and smaller sugar units necessitates a set of enzymes (exoamylases and endoamylases). Fig. 3 illustrates an overview of the enzyme-based breakdown of starch (Turner, Mamo, and Karlsson 2007). A large family of enzyme consisting of α-amylase, β-amylase, pullulanase, glucoamylase, and transglutaminase along with some lesser-known enzymes, is involved in the starch industry. Among these enzymes, α-amylase, and glucoamylase largely catalyze the transformation of the starch polymer into glucose residues. Sometimes, the use of additional debranching enzymes increased the glucose yield. β-Amylase is used to liberate maltose disaccharide that is commercially synthesized from barley grains. For instance, the use of transglutaminase has been associated with strengthening the texture of homogenized sausages, noodles, and yogurt (Kuraishi, Yamazaki, and Susa 2001).

Figure 2. The industrial potentialities of extensively studied enzymes in food industry.

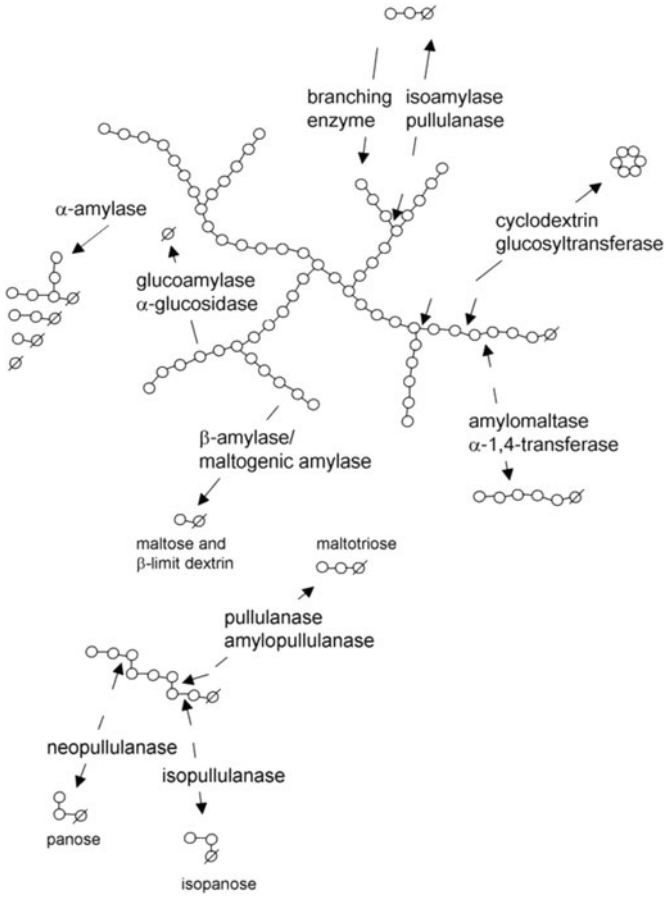

Figure 3. Enzymatic attack on part of an amylopectin molecule. Glucose molecules are indicated as circles and the reducing ends are marked by a line through the circle. Adapted from Turner, Mamo, and Karlsson (2007), an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0). Copyright (2007) Turner et al; licensee BioMed Central Ltd.

α-Amylases (EC 3.2.1.1) catalyze starch degradation by cleaving α-1,4 glycosidic linkages of polysaccharides resulting in the release of short-chain dextrin (Sindhu et al. 2017). In the starch processing industry, α-amylases find application for starch liquefaction in which starch is converted into fructose and glucose sirups. A typical enzyme-mediated starch conversion encompasses three steps, i.e. (1) gelatinization, (2) liquefaction, and (3) hydrolysis. In the gelatinization step, starch granules are disrupted to form a viscous suspension. After gelatinization, a liquefaction process, that reduces the viscosity by partial hydrolysis, readily liquefies gelatinized starch. Lastly, saccharification results in the release of maltose and glucose, employing thermally stable enzymes (Raveendran et al. 2018). It is demonstrated that starch hydrolysis (saccharification) is predominantly carried out by α-amylases from *Bacillus amyloliquefaciencs*, *Bacillus licheniformis,* or *Bacillus stearothermophilus* (Van Der Maarel et al. 2002). Fig. 4 illustrates a simplified scheme of starch-based substrate conversion into glucose sirup in the presence of an enzyme.

Glucoamylases (EC 3.2.1.3), known as saccharifying enzymes, are the exo-acting that hydrolyze starch polymer from the non-reducing end, liberating *β*-glucose monomers. Multiple strains of microorganisms can secrete this enzyme,

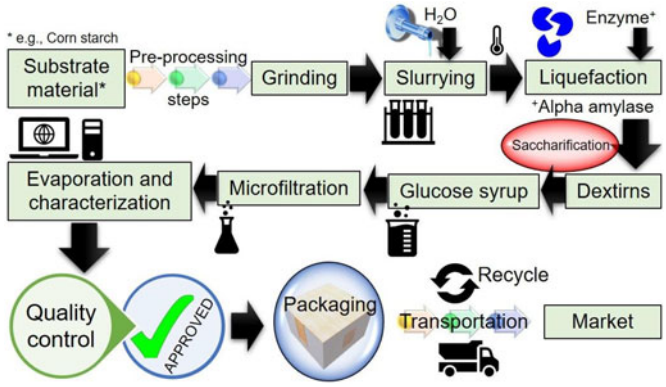

Figure 4. A simplified scheme of enzyme-based starch-based substrate conversion into glucose sirup, characterization and transportation to market cycle.

but the enzymes produced by *Aspergillus niger* and *Aspergillus awamori*, and *Rhizopus oryzae* have been recognized the most widely pursued enzymes for biotechnological applications (Coutinho and Reilly 1997). Notably, most of the glucoamylases are actively stable at a lower temperature and lose their activity at elevated temperatures due to conformational changes in a three-dimensional structure. Glucoamylases have found numerous applications in food processing setup, in particular, for the manufacture of high-fructose and glucose sirups (James, Simpson, and Marshall 1996). In addition to the conversion of flour starch into maltose and fermentable sugars, these enzymes have also been employed to produce glucose, which can yield ethanol upon fermentation with *Saccharomyces cerevisiae*. In this way, they play a key role in the manufacture of sake and soya sauce and light beer. They metabolize dextrins into fermentable sugars with low calorific value and alcohol-free or no-alcohol beer (Blanco et al. 2014). Enzyme-assisted lab-scale bioreactors have been used for continuous starch conversion, but pretreatment of the starch with α-amylase and clarification is indispensable avoiding the blocking/obstruction of the column.

