Food Production, Processing and Nutrition

REVIEW

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Microbial enzymes and major applications in the food industry: a concise review

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Abstract

The use of enzymes in the production of food products is an ancient practice. Microbes provide several enzymes that are involved in improving the taste, texture, as well as aroma of food items, offering several benefits to the food industry. Subsequently, the ease of availability of these microbial enzymes has increased their utilization in the food industry. This cost-effectiveness and ease of commercial-scale production make enzymes ideal tools for various industrial uses. Microbial enzymes are utilized in processing food products such as those associated with the brewery, dairy and bakery industries. In addition, the nutritional value, color, aroma and texture of food products can be improved by using microbial enzymes. With the progress in technology, several novel enzymes in various applications of the food and beverages industry have been developed and demand is constantly increasing. The present review provides a comparative narrative of the applications of some of the predominating enzymes, such as phytases, lipases, lactases, pectinases, and laccases, commonly used as processing aids in the food industry.

Keywords Eco-friendly, Food products, Food processing, Food quality, Shelf life

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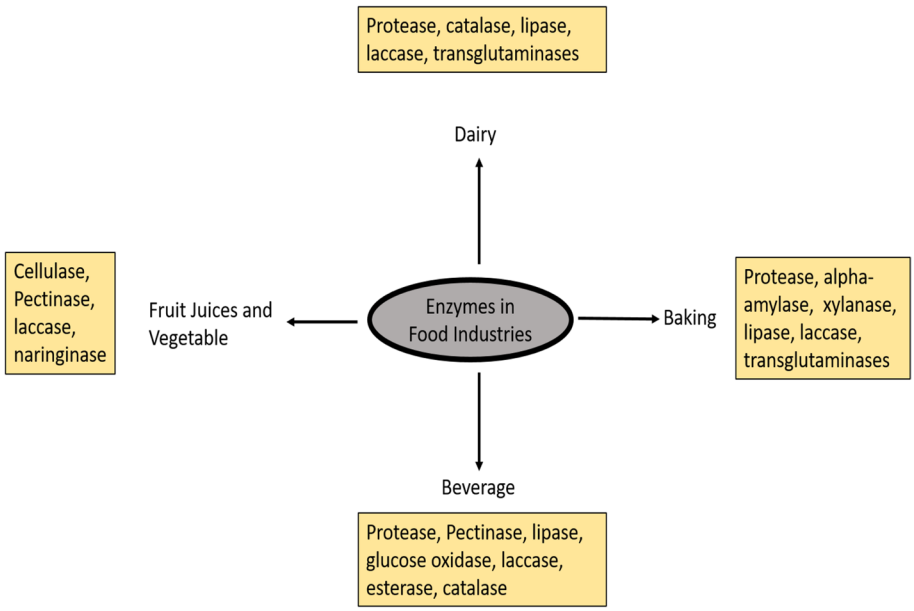
Graphical Abstract



Introduction

Enzymes are natural catalysts that facilitate the majority of chemical reactions in biological systems by enhancing the rate of reactions. Enzymes have been used to enhance the shelf-life of foods and beverages since ancient times. Still, their demand is constantly increasing in the food industry. The role of enzymes as catalysts was recognized in the 19th and early twentieth centuries. Initially, the role of yeast extract in the conversion of glucose to ethanol was discovered by Eduard Buchner in 1897. Later on, it was found that the enzyme present in yeast extract was responsible for the conversion. Firstly, James B. Sumner isolated and crystallized the pure enzyme, urease, and won the Nobel Prize (1946) for the same (Hau et al., 2018). In the food industry, enzymes are utilized to increase production and also improve texture as well as aroma, color, and flavor (Sharma et al., 2021). Also, the enzymes are utilized to provide raw materials such as dough in the baking for the production of standard bakery products of high quality. In the beverage industry, enzymes have been used to produce high-quality beer and wines by improving the brewing process (Kuddus, 2018). In addition to these, the food industry also involves the use of enzymes in meat and processing as well as the production of high-quality nutritious feed for cattle (Trono, 2019). The global industrial market for enzymes is estimated at USD 6.95 billion in 2022 and is expected to increase at a compound annual growth rate of 6.4% from 2023 to 2050. It is estimated that an increase in the consumption of bakery products and juice will further add to this demand for enzymes in the industry (Size, 2022).

Globally, certain microorganisms such as yeasts, fungi, and bacteria have been investigated for the biosynthesis of economically feasible formulations of different industrial enzymes (Nigam, 2013). Enzymes can hydrolyse complicated compounds into simple monomer fragments, such as carbs, into simple sugars, which are organic compounds that regulate various biological activities. Each enzyme is specific towards certain conditions, whether, it could be substrate, pH, and temperature specific for catalysing the reaction, thereby, transforming a reactant into a stable end product. Enzymes, unlike inorganic catalysts, are very selective, catalysing a particular substrate change or breaking a limited set of closely related molecules or a specific link. In large-volume processes, this reduces byproduct generation (Al-Maqtari et al., 2019). Processed foods' nutritional value and flavour are being improved using enzymes through food processing methods. At the commercial level, enzymes are successfully used in the food processing sector to make syrup, yogurt, wine and beer, leavened bread, and cheese (Singh & Kumar, 2019). The utilization of enzymes in several industries is attracting various researchers due to their eco-friendly nature and especially in response to consumer demands for reduced

use of chemicals in processed foods. Microbial sources offer a great choice to fulfil the increased demand of enzymes and their application in food industries. This is mainly because microbes are readily available, generate and reproduce at faster rates compared to animals and could be modified genetically to enhance catalytic efficiency that suits several conditions in an industrial environment. Enormous applications of microbial enzymes are reported in different disciplines such as pharmaceuticals, agriculture, textiles, leather, paper, pulp detergent, waste management, and food processing, emphasizing their need as essential tools in various industrial productions (Okpara, 2022). Several enzymes are used to depolymerize natural substances because of their hydrolytic action. The most dominating enzymes are the proteases, which have found extended uses in the dairy and detergent industries. Other important enzymes are carbohydrates, primarily amylases and cellulases, used in baking and the textile industries (Kirk et al., 2002; Ozatay, 2020). Gene modification is possible with the advancements in modern biotechnology which is very important for the production of novel enzymes. Recombinant DNA technology introduces a veritable approach to manipulating their structure and function, especially for commercial utilization (Srivastava et al., 2019). Microbial enzymes have garnered significant attention in the food industry due to their diverse functionalities and applications. This review paper provides a comprehensive overview of the role of microbial enzymes in food processing, highlighting their significance in enhancing food quality, flavor, nutritional value, and sustainability. This paper not only consolidates existing knowledge but also identifies knowledge gaps, facilitates decision-making, promotes innovation, and disseminates valuable insights into the field of microbial enzymes and their diversified applications in the food industry. This paper aims to elucidate the pivotal role of microbial enzymes in shaping the future of the food industry through a detailed exploration of various enzyme types, sources, mechanisms of action, and industrial applications. The paper also discusses key challenges, such as regulatory constraints, enzyme stability, substrate specificity, and consumer acceptance. Furthermore, future perspectives on the use of microbial enzymes, including emerging trends and technological advancements, are explored.

The demand for microbial enzymes in various industries

Advances in enzyme research suggest that industrial wastes can be converted into non-toxic, biodegradable products that are environmentally friendly. Only 5% of enzymes out of over 3000 are currently exploited as processing tools in various industries (Patel et al., 2023). This percentage can be enhanced with the help of modification of microbes through genetic engineering so that microbes can be improved to produce more stable enzyme catalysts on an industrial scale, which could enable the utilization of unexploited sources (Gurung et al., 2013). Certain harsh and non-favourable production conditions in industries for the performance of enzymes are one of the major drawbacks in utilizing enzymes, especially since these biological catalysts are proteins that could lose activity if denaturation occurs. For example, the presence of inhibitory compounds in the processing matrix and extreme temperature and pressure conditions could negatively affect enzyme action (Littlechild, 2015; Singh & Bajaj, 2016). Several animal and plant sources derived enzymes, in the absence of inhibitors, can perform catalysis optimally at 37 °C. Microbes as a source are utilized for enzyme production to overcome this issue and can be preferred in comparison to plant and animal sources for the production of industrial enzymes. The microbes are easily cultured in cheap growth media, their growth rates are higher, and the enzyme matrix is less complex when compared to plants and animals. Microbial enzymes are economically beneficial as they are easy to extract, consistent in different batches, easy to modify and production includes less variables in the production than from plant and animal sources. Microbial enzymes are more stable as compared to their counterparts in plants and animals. In addition, they provide more catalytic diversity for industrial applications (Sabu, 2003). Microbes provide several enzymes that are involved in improving the taste, texture, as well as aroma of food items, offering several benefits to the food industry. Nevertheless, there is a significant requirement for enzymes that exhibit optimal performance in challenging industrial environments across diverse sectors. These enzymes are sought after for their ability to facilitate the production of goods under extreme processing conditions, including high temperatures, varied pH levels, and elevated pressure (Okpara, 2022).

Techniques employed for enzyme purification

Enzyme purification represents a crucial step in the industrial application of enzymes. The enzyme mixture obtained after microbial fermentation to produce a specific enzyme often contains impurities and solvents that can impact enzyme kinetics, compromising the quality and final yield of the enzyme. Purified enzymes, however, exhibit increased stability and higher specificity towards their substrate. One of the simplest methods for enzyme purification, applicable under various conditions, is precipitation. This involves the addition of an organic solvent or salt, leading to the precipitation of the enzyme—a technique commonly referred to as salting out. The resulting precipitate can be collected through

centrifugation or filtration (Larsson & Mosbach, 1979). Another widely used technique for enzyme purification is chromatography. This method involves the separation of a protein mixture through a column containing a polar stationary phase and a non-polar mobile phase to homogeneity. Various chromatography techniques can be employed based on the size, charge, and affinity of the enzyme. The choice of column type is tailored to the specific requirements of the purification process (Rodriguez et al., 2020). In the food industry, electrophoresis is another technique employed by industries for enzyme extraction. This method involves the separation of proteins based on their charge and size through an electric field (Cermeño et al., 2020). Other techniques such as ultrafiltration has been employed for the separation of enzymes such as lipases, pectinases and amylase (Li et al., 2021; Perreault et al., 2021; Sharma et al., 2001). The enzymes can be purified through a meticulous process, ensuring high quality, which enables their study of kinetics and facilitates the development of new enzymes with enhanced properties.

Microbial enzymes in the food industry

Enzymes contribute to improved human health due to their applications in food processing, which enables the manufacture of high-quality and nutritious food products. The contributions of enzymes to food product manufacture date back to ancient times (Okpara, 2022) and the market sale of food enzymes in India is depicted in Fig. 1. Natives of some Pacific islands practiced the use of papaya to tenderize the meat, and it was among the initial applications of enzymes in food processing (Gomes et al., 2018). The ability of enzymes to convert substrates into products is considered a relevant indicator for their standard use in food processing operations. For example, enzymes can be used along with biosensors to detect the freshness of milk vegetables and quality changes during processing as well as an intrinsic component of smart packaging (Kaur & Kaushal, 2019). Table 1 provides an overview of the applications of some enzymes that are commonly used in the food industry. Technology development has led to the creation of novel enzymes with various uses and specificities, and new application fields are still being investigated. Microbial enzymes are favoured over plant and animal sources owing to better stability, ease of production, lesser space, and lesser time requirement for their cultivation, cost-effectiveness, high consistency, and amenability to process modification and optimization. (Gurung et al., 2013; Raveendran et al., 2018) Microorganisms such as bacteria, yeast, and fungi and their enzymes are widely used in several food preparations to improve taste and texture with substantial economic benefits to industries (Raveendran et al., 2018). Applications of microbial enzymes have been found in several areas of the food industry, including dairy, baking, food processing, livestock feed, fruit and veggie juice, beverages, and confectionery (Okpara, 2022). The following sections provide in-depth and recent updates on different food enzymes of microbial origin.