Belonging to the class of transferases, transglutaminase (EC 2.3.2.13) carry out the development of cross-linking within a protein moiety and among molecules of diverse proteins (Kieliszek and Misiewicz 2014). This characteristic feature transglutaminase enzyme has a significant influence in modifying the physicochemical properties of protein such as solubility, viscosity, thermo-stability, pliability, emulsifying capability, gelation, and foaming properties. Apart from this, these enzymes can also mediate the deamination reactions without the presence of free amine groups, where water serves as an acyl group acceptor (Kuraishi, Yamazaki, and Susa 2001).

In glycogen or other interrelated polymers, the hydrolysis of α-1,6-glycosidic linkages can be achieved by the action of debranching enzymes. Affinity towards α-1,6-bond differentiates the debranching enzymes from other amylases, which typically exhibit affinity for α-1,4-glucosidic bonds. These enzymes are divided into two major groups, i.e. (1) direct and (2) indirect (Fogarty and Kelly 1990). Based on their substrate preference, these enzymes may be categorized into three major categories (a) microbial pullulanases (b) isoamylases, and (c) amylo-1,6-glucosidases (Nakamura 1996). The

indirect debranching enzymes are generally found in animal and yeast. Prior to debranching action, these enzymes require substrate modification by some other enzyme(s) (Nakamura 1996). For example, the amylo-1,6-glucosidase first requires the action of transglucosylase to eliminate an oligosaccharide, leaving a glucose molecule attached to a tetra-saccharide by 1,6-bond (Nair, Singhal, and Kamat 2007). This enzyme can hydrolyze 1,6-α-glucosidic linkages only if the side chain contains a single glucose residue. The direct debranching enzyme (i.e. isoamylases and pullulanase), present in bacteria and plants, can directly break α-1,6-branching point of the substrate without any prior modifications. According to the substrate preference, these enzymes can be classified into pullulanases, isoamylases, or R-enzymes. Pullulanase (EC 3.2.1.41) is produced by a large number of microorganisms such as *Klebsiella planticola*, *Bacillus acidopullulyticus*, *Bacillus deramificans* (Nair, Singhal, and Kamat 2007), thermophilic *Bacillus sp*. AN-7 (Zareian et al. 2010), *Bacillus cereus* FDTA-13, and *Geobacillus stearothermophilus* (Zareian et al. 2010). Among various direct debranching enzyme, microbe-derived pullulanase has geared a deal of attention due to its explicit action on α-1,6 bonds in pullulan.

Food enzymes—baking industry

Bread is considered the most traditional and common food, worldwide having a close association with the enzymes. Given the booming demand for natural and healthier bio-products, enzymes have achieved insightful significance in the preparation of bread to upgrade dough and bread quality, which in turn promote the dough flexibility, machinability, steadiness, loaf volume, crumb structure, and shelf life of foodstuffs (Baillet, Downey, and Tuohy 2003). Cellulases and xylanases are hydrolytic enzymes involved in the random cleave of the *β*-1,4 backbone of xylan (Fig. 5) and can increase the strength and elasticity of the gluten network either directly or indirectly with an ultimate improvement of the bread quality (Demain 2000).

Xylanases, produced by different species of *Aspergillus* and *Trichoderma*, are extremely valuable in the baking industry to increase the bread volume, crumb structure, and reduce stickiness (Kirk, Borchert, and Fuglsang 2002). They also play a noteworthy role in extending the shelf life of bread and reducing bread staling, when used at optimum

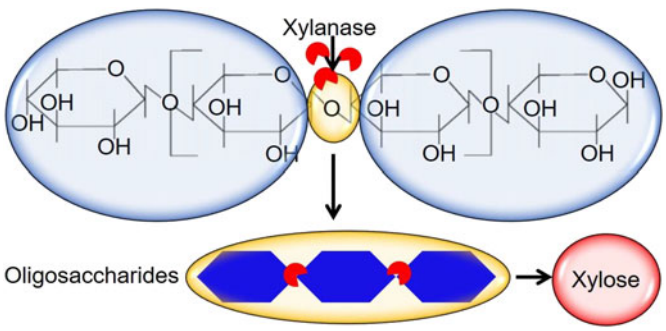

Figure 5. Xylanases involved in the random cleave of the *β*-1,4 backbone of xylan.

levels. Therefore, increasing interest has been directed toward the use of xylanases in the baking industry for bread manufacture (Butt et al. 2008). Application of these enzymes moderates the water requirement in baking that leads to a more stabilized dough by reducing the water absorption. Xylanases are specifically utilized in dry crisps and whole-meal rye baking common in Scandinavia. Microorganisms such as bacteria and fungi, as well as, animal and plant cell can produce the xylanase enzymes either extracellularly or intracellularly (Nair and Shashidhar, 2008; Bajaj and Singh, 2010; Sanghi et al. 2010; Sharma and Chand, 2012; Mandal, 2015). Nevertheless, interest in microbial xylanases has grown markedly to encounter the global demand of contemporary energy crisis together with the incapability of the animal and plant-based xylanases. To date, two strategies using either native or genetically engineered microbial strains have been applied for the synthesis of microbial xylanases.

Proteases are classified as peptide hydrolases (EC 3.4) with a subdivision into endopeptidases (EC 3.4.21-99) and exopeptidases (EC 3.4.11-19) (Gomes and Steiner 2004). They can also be classified according to the pH range, i.e. (1) acidic proteases (pH 2.0–6.0), (2) neutral proteases (pH 6.0–8.0), and (3) alkaline proteases (pH above 8.0) (Pereira et al. 2018). They belong to a large group of hydrolytic enzymes that catalyze the peptide bonds in the polypeptide chain. Exo- and endopeptidases catalyze the hydrolysis of peptide bond proximal to the amino or carboxyl terminus, and distant from the termini of the substrate, respectively (Rao et al. 1998; Dipasquale et al. 2009). Proteases are routinely employed on large-scale in the manufacturing of baked goods, bread, crackers, and waffles. Also, proteases can be incorporated to reducing mixing duration, assuring dough, consistency, controlling gluten strength in bread, maintaining bread texture, and enhancing flavor (Mohapatra, Bapuji, and Sree 2003; Goesaert et al. 2005; Aguilar 2008). Interestingly, proteolytic enzymes have appeared as a promising alternative to bisulfite used earlier to control consistency through the reduction of disulfide bonds in gluten protein.

Due to potential starch degrading properties, α-amylases have gained wider attention in the baking industry. In the bread-baking process, the addition of α-amylase in the dough causes the hydrolysis of starch into small dextrin's, which can subsequently be fermented by yeast. Moreover, the enzyme-based hydrolysis of starch reduces the dough viscosity and thus improving its volume, texture, and softness of the bread. α-Amylases have also shown a promising antistaling activity that helps to improve the softness retention and shelf life of the baked products (Van der Maarel et al. 2002). However, a slight overdose of this enzyme may lead to gumminess of the bread due to the formation of branched dextrin's. Use of pullulanase in a blend with α-Amylase may overcome these conditions by explicitly hydrolyzing compounds involved in gumminess of amylase-treated bread (Sundarram and Murthy 2014).

Food enzymes—fruit juice processing industry

The consumption of popular fruit products (i.e. fruit juices, beverages, and nectars) has tremendously increased resulting

in an upswing in fruit juice bioprocessing in fruit-cultivating countries. Enzymes are considered highly imperative candidates in food processing since they simplify intermediate bioprocesses during food manufacturing (Ramadan 2019). Among various groups of enzymes, cellulases, pectinases, and tannases are the most important and widely used enzymes in industrial fruit processing purposes (Yao et al. 2014; Sharma et al. 2016; Amin et al. 2017a; Amin et al. 2017b). In contrast to traditional processing, enzyme-assisted treatment of fruit juices presents the advantages of increased fruit juice yield, superior clarification, increased pulp liquefaction, and diminished viscosity and turbidity (Kaur and Sharma, 2013; Amin et al. 2017c). Recently, Bilal, Asgher, Iqbal, et al. (2017) used an alginate-chitosan-immobilized ligninolytic cocktail that comprised on manganese peroxidase (MnP) (EC 1.11.1.13), lignin peroxidase (LiP) (EC 1.11.1.14), and laccase (EC 1.10.3.2), and used for fruit juice clarification purposes. From the turbidity viewpoint, a significant reduction was recorded for apple, grape, orange, and pomegranate juice when treated with free and immobilized cocktail (Fig. 6). Pectinases, a class of versatile enzyme with worldwide interest as a biological catalyst, depolymerize pectic substances by de-esterification (esterases) and depolymerization (hydrolases and lyases) reactions for attaining stability and clarification (Kohli and Gupta 2015). Use of these enzymes in fruit juice clarification is a prerequisite because they reduced cloudiness, bitterness, and viscosity of fruit juices, as well as augment pulp pressability and destabilize jelly-like pectin (Amin et al. 2017c). Reports have revealed the application of pectinase treatment in different fruits including apple, blackberry, raspberry, grape, orange, and strawberry, for increased chromaticity and stability of fruit juice (Kohli and Gupta 2015). Similarly, cellulases exhibit the remarkable capability to degrade/depolymerize cellulosic-based materials for converting into valuable bioproducts using some suitable microbial strains (Sharma et al. 2016). Tannases are another a very important class of enzymes with the potential to be used in diverse food industries. A range of microorganisms, such as *Aspergillus*, *Bacillus, Lactobacillus*, and *Paecilomyces* can produce tannases with functionalities in wide-ranging pH and temperatures conditions (Yao et al. 2014). The reduction in fruit juice bitterness by treating with tannases displays the advantages of the high fruit juice quality due to the lower haze and non-deteriorated effects (Beniwal et al. 2013). A high level of tannin in new fruit juices including raspberry, pomegranate, cranberry, and iced tea causes sediment formation, as well as associated with color, astringency, and bitterness of these fruit juices during storage. In this context, the inability tannases have been proved as promising candidates to effectively eliminate the bitterness of the fruit juices than that to traditional debittering approaches. It is demonstrated that tannase-treated high concentrations of fruit juices and black tea can be preserved for longer periods without showing any precipitation and clouding, thus demonstrating superior quality (Beniwal et al. 2013). Rout and Banerjee (2006) reported that tannase treatments caused 25% tannin degradation, whereas, the equal combination of gelatin and

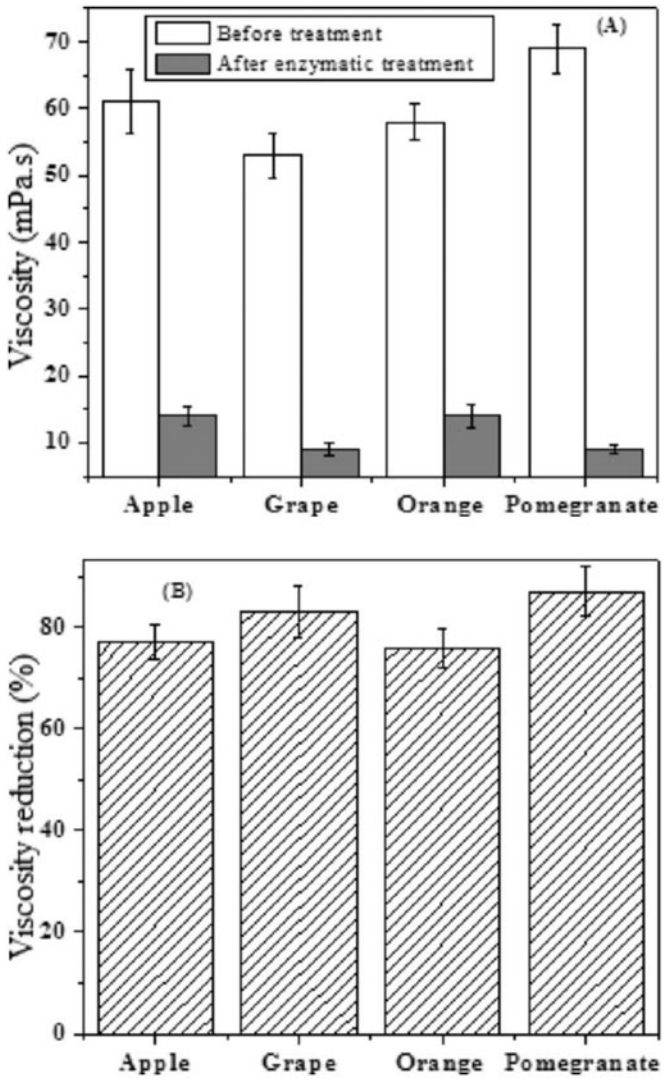

Figure 6. Viscosity (A) and viscosity reductions (B) in different fruit juices treated by alginate-chitosan immobilized ligninolytic enzymes. Reprinted from Bilal, Asgher, Iqbal, et al. (2017), with permission from Elsevier. Copyright (2017) Elsevier Ltd.

tannase led to 49% depolymerization of tannin, in pomegranate juice. In rennet years, the development of fruit juice processing industry has strongly associated with the enzyme, where enzymes are regarded as indispensable tools in terms of quality enhancement and cost savings. The hemicellulose hydrolyzing enzymes, hemicellulases, showed best working performance along with pectinase combination for clarification of juices. These enzymes also improve color and brightness properties (Martín-Sampedro et al. 2012).