Dairy industry

Various enzyme types, including catalase, aminopeptidase, proteases, lacto peroxidase, lipases and transglutaminase have found applications in the dairy industry.

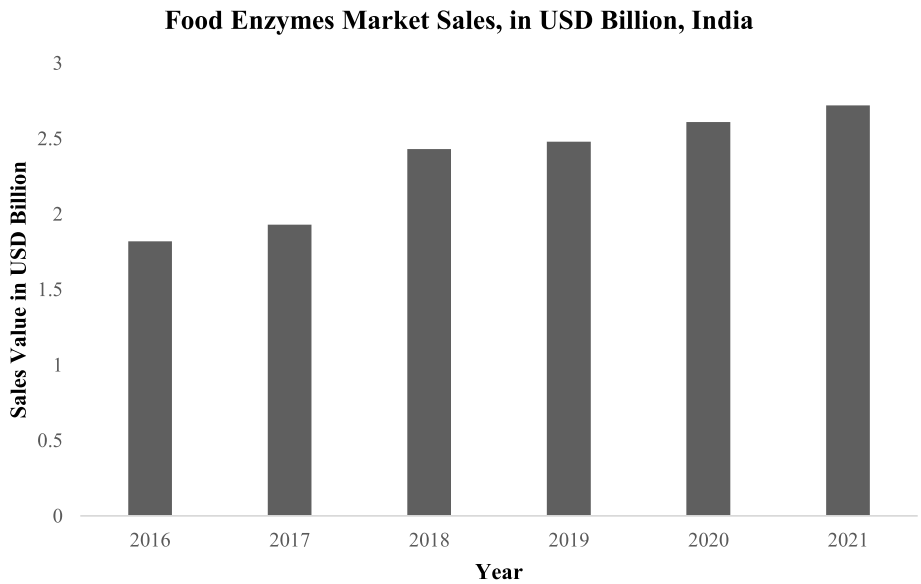


Fig. 1 The sales figures for the Food Enzymes Market covering the period from 2016 to 2021, obtained from the USDA Foreign Agriculture Service

Table 1 Sources and applications of important enzymes derived from bacteria and fungi in food industry

Enzyme	Source Microorganisms	Area and form of Application	References
Amylase	**Bacteria:** *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus coagulans* **Fungi:** *Aspergillus oryzae*, *Aspergillus niger*, *Rhizopus sp.*	· Bakery industry · Production of fructose and glucose by the enzymatic conversion of starch · Detergent industry	(Patel et al. 2023; Sundarram & Murthy, 2014)
Lactase	**Bacteria:** *Bacillus licheniformis, Bacillus subtilis, Lactobacillus acidophilus,* **Fungi:** *Kluyveromyces*, *Sacchromyces fragilis*	· Dairy Industry · Production of sweeteners and hydrolysed whey syrups. · Whole milk concentrates	(Patel et al., 2023; Bosso et al., 2020)
Cellulase	**Bacteria:** *Acetivibrio cellulolyticus*, *Bacteriodes cellulosolvens*, *Bacteriodes succinogenes*, *Clostridium thermocellum* **Fungi:** *Trichoderma, Penicillium, Aspergillus niger*, *Phylosticita sp.*	· Biofuel production · Deinking of papers for recycling paper · Fruit and vegetable juice extraction and clarification.	(Patel et al., 2023; Saranraj & Reetha, 2012)
Pectinase	**Bacteria:** *Bacillus macerans*, *Bacillus pumilus*, *Bacillus subtilis* **Fungi:** *Aspergillus japonicus*, *Aspergillus giganteus*, *Penicillium italicum.*	· Fermentation of coffee beans, coffee concentrates in the coffee industry. · Pressing, clarification, and filtration in the wine industry.	(Patel et al., 2023; Pedrolli, 2009)
Esterases	**Bacteria:** *Bacillus subtilis*, *Clostridium thermocellum*, *Lactococcus lactis*, *Rhodococcus sp.* **Fungi:** *Aspergillus awamori*, *Saccharomyces cerevisiae*	· Synthesis of optically pure compounds · Degradation of organophosphorus compounds. · Pulp, paper, textile, leather and baking industry	(Panda & Gowrishankar, 2005)
Xylanases	**Bacteria:** *Bacillus circulans*, *Bacillus polymyxa*, *Bacillus sp.*, *Micrococcus* **Fungi:** *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium purpurogenum*, *Geotrichum candidum*, *Sterptomyces sp.*	· Used in bread making and bread improvers · Improving texture, palatability, and uniformity of wafers. · Employed in the initial processing of forage crops to enhance the digestibility of feeds for ruminant animals.	(Harris & Ramalingam, 2010)
Lipases	**Bacteria:** *Alcaligones sp.*, *Pseudomonas aeruginosa.*, *Bacillus stearothermophilus* **Fungi:** *Aspergillus oryzae*, *Mucor pusillus*,	· Development of flavouring agent in milk, cheese, and butter · Eliminating stubborn stains and breaking down oil and grease through hydrolysis. · Biodiesel production	(Mehta et al., 2021)
Laccases	**Bacteria:** *Azospirillum lipoferum*, *Aquifex aceolicus*, *Streptomyces psammoticus*, *Escherichia coli* **Fungi:** *Trametes versicolor*, *Trametes ochracea*, *Cerena maxima*	· Decolorization and detoxification of azo dyes. · Treatment of wastewater from beer factory. · Pulp bio-leaching · Phenolic compounds removal	(Mehta et al., 2021)

These enzymes help improve shelf life and are different from coagulants. In the dairy industry, various valuable items like cheese, yogurt, syrup, and similar products have been produced through the utilization of enzymes, which can be engineered to enhance the quality of such food products (Abada, 2019). Rennet is regarded as the most renowned exogenous enzyme complex utilized in dairy processing and dates back to 6000 BCE. Rennin is the main protein clotting factor in the rennet and has enzymatic and non-enzymatic actions that cause the casein component of milk to coagulate (Ozatay, 2020; Abada, 2019). Dairy calves were the sources of rennet but cannot meet current-day demands due to the increase in global demand for cheese, which has led to the exploration of other means of producing rennin (Nicosia et al., 2022), resulting in the synthesis of chymosin, a genetically engineered version of renin produced in bacteria. Up to 70% of cheeses are thought to be produced with bioengineered chymosin (Furtado, 2022). The availability of chymosin has become very important as it reduces the number of calves that are sacrificed for rennet production and meets consumer demand for the ethical manufacture of food products. The other enzymes utilized in dairy products include proteases, lipases, and lactases, which are all members of the hydrolase family (Zaheer & Gupta, 2019).