Food enzymes—brewing industry

Among key enzymes involved in the brewing industry, beta-glucanase, protease, and amylases (both alpha and beta) are some examples (Johnson 2013). The brewing industry implicates some batch-type fermentation operations in processing the raw feedstock materials. In the Brewing industry, the use of enzymes is generally categorized into four processes, i.e. (1) germination, (2) mashing, (3) fermentation, and (4) clarification. Each of these processes requires different

working pH and temperature conditions. Enzymes either can be within the system or commercially acquired. Sometimes, within the processing system, the enzyme level went low when barley mashing does not liberate enough enzymes (Beta-glucanase endogenously found in barley are referred to as endo-β1,3-1,4-glucanases) which are required for starch hydrolysis. Overall, this limit or lead to the low-quality beer with less yield. Thus, to overcome these drawbacks, commercially available enzymes are incorporated in the processing system to induce attributes such as clarification, color, texture, or flavor. In the beer brewing process, the hydrogenation of 1-3 β-glycosidic bonds between glucose molecules in glucans in the presence of beta-glucanase lowers the viscosity of warts.

In the malting process, amylases, both α- and β-amylases, are mainly used to break down starch into dextrins, oligosaccharides, maltose, and glucose molecules as part of the malting process. Following beta-glucanase and xylanases assisted hydrolysis, both α- and β-amylases are liberated and hydrolase starch solution within wort during mashing phase into simple sugars (Guerra et al. 2009; Sammartino 2015). Alpha-amylase is used in light beers after malting and mashing to increase the carbohydrate yield for fermentation. High sugar in wort leads to the high alcohol content in beer and vice versa (Guerra et al. 2009). The alpha and beta amylase have different pH and temperature optima values (Sammartino 2015). Proteases are mainly used in brewing for protein digestion in the clarification and facilitation of malting (Hui and Sherkat 2005). These enzymes primarily decrease beer viscosity by lowering proteins solubility and create favorable condition for yeast growth by providing an adequate presence of amino acids, which is a vital factor for yeast growth (Hui and Sherkat 2005; Lei, Zhao, and Zhao 2013). Proteases hydrolyze the cell wall during mashing, thus softening the kernel and exposing the starch for wort fermentability. The working temperature condition for proteases is pH 10 and 52 °C, and this enzyme is denatured at the higher temperature of 70–75 °C, that necessitate the close monitoring while operating protease enzyme (Sarker et al. 2013; Sammartino 2015). With the rapid advances in modern biotechnology, some other enzymes are also being used in the beer manufacture process to enhance its quality and facilitate proper storage and transportation.

Food enzymes—dairy industry

Enzymes have gained a special place as the most pliable biocatalysts in the dairy industry for food processing purposes. Several types of enzymes such as lipase, protease, esterase, catalase, and lactase played a crucial role in the bioconversion reactions such as hydrolysis, aminolysis, esterification and interesterification, acidolysis, and alcoholysis, etc. More specifically, at the early stage of the cheese production process using raw milk, a mixture comprised of chymosin and pepsin (collectively known as rennet) is used for milk protein coagulation. Additionally, this also enhances/induce other cheese-related processes such as accelerated cheese ripening, modification or imparting functional properties, and structural and functional alteration in the milk proteins. This process of cheese production using raw milk as the main source is schematically shown in Fig. 7 (Kamal, Rehman, and Iqbal 2017). Moreover, in the cheese ripening process, lipases are mostly used to develop a lipolytic flavor (Ray 2012). Sometimes, bitter peptides appear in the ripened cheese as it matures, which attributed to the formation of unpleasant flavored peptides from milk proteins. Cleavage of these bitter flavored peptides by peptidases-assisted hydrolysis can help to limit the bitter taste and maintain the traditional cheese flavor (Budak et al. 2018; Maitan-Alfenas and Casarotti 2018). Various kinds of proteolytic enzymes are used to limit or lower the allergenic characteristics of milk-based products for infants/babies (Kolok et al. 2018). Microbial lactases or β-galactosidases hydrolyze lactose into galactose and glucose, enabling products more digestible which can be used by lactose intolerant individuals.

Lipases and phospholipases represent versatile biocatalysts with great capability to degrade ester bonds of triglycerides and phospholipids, respectively. Lipases also carry out esterification, transesterification and interesterification activities, whereas acyltransferase, transacylase, and transphosphatidylation reactions are catalyzed by phospholipases (Ozturkoglu-budak et al. 2016; Rehman, Bhatti, et al. 2017; Rehman, Wang, et al. 2017). In spite of their ubiquity (i.e. bacteria, fungi, yeasts, animals, and plants), microbial lipases and phospholipases are preferred due to abundant availability and easier biosynthesis (Choudhury and Bhunia 2017; Guerrand 2017). Lipases are applied in the cheese industry for producing and ripening of cheese (Law 2009). Cured or ripened cheese is not readily consumable after manufacture and held for some time at temperature, and conditions resulting in necessary physical and biochemical changes. Particularly, many mold-ripened cheeses are characterized by extensive lipolysis. Such verities of cheeses often have pronounced flavor and aroma from both proteolysis and lipolysis (Budak et al. 2018).

Lactases (EC 3.2.1.23) are specific to hydrolyze lactose into glucose and galactose, thus improve the solubility and sweetness of a variety of dairy products. Many people lack an adequate level of lactase to consume milk sugar (Pereira et al. 2018), so lactose hydrolysis helps these lactose-intolerant people to eat and drink various dairy products. Among a large quantity of whey generated as a byproduct by the cheese manufacturing industry, merely lactose represents 70–75% of the whey solids. This huge amount of lactose can be hydrolyzed by lactase into an array of high-value food ingredients. Also, these enzymes have also been utilized in the bioprocessing of dairy wastes (Erich et al. 2015). As a substitute to pasteurization with heat, H_2O_2 acts as a stable and effective chemical sterilant. Catalase (EC 1.11.1.6), an efficient H_2O_2 decomposer, is used to eliminate the traces of residual peroxide (Sîrbu, 2011). In addition to the major role of all the enzymes mentioned above, a list of other enzymes including glucose oxidase, sulfhydryl oxidase, superoxide dismutase, lysozymes, and lactoperoxidase are also associated with dairy processing industry but with restricted applications (Patel, Singhania, and Pandey 2016).

Figure 7. Schematic representation of cheese production using milk as a raw material. Reprinted from Kamal, Rehman, and Iqbal (2017), with permission John Wiley and Sons. Copyright (2016) American Institute of Chemical Engineers Environ Prog.

Food enzymes—animal feed (poultry industry)

Poultry-based products such as eggs and poultry meat are considered an important protein source in the diet. Since the 1980s, the broiler production, and consumption have profoundly increased. This high consumption hiked the poultry industry, which supplies processed products easier to prepare for the customers (Martínez-Alvarez, Chamorro, and Brenes 2015). Phytase is one of the most readily acknowledged enzymes by monogastric nutritionists. However, to date, it constitutes not more than 20% of the usage of the entire commercial enzymes. At present, only a few numbers of microorganisms, *Aspergillus sp*. being the leading source, commercially produces this enzyme. Whereas, it can also be produced by numerous species of bacteria, fungi, yeast, and plants. These sources are believed to become the commonest and cheapest methods of phytase manufacture that suffer from source-to-source variations (Bedford and Schulze 1998). Phytic acid, a substrate for phytase, is challenging to the animal because it readily binds amino acids and minerals, and thus becoming unavailable to the animal (Selle and Ravindran 2007; Shah et al. 2015; Habib et al. 2018). This results in excretion of useful nutrients into the environment, leading to a significant loss in performance. For more than a decade, phytases have been supplemented to monogastric diets to reducing the excretion of phosphorus, and increasing cost savings of diet (Ashraf, Rahman, and Abdullah 2018).

Carbohydrase (e.g. amylases, glucanases, and xylanases) have found a prominent place in feed industry by catalyzing the degradation of carbohydrates (e.g. starchy, and non-starchy polysaccharides, and fiber) into simple sugars which in turns used an efficient and readily available energy source (Ashraf, Rahman, and Abdullah 2018). Xylanase, one of the most commonly used carbohydrases, splits the arabinoxylans structure of wheat or corn, enabling their components to be easily absorbed by the animal as energy sources. This reduces the inevitable supplemental fat or energy requirement in the final diet (Van Dorn, Shanahan, and Ciofalo 2018). Proteases cleave anti-nutritional factors associated with various kinds of animal or vegetable proteins. In this way, these enzymes help to liberate beneficial nutrients by increasing proteins digestibility and improving the availability of amino acid (Hanbury et al. 2000). Proteases are important to aid to manage the nutritional risks associated with feedstuff quality and thus allow to utilize all accessible feed constituents. Animals consuming a traditional corn-soybean meal diet are unable to utilize the complete protein fraction, and supplementing proteases in this diet enhances amino acid digestibility and thereby animal performance.

The poultry products, such as dried egg albumen and/or dried egg white, important raw materials for the food industry, are susceptible to undesirable browning, so-called "Maillard reaction", which involves reducing sugars (such as glucose) and functional amino groups. Such undesirable reactions are considered a major cause of either discoloration or off-flavor development with time in egg albumen (Quan and Benjakul 2019a). Additionally, the reaction also adversely influences the protein solubility (Wu 2014). The enzymatic desugarization is considered a potent solution to tackle this issue effectively. In this context, Sisak et al. (2006) reported the elimination of glucose in egg white using immobilized glucose oxidase. For a said purpose, a commercial glucose oxidase containing catalase (NOVOZYM 771) was used to immobilize on Amberlite UP 900 resin using glutaraldehyde as a cross-linking agent. As developed immobilized catalyst was then employed to remove glucose from egg white in a pilot-scale three-stage fluidized-bed bioreactor with mechanical mixing, equipped with large porous surface for bubble-free transport of oxygen as co-substrate (Sisak et al. 2006). Glucose oxidase containing catalase converts glucose into gluconic acid (Woods and Swinton 1995). Further to this, glucose oxidase catalyzes the oxidation of β-$_{D}$-glucose by oxygen to $_{D}$-glucono-1,5-lactone. Earlier, Quan and Benjakul (2019b) reported impacts of desugarization and drying methods on physicochemical and functional properties of duck albumen powder. The response surface methodology (RSM) was used to investigate the optimum desugarization conditions, which were found to be: glucose oxidase (31.24 units/mL), catalase (781 units/mL) and an incubation time of 6.55 h at 30 °C. Prior desugarization could lower the browning issues (Quan and Benjakul 2019b).

Engineering strategies for widening the scope of natural enzymes

It is not surprising that most of the enzymes in their original form do not catalyze reaction efficiently under harsh industrial milieus. Moreover, the free enzyme forms suffer from a low level of activity, selectivity, specificity, stability, shelf-life, and volumetric outputs. In this context, biocatalytic, biophysical, and physiochemical attributes of natural enzymes, including catalytic power, stability, and substrate specificity, should be improved to widen their industrial scope further (Bilal, Asgher, Parra-Saldivar, et al. 2017; Bilal, Iqbal, et al. 2018; Bilal and Iqbal 2019b; Bilal, Adeel, et al. 2019). In particular, biocatalytic stability against heat and organic solvents is contemplated an extremely critical aspect due to the harsh industrial environment. Thermal stability is often related to enzyme resistance against various organic solvents and destabilizing agents, and the improvement in thermal stability is a prerequisite for industrial applicability. Aiming to overcome these significant limitations, several engineering strategies have been developed and reported for widening the catalytic scope of natural enzymes (Fig. 8) (Bilal, Iqbal, et al. 2018; Bilal and Iqbal, 2019c; Bilal and Iqbal, 2019d; Bilal, Asgher, et al. 2019). Following robust strategies (discussed in the following subsections), enzyme-based biocatalysts are being re-engineered to achieve a high level of catalytic efficacy to deliver optimal productivity with an overall high cost-effective ratio (Bilal, Iqbal, et al. 2018; Bilal and Iqbal, 2019d; Bilal, Cui, et al. 2019).

Figure 8. State-of-the-art strategies for widening the scope of enzymes.