Protease

Proteases are hydrolases that hydrolyse the bonds between peptides and amino acids (Gurumallesh et al., 2019). Proteases constitute a diverse and intricate group of enzymes characterized by differing substrate specificities, multiple active sites, diverse catalytic processes, optimal pH ranges, temperature preferences, and stability

profiles. Certain proteases, such as pancreatic trypsin, chymotrypsin, pepsin, and chymosin, are sourced from animals, while others, like pawpaw papain, bromelain, actinidin, and ficin, are derived from plants (Martnez-Medina et al., 2019). Recently, there has been a growing interest in producing these proteases from genetically modified microbial sources due to the industry's escalating demand for enzymes (Sanroman & Deive, 2017; Martnez-Medina et al., 2019; Kocabas et al., 2022)). These enzymes may be exogenous (amino or carboxy peptidases, which break down the chain's final amino acids) or endogenous (proteinases, which break down peptide bonds inside the polypeptide chain. Classes of proteases that have serine, thiol, metallic ions, or aspartic acid in their active sites are serine, thiol, metallic, and acid proteases.

Proteases have been used to produce appealing flavours and texture evolution during the maturation of cheese and minimize the allergenic properties of products derived from bovine milk. (Ardo et al., 2017). Other proteases are very potent in hydrolysing peptide bonds in kappa-casein in the process of producing curds for cheese manufacture. Additionally, proteolytic enzymes have been found to reduce the allergic properties of some fermented milk products. Fruit pulp and husk of plant *Withania coagulans* have protease activity. The plant preparations from dried fruit have been used for cheese-making at a pH ranging from 4–6 (Kazemipour et al., 2017). A recent study proved that the milk-clotting protease from *Aspergillus oryzae* strain DRDFS13MN726447 could improve the sensory characteristics or qualities of cheese as a whole (Mamo, 2020).

Lipase

Lipases are the catalysts that facilitate the degradation of long-chain triacyl glycerides and are highly versatile tools for use in several industrial processes. Lipase is primarily employed to facilitate the separation of milk and fat, thereby contributing to the flavor profile of cheese and its qualities (Jooyandeh et al., 2009). Lipase is used as a processing aid to improve cheese flavor (Selamoglu, 2020). The flavor resulting from the hydrolysis of milk fat is imparted by liberated unsaturated fats. Lipases utilized in the production of specific cheese varieties often hydrolyze a wide spectrum of triglycerides. Phospholipase A1, which breaks down phospholipids into fatty acids and other lysophospholipids, is recommended for use in dairy processing due to its high specificity and minimal to no activity toward di- or triglycerides. (Kocabas et al., 2022). Furthermore, lipases find application in butter and margarine to improve flavor and prolong the shelf life of a variety of baked goods (Aravindan et al., 2007). Lipases are also used to produce structured lipids with comparable organoleptic qualities to cocoa butter by having a melting point of 37 °C, which is because of the existence of palmitic and stearic acids as the dominant fatty acids on the triacylglyceride (Jaeger & Reetz, 1998; Sharma et al., 2001). Animal lipases are produced by calves and sheep, whereas microbial lipases are produced by mainly yeasts and fungi (*Candida, Yarrowia, Aspergillus, Penicillium, Rhizopus, Rhizomucor*, and *Thermomyces*) (Hasan et al., 2006). Lipases obtained from animal sources hydrolyze short and medium-length fats, while microbial lipases, used in cheese manufacturing, exhibit less specialization in hydrolyzing specific fats. The hydrolysis of shorter-length lipids is more favorable than longer-chain unsaturated fats, as the former enhances the flavor of various types of cheese. Longer-chain unsaturated fats, after hydrolysis, can result in a soapy or tasteless quality (Alkan et al., 2007). Lipases catalyze the hydrolysis of triacylglycerols (TAG), forming monoacylglycerols (MAG), diacylglycerols (DAG), glycerol, and free fatty acids during the process of bread making (Zheng et al., 2023).

Lactase (β-galactosidase)

Lactase hydrolyzes lactose into glucose and galactose sugars. In addition to milk, whey, a by-product of cheese production can be utilized by β-galactosidase as a source of lactose. This enzyme can convert whey lactose into syrups which are useful in the bakery and confectionary industries (Movahedpour et al., 2022). There are two different kinds of lactases available on the market: neutral lactases and acid lactases, which are used in dairy products and dietary supplements, respectively. These enzymes, which are members of various families of glycosyl hydrolases, differ greatly in their sequence, structure, and metabolic characteristics (Dekker et al., 2019). In the commercial setting, lactase is employed to produce lactose-free products tailored for individuals with lactose intolerance. Moreover, it is utilized in the production of frozen yogurt to enhance creaminess and improve the overall taste of the product (Okpara, 2022).

In dairy products, lactase is utilized not only to hydrolyze lactose but also to make the product sweeter. Lactase makes milk sweeter because lactose possesses just 20% of the sweetness of sucrose, in contrast to the blend of glucose and galactose generated through hydrolysis, which possesses approximately 70% of the sweetness of sucrose (Horner et al., 2011). Earlier. lactase was utilized in dairy industries to produce milk-based products (such as whey). This is one of the attractive approaches for improving their digestibility, best suited for lactose-intolerant patients, thereby managing dehydration and diarrhea (Mahoney, 1997). Lactase plays an important role in whey treatment in

dairy industries as during whey treatment, lactose is associated with critical environmental issues such as higher biological oxygen demand (BOD) and chemical oxygen demand (COD) of whey (Khider, 2004; Johansen et al., 2002). Galactooligosaccharides (GOS) generation due to hydrolytic cleavage of lactose is a relevant application of this enzyme. The transglycosylation activity of β-galactosidase is mainly responsible for GOS generation, which is used as prebiotic food ingredients as previously reported (Gibson & Wang, 1994).

Commercial beta-galactosidase or lactase (EC 3.2.1.23) is frequently utilized in the production of lactose-free milk products, particularly in processes involving relatively low temperatures. An evolved variant of beta-galactosidase, achieved through directed evolution, exhibits high activity under industrial conditions relevant to milk processing—specifically, with substrate lactose, a buffer pH of 6.75, and a temperature of 8 °C (Mamo et al., 2020).

Enhancements to the transglycosylation activity of this enzyme were achieved by obtaining a thermoresistant variant of *Thermotoga maritima* beta-galactosidase through rational design. This modified enzyme demonstrates improved specificity for the biosynthesis of galacto-oligosaccharides, a prebiotic compound (Ramadan, 2019). In addition to the above enzymes, transglutaminase (TG) has been considered a widely acceptable enzyme in producing yogurt, frozen desserts and ice cream for improved texture and storage stability.

Beverage industry

Improvement in juice yield, clarification, and pulp liquefaction is possible with fruit juice's enzymatic treatment, which is more advantageous than traditional processing (Ramadan, 2019). A previous study investigated the fermentation of high- fibre pineapple wastes for enzyme production. Fermentation of the pineapple fruit waste was achieved with *Lactobacillus casei* and led to the production of xylanase and cellulase (Sivanesan et al., 2022). Such an approach enables the valorisation of fruit waste using microbial enzymes to produce other useful industrial catalysts. Amylases have found several applications in the brewing industry, with extensive application to malted barley for enhanced production of sugars that can be fermented into alcohol (Meshram et al., 2019). Production of beverages such as beer, wine, and fruit juice are attributed to various issues such as haze formation, turbidity, and browning due to the presence of phenolic compounds which have problems. Laccase, manganese peroxidase, and lignin peroxidase are promising enzymes to improve the colour of beverages by eliminating the phenolic compounds to produce clear beverages (Chowdhary et al., 2019).

Pectinases

These enzymes catalyse a variety of processes in the pectic compounds found in plant cell walls (Dey & Maity, 2023). Pectin esterase catalyses the deacetylation and demethoxylation of pectin, pectolyase catalyses the trans-eliminative cleavage of 1,4-D-galacturonan methyl ester, and polygalacturonase catalyses the hydrolysis of 1,4-glycosidic bonds (Satapathy et al., 2020).

Pectinases play a crucial role in the fruit juice industry by breaking down the pectin present in the cell walls of fruits. This process enhances juice extraction, improves flavor, clarity, filterability, and overall yield. In the beverage industry, particularly during the curing step in wet processing for coffee manufacturing, pectinases are utilized to increase yield, enhance fragrance and flavor, and reduce processing time. Additionally, in cocoa processing facilities, pectinases are employed to ensure the complete removal of mucilaginous layers surrounding cocoa beans, improve aroma and flavor, and shorten processing time, thereby enhancing the quality of the resulting coffee. Tea leaves are treated with pectinases to lessen their pectin content to allow a somewhat faster fermentation process while brewing tea. Several recombinant pectinases from several sources such as *A. niger* (Xu et al., 2015), and from *Penicillium purpurogenum* (Perez-Fuentes et al., 2014), and an acidic endopolygalacturonase from *A. niger* (Jiang et al., 2013), expressed in *P. pastoris* have been described in the literature to produce juices.

Presently, pectinases from different sources are combined to form recombinant enzymes for their utilization in the juice industry. For example, Novoshape, a recombinant pectin methylesterase from *A. aculeatus*, is expressed in *A. oryzae*. Other preparations include pectin lyases from *A. niger* and *P. purpurogenum,* pectin methyltransferase and endopolygalcturonase from *A. niger expressed in P. pastoris* (Trono, 2019). A biofunctional enzyme (S6A), formed by combining the catalytic domains of pectin methyltransferase (S6PE) and polygalacturonase (S6PG) in *Penicillium oxalicum* and expressed in *P. pastoris*. This enzyme had properties of both enzymes and, therefore found to break pectin walls as well as clear the juice (Tu et al., 2014).

Esterases

The hydrolysis of esters into alcohol and acid is catalysed by a class of hydrolase enzymes called esterases. Short-chain glycerides can be hydrolysed into fatty acids and glycerol by this enzyme (Raveendran et al., 2018). In the beverage industry, feruloyl esterase is used to produce

ferulic acid, which is then used to manufacture fragrance compounds. Vanillin is a key element in Vanilla, which is used to improve the flavor of beverages. Esterases are also utilized in fruit juices to alter and enhance the flavour of the fat (Gallage et al., 2014). Esterases are primarily produced on a large scale by microbes, such as *Bacillus licheniformis, Lactobacillus fermentum*, *Lactobacillus farciminis*, *Lactobacillus acidophilus*, and *Lactobacillus amylovorus*. *Bacillus licheniformis* produced a thermostable esterase that was heterologously expressed in *E. coli* for ester hydrolysis (Macarie & Baratti, 2000).