Genetic engineering—tapping for improved biocatalysts

The application of genetic engineering or recombinant DNA technology has led to a significant improvement in the diverse microbial strains, as well as, the biocatalysts to fulfill the needs of the food processing industry, i.e. enrichment of food flavors, colors and other enhancements in food properties (Kapoor Rafiq, and Sharma 2017). A wide variety of food processing enzymes has been engineered/tailored by this revolutionary technology to develop industrial biocatalysts with explicit specificity and sensitivity accompanied by the reduction of overall production cost. The development of recombinant strains improved the enzyme titers as well as widened the scope of these enzyme applications in food technology. Effective implementation of genetically improved strains can substantially reduce the requirement for labor-intensive and expensive extraction and purification processes, respectively (Rastogi and Bhatia 2019). The recombinant strains are utilized in the manufacture of food processing enzymes and ingredients such as amino acids, monosodium glutamate, and polyunsaturated fatty acids. Bovine chymosin was the first commercial recombinant enzyme approved by the US Food and Drug Administration (Flamm 1991). Enzymes associated with starch degradation such as α-amylases and pullulanases are in essence produced using engineered strains. The genetically modified α-amylase are heat-stable with great capability to produce high-fructose corn sirups (Olempska-Beer et al. 2006). The phospholipase A1 enzyme produced by expressing a phospholipase A1 gene from *Fusarium venenatum* in GM *Aspergillus oryzae* is

used in the dairy industry to boost process efficacies and cheese yields (Olempska-Beer et al. 2006). Nevertheless, the use of these modified strains is tempered in some food technologies, due to the lack of safe status for consumption, incongruity with production/purification techniques, and increasing public awareness regarding the non-consumption of genetically modified microbial strains or associated products (Olempska-Beer et al. 2006; Agarwal and Sahu 2014).

Protein engineering—designing tailor-made biocatalysts

Protein engineering strategies entail the designing and development of tailor-made biocatalysts with multifunctionalities (Fig. 9) (Bilal, Iqbal, et al. 2018; Bilal, Zhao, et al. 2019). Rational design by site-directed mutagenesis involves the point mutations. Under such reaction, any of the standard amino acids substitute a particular amino acid at a specific location. However, a rational design strategy necessitates prior information of the amino acid sequence, three-dimensional structure, and function of the target proteins (Kapoor, Rafiq, and Sharma 2017), to introduce the desired traits. On the other hand, these qualities can be achieved by constructing mutant enzymes libraries and screening desired attributes using directed evolution (molecular or in-vitro evolution). In directed evolution approach, genome mutations are randomly introduced by error-prone PCR, DNA shuffling, or chemical mediation methods without prior information of the protein, and the screening/selection of resulting target mutant with improved properties is carried out based on the desired trait (Packer and Liu 2015). This procedure is reiterated for several rounds until the achievement of desired modifications with genetic diversity. Protein engineering strategies have been widely employed to improving the catalytic properties of industrially pertinent enzymes including, thermal tolerance/thermo-stability, substrate preference, catalytic efficiency, and the development of novel enzyme with previously non-existed activity (Garcia-Ruiz et al. 2012; Zhou, Xue, and Ma 2015; Bilal and Iqbal 2019d; Bilal, Zhao, et al. 2019). Among these parameters, the thermal stability of the enzyme is one of the most important and best-studied aspects from economic and as well as engineering perspective. Majority of the enzymes tend to be inactive at higher temperatures and are no longer capable of carrying out the desired tasks. Rational design and directed evolution approaches have shown great promise in improving the thermal stability of the enzymes (Bilal and Iqbal 2019d; Bilal, Cui, et al. 2019). Lin et al. (2008) created amylase mutants from *Bacillus* sp. *strain TS-23* by adopting a rational design approach for improving the thermostability of the enzyme. By site-specific mutagenesis strategy, the thermal stability of xylanase recovered from *Trichoderma* sp. was increased about 2000-times at 70 °C. Also, improvement in half-life from 60 s to 4 min was attributed to the stabilization (Fenel et al. 2004). Similarly, a range of food biocatalysts, including α-amylases, glucoamylases, cellulases, lipases, and proteinases, has also been engineered following the directed evolution strategy (Singhania, Patel, and Pandey 2010).

Immobilization approaches—engineering stabilized and reusable biocatalysts

Immobilization of enzymes has numerous merits, such as hyperactivation, enhance enzyme stability via multipoint or