Laccase

Laccase is a blue-copper oxidoreductase that represents the largest subgroup of multicopper enzymes and is among the most researched enzymes worldwide (Raveendran et al., 2018). It is ideal for various commercial uses due to its poor substrate specificity but strong potential to utilize oxygen to breakdown phenolic compounds (Mayolo-Deloisa et al., 2020). Many different compounds, including phenolics, aromatic amines, and ascorbate, are oxidized by laccases (Okpara, 2022). Fungi, bacteria, soil algae, and a few insects have been reported to produce laccase (Janusz et al., 2020). Owing to its adaptability, stability, and variety of substrates, numerous laccase commercial uses have been investigated in recent years. It is widely utilized in a variety of food industry activities, including the gelation of sugar beet pectin, wine and beer stabilization, and beverage processing (Deloisa et al., 2020). Phenolic substances in juices degrade beverage quality, color, and taste, lowering economic value. Laccase has been reported to have high efficacy in removing these phenolic compounds in juices. The wine industry employs laccase to stabilize the wine by regulating its phenolic compounds (Osma et al., 2010). Laccase has been employed to eliminate oxygen to extend the lifespan of wine (Okpara, 2022). Laccase can be used to extend the half-life of beer by introducing the enzyme at the end of the brewing process, which oxidizes undesirable phenolic chemicals (Osma et al., 2010). Like the wine process, this enzyme also eliminates oxygen in the last step of beer production, thus prolonging its life span and drinking quality.

Proteases

Proteases may also aid in the prevention of haze development during wine fermentation. The acid proteases from *Saccharomyces fibuligera* and *Torulopsis magnolia* could be utilized for haze inhibition while acid proteases from *Aspergillus niger* make beer free from haze (Mamo & Assefa, 2018).

Bakery industry

Baking is a universal process in manufacturing products like bread, pies, cookies, and pastries. Even though the utilization of enzymes in the bakery industry is not new, current advancements in enzyme technology, biotechnology, and biochemistry have transformed enzyme potential as a processing tool for baked goods production. Extension in the shelf life of bread can be achieved by utilizing glucose oxidases, xylanases, and amylases (Dahiya et al., 2020). The main attractive attributes of bakery products are their long-term freshness and softness. These enzymes either can be used individually, or mixtures of these enzymes could be incorporated into flour and dough to improve the flavour and texture and impart major contributions towards baking operations (Avendano et al., 2016). The incorporation of diverse enzymes not only enhances the flavour but also facilitates the digestion of proteins in wheat. This process concurrently reduces maturation time and enhances consistency (Mamo & Assefa, 2018).

α-Amylases

α-Amylases are natural starch-degrading enzymes that hydrolyse polysaccharide α-1,4 glycosidic linkages, resulting in the formation of short-chain dextrins (Sindhu et al., 2017). α-amylases are widely used in the food sector for starch liquefaction, cooking, brewing, and digestion enhancers. They are often used in the baking industry as flavour boosters and anti-staling agents to improve bread quality (Sindhu et al., 2017). α-amylases are added to the dough during baking to assist with starch break down into smaller dextrin, which yeast subsequently ferments. It improves bread flavour, appearance, and stability (Couto & Sanromán, 2006). α-amylases from *Bacillus licheniformis, Bacillus stearothermophilus,* or *Bacillus amyloliquefaciens* are employed for the bulk of starch saccharification (Van der Maarel et al., 2007). However, the α-amylase isolated from these sources was sensitive to acidic conditions and was replaced by genetically engineered (Histidine residues in active site were replaced with Arg and Asp residues) one which had high stability and activity as compared to wild-type (Liu et al. 2017). This enzyme enhanced the various processes including starch liquefaction, saccharification and fermentation in which slurry has a usual pH of < 6.0 (Zhang et al., 2019). For high maltose syrup production, α-amylase from *Rhizopus oryzae* was modified using site-directed mutagenesis to improve resistance at a high temperature (60 °C) and stability at lower pH levels (4.0–4.5) in comparison to the wild-type enzyme (Li et al., 2018).

Protease

There are two types of proteases: exopeptidases and endopeptidases. Endopeptidases break peptide bonds far from the amino or carboxy termini of the substrate, while exopeptidases cleave peptide bonds near to those termini (Ganzle et al., 2008). The majority of the proteolytic activity in wheat and rye flours is attributed to aspartic and carboxypeptidase enzymes, both of which are active at acidic pH levels (Bombara & Anon, 1997). Additionally, gluten is partially correlated with wheat aspartic proteases. Commercial manufacturing of waffles, crackers, baked products, and bread utilizes proteases. These types of enzymes can be used to reduce the amount of time required to mix the dough, ensure consistency, manage the texture of the bread, and enhance flavour (Raveendran et al., 2018). Furthermore, while proteolysis breaks down peptide links, proteases have essentially taken the position of bisulfite, which was originally employed to regulate consistency by reducing the disulfide bonds in gluten proteins. A fungus-derived acid protease is employed to alter mixes with a high gluten concentration in the manufacture of bread. When proteases are added to the mixture, they partially hydrolyses, becoming pliable and simple to pull and knead (Di Cagno et al., 2003; Salleh et al., 2006). Proteases obtained from microbes like *Aspergillus oryzae* and *Aspergillus niger* can break down gluten into water-soluble peptides. This enzymatic action results in a reduction of immunogenic gluten peptides, which are the primary contributors to gluten allergies, while concurrently increasing the concentration of amino acids (Heredia-Sandovan et al., 2016).

Xylanases

Xylanases are hydrolytic enzymes, produced by various *Aspergillus* and *Trichoderma* species. In baking, xylanases are extremely useful because they have been shown to increase bread volume, improve crumb structure, and lessen stickiness. Xylanases are used to prolong the shelf life of bread and mitigate bread staling. Moreover, they break down non-extractable arabinoxylan, producing water-extractable xylan. This process leads to a redistribution of water within the dough, enhancing the formation of the gluten network. As a result, the dry dough exhibits improved elasticity, extensibility, and softness. Owing to these properties, the demand for this enzyme continues to rise in the baking industry (Paucean et al., 2016). Xylanases particularly work during the gluten re-agglomeration followed by the breakdown of gluten thus, affecting the rheological properties of gluten (Ghoshal et al., 2017). Variation in the original xylanase through site-directed mutagenesis showed an increase in specificity and thermal stability (50°-60 °C) and can be efficient in alkaline conditions (pH 7–10) to produce xylooligosaccharides from wheat straw (Faryar et al., 2015). The water-extractable arabinoxylan is broken down by *Aspergillus niger* xylanase, which also lowers the molecular weight and viscosity of the dough (Romanowska et al., 2003). Similar to this, *Bacillus subtilis* xylanase preferentially solubilizes the water-insoluble arabinoxylans and boosts dough viscosity while negatively affecting gluten aggregation (Courtin & Delcour, 2002).

Lipases

Lipases catalyze the hydrolysis of triacylglycerols (TAG) resulting in free fatty acids, glycerol, monoacylglycerols (MAG), and diacylglycerols (DAG) during the bread-making process (Sandoval, 2018). Lipases boost the bread loaf's volume and dough tolerance. Chemical dough enhancers such as diacetyl tartaric esters of monoglycerides (DATEM) can be replaced with microbial lipases (Gerits et al., 2015). First-generation lipases improve dough machinability by increasing strength and stability and improving rheology. When lipases are added, they not only increase the volume of the bread but also improve the shape of the crumbs and make the crumbs softer (Dahiya et al., 2020).

Laccase

Laccase is used in the baking industry because of its ability to cross-link with biopolymers. Laccase use has increased bread batter's machinability in the baking industry through improved dough consistency, strength, and reduced stickiness (Mayolo-Deloisa et al., 2020). Additionally, it boosts the product's suppleness and volume (Labat et al., 2000).

Microbial enzymes in food processing

The desirable enzymes are either not available or are unable to carry out the necessary transformation effectively. Search and discovery technologies may be used to create an optimum library of enzyme preparations suitable for use in organic synthesis, ensuring continued progress. Technology development has led to the creation of novel enzymes with various uses and specificities, and new application fields. Microbial enzymes are preferred among animal and plant enzymes due to their higher stability, ease of production, lesser space, and lesser time requirement for their cultivation, cost-effectiveness, high consistency, and amenability to process optimization and modification (Gurung et al., 2013; Raveendran et al., 2018). Microorganisms such as fungus, yeast, and bacteria, as well as their enzymes, are commonly utilized to improve flavour and texture in a variety of food preparations, with significant economic advantages to industries (Raveendran et al., 2018). Microbial enzymes (Table 2) have been identified to be useful in a variety

Table 2 Chemical properties and applications of certain enzymes derived from plants and microorganisms

Sources	Enzyme	Chemical properties	Food applications	References
Wheat starch, edible oil, sunflower oil, *Bacillus subtilis*	α- Amylases	α-(1 → 4)-D- glycosidic [endo], liberating α-dextrin, requires calcium ions, possess an TIM barrel structure	Bread volume increases, fermentation time decreases, and dough viscosity improves	(Al-Maqtari et al., 2019; Zhao et al., 2015)
Gluten proteins, Gliadin, *Rhizomucor or Cryphonectria*	Proteases	Hydrolyses peptide bonds	Reduced dough time, enhances dough extensibility; production of amino acids and flavors, provides crispiness features	(Al-Maqtari et al., 2019)
Aspergillus awamori, A. oryzae, A. sojae, A. terreus, Streptomyces, Penicillium capsulatum	Xylanases	Single chain glycoproteins, initiates conversion of xylan into xylooligosaccharides	Decreases dough firmness; decreases dough firmness	(Harris & Ramalingam, 2010)
Alcaligones sp., Pseudomonas aeruginosa., Bacillus stearothermophilus	Lipases	Liberation of free fatty acids	Increases dough stability; develops flavours; and formation of emulsifiers	(Mehta et al., 2021)

of food industry applications, including food processing and packaging, baking, dairy, livestock feed, fruit and vegetable juice, oil refinery, confectionery, and beverages (Okpara, 2022). The following sections provide in-depth and recent updates on different food enzymes of microbial origin.

Lipases

Lipases are highly adaptable enzymes that are utilized in a variety of industrial processes. They aid in the hydrolysis of long-chain triacylglycerides. Lipases can be extracted from various microbiological, vegetable, and animal sources. Microbial lipases had a market value of USD 425.0 million in 2018 and is expected to reach USD 590.2 million by 2023, increasing at a compounded yearly growth rate of 6.8% (Chandra et al., 2020). Because of the wide variety of catalytic activity and higher manufacturing yield, microbial lipases are regarded more useful than those derived from plants or animals. Furthermore, microbial lipases provide ease of genetic modification, not affected by seasonal changes, consistent supply, better stability, safe to use and ability to be produced from microorganisms that grow rapidly on cheap media (Reetz, 2013; Mendes 2012). Bacteria, fungi, and yeast are all lipase producers, accounting for about 90% of the world's lipase market (Guerrand, 2017).

Lipases are frequently employed in the fruit juice, baking, brewing and dairy sectors (Raveendran et al., 2018). Commercial lipases are mostly used in the processing of meals that contain fat and to flavor dairy products. Companies that produce wine and beverages use lipase to enhance the fragrance of the products (Okpara, 2022). Lipases are also utilized in butter and margarine to improve flavor and increase the shelf life of many baked goods (Aravindan et al., 2007). *Fusarium oxysporum* recombinant lipase produced in *Aspergillus oryzae* significantly enhanced loaf volume and bread volume during bread preparation. (Melis et al., 2017a, 2017b). Novoren (Novo Nordisk) is a recombinant aspartic protease from *Rhizomucor miehei* that has been produced in *A. oryzae* (Trono, 2019). Recombinant *Saccharomyces cerevisiae* with lipase A from *Bacillus subtilis* was added which increases the bread's leavening and overall aroma (Paciello et al., 2015). When exogenous lipase from the *Rhizopus oryzae* (Belpan LIPO B) was added to flour, it was observed that the quality of the bread was improved due to the increased loaf volume, higher stability, and improved crumb qualities as well as the preservation of freshness (Sîrbu & Paslaru, 2005). When Melis et al. (2017a, 2017b) investigated the impact of *Fusarium oxysporum* recombinant lipase expressed in *Aspergillus oryzae* during bread production, they discovered a considerable increase in the loaf volume and bread volume.