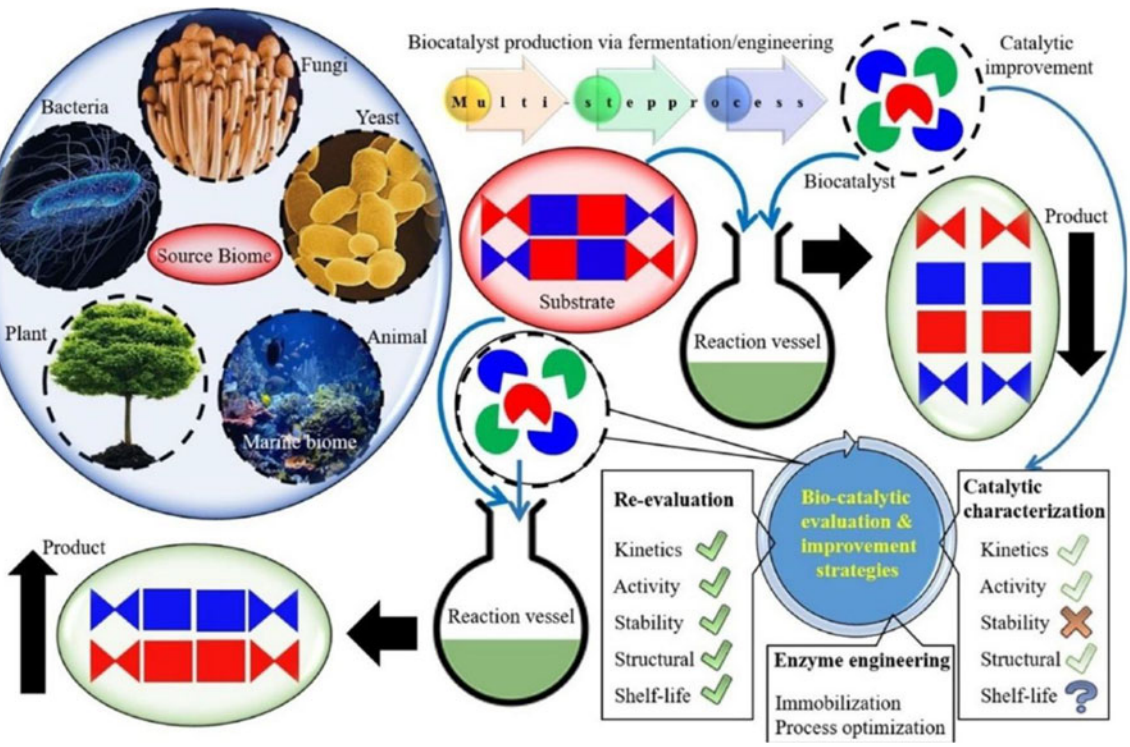

Figure 9. Biocatalytic evaluation and improvement strategies. A complete overview from biocatalyst (enzyme) production via fermentation and/or engineering to catalytic pathway. The low substrate conversion can be significantly induced following enzyme engineering.
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multisubunit immobilization, may improve activity, specificity or selectivity, enhanced thermal and operational stability, and storage, etc. All these features facilitate their employment at the adverse and inconsistent industrial environment (Fig. 10) (Bilal, Asgher, et al. 2019). Moreover, immobilization simplifies the downstream processing of product separation and purification from the biocatalyst. Immobilized biocatalysts can be easily recovered from the complex reaction media, and reprocessed, resulting in improved productivity along with least substrate inhibition (Sheldon 2007; Rehman et al. 2016; Bilal, Asgher, Parra-Saldivar, et al. 2017; Bilal, Rasheed, et al. 2018). Potential applications of immobilized biocatalysts-based bioreactors routinely employed in food processing industries (Nakakuki 2003; Walsh 2007; Fernandes 2010; Rastogi and Bhatia 2019). Some typical examples are given below:

1. Manufacture of high-fructose corn sirup by immobilized glucose isomerase
2. Biosynthesis of amino acids by aminoacylase
3. Production of whey hydrolysates and tagatose by lactase
4. Manufacture of inverted sugar sirup by invertase
5. Interesterification of edible oils to produce trans-free oils, cocoa butter equivalents by lipases
6. Synthesis of fructooligosaccharides by β-fructofuranosidase
7. Production of isomaltulose by isomaltulose synthase

Selection of immobilization technique and supporting materials is critical to developing reusable, stable, and durable immobilized biocatalytic systems. Therefore, the supporting material should be judiciously considered for immobilization (Bilal, Zhao, et al. 2018; Bilal and Iqbal 2019c; Bilal, Adeel, et al. 2019). For example, lipases are demonstrated to be hyper-activated upon immobilization onto hydrophobic materials (Manoel et al. 2015), whereas, β-galactosidases lost their catalytic activities upon immobilization on hydrophobic materials (Wong et al. 2014). Hydrogel-based beads/microspheres exhibited great promise for activity retention of β-galactosidase, but problems arise with the migration of the biocatalyst from the immobilization matrix (Zhang et al. 2016). Another important influence of enzyme immobilization is the potential to shift the optimal working conditions of the enzyme. It is revealed that the local pH environment surrounding enzymes can be manipulated by immobilization/encapsulation in various polymeric networks (with cationic and anionic materials shifting pH optima more acidic and alkaline (Wentworth et al. 2004; Bilal, Cui, et al. 2019), respectively. Introduction of stabilizers or stabilizing groups, i.e. polyethylene glycol within a support carrier can also affect enzyme activity retention (Iyer and Ananthanarayan 2008). Enzyme immobilization to a support material can be categorized as covalent bonding, adsorption, entrapment, and crosslinking. Multipoint covalent bonding, where the enzyme molecule is associated with the functionalized carrier support through different amino acid residues, can attain high biomolecules stabilization. Effective covalent binding is accomplished via glutaraldehyde, genipin, epichlorohydrin, or glyoxyl-containing compounds that results in exceptional stabilization and repeated usability (Bernal, Rodríguez, and Martínez 2018). Further, the amalgamation of adsorption and covalent bonding has appeared as a noteworthy approach to control enzyme orientation and promoting enzyme-support interactions during the immobilization process (Mateo et al. 2007). Similarly, the co-localized immobilization of biocatalytic cascades comprising two or more types of enzymes exhibit short diffusional distances and consequently accelerate the rate of the reaction leading to the enhanced catalytic performance of the cascade than their soluble counterparts

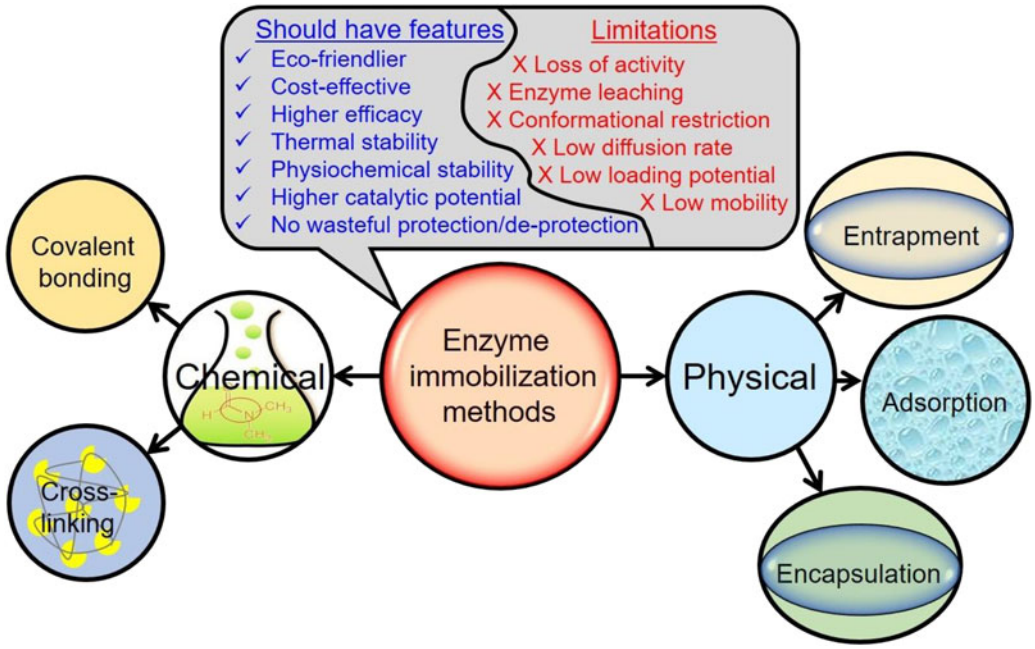

Figure 10. Physical and chemical based enzyme immobilization methods along with considerable limitations and potentialities. Reprinted from Bilal, Asgher, et al. (2019), with permission from Taylor & Francis. Copyright (2018) Informa UK Limited, trading as Taylor & Francis Group.

(You, Myung, and Zhang 2012). Despite the proven potential of immobilization, however, some intrinsic drawbacks have been identified that vary from various immobilization strategies to various categories of biocatalysts used. Immobilization methods result in loss of enzyme activity presumably due to restricted mass transfer, steric hindering, and possibilities of enzyme leakage from the support material during the operation. Nevertheless, considering the economic viewpoints, the immobilization of the expensive catalysts renders the overall process cost-efficient (Fernandes 2010; Adeel et al. 2018).

Computational design for biocatalyst engineering

Computational-based tools and modeling increasingly contribute to improving the structure-function relationship and engineering important properties of the enzymes, such as activity, substrate specificity, and stability for expanding their applications. Two major approaches are (1) de novo designing and (2) redesigning. De novo designing is a promising approach, where novel proteins can be designed, and bioinformatics tools can help to deduce their functions (Huang, Boyken, and Baker 2016; Khan et al. 2018). Whereas, the catalytic activity and functional dynamics of the natural enzymes are redesigned in the second approach (Penning and Jez 2001). The resulting designed biocatalysts can carry out non-native catalytic reactions, including retro-aldol conversion, proton transfer, and Diels-Alder cycloaddition (Kries, Blomberg, and Hilvert 2013; Hilvert 2013). Adopting computational-guided protein engineering approach, Gordon et al. (2012) redesigned the specificity of endopeptidase kumamolisin-As using Rosetta Software Suite based computational modeling. The experimental analysis revealed the improved proteolytic activity of 50% of the resulting variants toward the new substrate PQLP. The variants having seven substitutions displayed 116-folds greater activity and 877-folds shift in the specificity towards the substrate in contrast to the original enzyme. Likewise, Joo et al. (2010) used a computational design FRODA to select the flexible residues on the protein surface to improve the thermal stability of xylanase Bcx from *Bacillus circulans*. Among the eight substitutions predicted by RosettaDesign algorithm (Liu and Kuhlman 2006), three substitutions were experimentally corroborated to evaluate thermal stability.

Concluding remarks

In conclusion, the use of efficient and robust enzyme-based biocatalytic systems represent a notworhy approch to valourize various food processing strategies and augmenting economic and environmental sustainability of food production. Comparative to this, food processing using conventional methods can affect the overall quality of the final product by limiting or reducing the available ingredients of the raw materials. These facts certainly demand an advanced mechanism for processing products without deteriorating their ideal quality. Owing to high specificity, elevated yield, maintenance of the nutritional property, easy treatment, process control and safety, sustainable and eco-friendly nature, application of enzymes or enzyme-based processes has recently gained a broader interest in the food biotechnology industries. The data reviewed above suggest that the enzyme-mediated processes have a great impact on controlling the characteristics such as (1) physiochemical properties, (2) rheological functionalities, (3) facile process as compared to the chemical-based processing, (4) no or minimal consumption of harsh chemicals, (5) overall cost-effective ratio, (6) sensory and flavor qualities, and (7) intensifying the stability, shelf life and overall quality of the product, etc. in the food industry. However, intensive care, expertise, and ingenuity are required to acclimatize biocatalysts in diverse industrial bioprocesses. Consistent research activities are of great significance to make the application of the enzyme more promising and diversified in food biotechnology. From the industrial perspective, special emphasis should be given on screening for high enzyme producers along with designing a short protocol for enzyme extraction for commercial-scale implementations. However, significant challenges remain with their larger-scale applicability requiring additional comprehensive research efforts to overcome these obstacles. In addition to differential behaviors of numerous enzymes upon immobilization, regardless of the immobilization type and supporting matrices, the cost is another significant hurdle in adopting immobilized biocatalytic systems at industrial level. The deployment of purified enzyme forms instead of whole-cell or crude extract additionally raises the overall cost of biocatalysis process. Moreover, a proper selection and exploitation of ideal immobilization method combined with various operational requirements is another big concern for food stream valorization. Besides the exploitation of low-cost support materials, immobilized biocatalyst must preserve activity over multiple successive cycles to diminish the overall cost per use. Thus, the use of advanced tools along with a rigorous experimental characterization of immobilized enzyme systems against activity loss, stability retention under harsh industrial reactions, and leaching are essential to reveal their true performance in an industrial application.

Future perspective and directed trends

Undoubtedly, in the above-discussed food industries, the enzyme-based processes have driven a new way and significantly contributed to revising the, in practice, traditional processes. The use of enzymes as an environmental friendliness processing aid or additive in the food industry have also strengthened the process simplification, enhanced the overall competitiveness, improved the product novelty. The incorporation of the biocatalyst is a well-established approach. However, there remains plenty of scopes to improve the overall enzyme traits with multiprocessing functionalities further. The scientific and industrial research community is devoting consistent efforts to make enzymes more robust, effective, and diversified, and withstanding enzyme structure-function activities under variable and aggressive environmental conditions (i.e. pH, temperature,

organic solvents, and inhibitory compounds) of the bioreactor. Recent developments in biotechnological tools such as genetic manipulations, protein engineering, immobilization, and computational modeling have profoundly extended the scope of the enzyme in myriads of food and other industries. Multidisciplinary studies associated with enzymes should be carried out to establish a new process, or upgrade available process. There is also a dire need to engineer or redesign enzymes with synergistic functional properties. Also, the development of newer strategies is needed for cheaper and easier production of these enzymes to accomplish the ever-increasing demands for fruit/vegetable processing markets. Additional, comprehensive research is necessary to optimize operating parameters for the pilot plant as well as commercial scale applications. To achieve all these directed trends, the physiochemical, structural, rheological, and functional attributes of food components and their enzyme-based catalysis and hydrolysis into the product of interest are to be well studied. The future studies should also be based on multi-enzymatic biocatalytic systems instead single-enzyme processes, which could be promising for effectively catalyzing value-added bioconversion processes. Additionally, the meticulous implementation of multi-enzymatic biocatalytic systems will update the essential apprehension of the immobilized enzyme systems for real-time practical applications. In this perspective, rational design approaches and engineered multi-enzymes are believed to have a profound potential to improve food sustainability by producing an array of valuable products of industrial interests. Given careful consideration, the food sector will have an excellent means in developing value-added food products with high health-value.

Acknowledgments

Authors thankfully acknowledge the literature services provided by their representative universities/institutes.

Disclosure statement

The authors declare no conflicting interest in any capacity.

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