Laccase

The largest subgroup of multicopper enzymes is represented by the laccases, a collection of oxidases. Laccases oxidize several compounds including phenolics, aromatic amines, and ascorbate. Laccase is widely utilized in numerous food industry operations, including sugar beet pectin gelation, wine and beer stabilization, and beverage production (Mayolo-Deloisa et al., 2020). These enzymes exhibit a broad range of substrates, and the diversity depends on the specific microbial sources (Madhavi & Lele, 2009). Several fungi secreted laccases as secondary metabolites during the fermentation process (Morozova et al., 2007). Some of the common fungal producers of laccases are Deuteromycetes, Ascomycetes and Basidiomycetes (Gochev & Krastanov, 2007; Sadhasivam et al., 2008). Through absorbent fermentation, the white rot fungus *Funalia trogii* produces laccase. *F. trogii* could produce 11,900 U/L (Li et al., 2017). Recombinant laccases are another product of *Bacillus licheniformis* that are utilized in industry (Tonin et al., 2016). Recently, Recombinant DNA technology has recently been used to generate heterologous laccase protein production. For example, *B. vallismortis* fmb-103 genes were cloned and heterologously expressed in *E. coli* BL21 (DE3) cells to produce this enzyme (Sun et al., 2017).

Laccases are employed in clearing haze formation which is a common problem in the brewing industry. Flavourstar, a commercial laccase made by Novozymes, is used in the brewing industry to remove off-flavour development (Raveendran et al., 2018).

Asparaginase

Among the many enzymes used in the food industry, asparaginases are an extensive class of industrial, nutraceutical, and pharmaceutically important enzymes (Krishnapura et al., 2016). Asparaginase, also known as the asparagine-depleting enzyme, is an enzyme that catalyses the degradation of asparagine into the subsequent acid derivatives, aspartic acid, and ammonia (Raveendran et al., 2018). Asparagine is a non-essential amino acid for humans, but it is crucial for the growth of cancerous cells. As a result, asparagine deficiency significantly impacts the development of cancerous cells, which is why this enzyme functions as a potent anticancer agent (Krishnapura et al., 2016).

In the food sector, asparaginase is widely utilized to counter acrylamide, a substance that may cause cancer in humans when foods high in carbohydrates are cooked at high temperatures. Asparagine is transformed into acrylamide by many foods processing techniques, including baking and frying in oil. One such instance is the high-temperature cooking of potatoes used to make potato chips (Adebo et al., 2017). This reaction applies

to all foods high in carbohydrates cooked at high temperatures and is not limited to potato chips only (Muneer et al., 2020). For the millions of people who consume these food items every day, there are significant risks and grave worries regarding acrylamide exposure (Adebo et al., 2017). As mentioned earlier, the Maillard reaction primarily occurs at elevated temperatures, involving the interaction of asparagine and carbonyl compounds found in high-carbohydrate diets, leading to the formation of acrylamide (Krishnakumar & Visvanathan, 2014). Asparaginase effectively lowers acrylamide levels in potatoes and various baked goods by hydrolyzing asparagine into ammonia and aspartic acid (Borda & Alexe, 2011). Asparaginases derived from *Aspergillus oryzae* and *Aspergillus niger* cultures, respectively, are widely marketed under the names Acrylaway® (Novozymes, Denmark) and PreventASeTM (DSM Food Specialties, Denmark) (Adebo et al., 2017). According to the United States Food and Drug Administration (FDA), some microbial sources, including *B. subtilis*, produce enzymes deemed acceptable for food preparation. Recombinant enzyme from microbial sources, such as *B. subtilis*, have been recommended by the FDA to be safe for use in food preparation (Baskar & Renganathan, 2019). As a result, asparaginase is considered to be a good processing aid for preventing the formation of acrylamide in food items. It should be emphasized that the extremely thermostable form of L-asparaginase is necessary in the food business since baking and frying involve high temperatures (Zhang et al., 2019). L-asparaginase is exposed to incubation and pre-treatment at high temperatures, so its stability and reusability are both crucial from a commercial perspective. Because of its low stability and lack of recyclability, the price of free L-asparaginase raises the expense of food manufacturing and hence, restricts its widespread use. However, L-asparaginase has been immobilized using a variety of substances to improve its enzymatic stability and recyclability, including chitosan, agarose spheres, magnetic nanoparticles, and aluminium oxide pellets which could enhance affordability and lower food processing costs (Jia et al., 2021).

Lactase (β-galactosidase)

Lactase is a hydrolase that facilitates the breakdown of lactose to produce simpler monomeric sugars such as glucose and galactose (Okpara, 2022). A wide variety of resources like animals, fungi, and plants, and their ease of availability is one of the major advantages in obtaining lactase, also called β-galactosidase (Panesar et al., 2010). Microorganisms are preferred for producing β-galactosidase because of their cost-effective approach and higher yield (Santos et al., 1998; Zadow, 2012). Lactase is commonly obtained from the fungus *Aspergillus* and the yeast *Kluyveromyces* (Wilkinson et al., 2003). In industry, two main classes of lactases are of common use. These are thermostable and active at low temperatures (Wołosowska et al., 2004). Lactase is exclusively manufactured commercially using GRAS-certified microorganisms for use in milk and dairy products. Enzyme engineering can convert the β-galactosidase into glycosidases. For example, DNA shuffling was used to convert β-galactosidase into β-glucuronidase which has higher substrate specificity toward p-4-Nitrophenyl β-D-glucuronide than wild-type enzyme (Lu et al., 2020). The mutants of β-galactosidase from *Bacillus circulans* ATCC 31382 (BgaD), R484S and R484H produced by site-saturated mutagenesis resulted in altered product specificity and catalyzed the synthesis of new GOSs (Yin et al., 2017). Site-directed mutagenesis of this enzyme produced triple mutants (K166P, G307P and A833P) which had higher thermal stability of 60 °C (Ishikawa et al., 2015). The rational mutation was used to improve the half-life as well as enhance the catalytic efficiency of c *Kluyveromyces lactis* through the addition of cysteine residues at subunit interfaces (Rico-Diaz et al., 2017). β-galactosidases are generally inhibited by their product, galactose produced during hydrolysis of lactose. This product inhibition was overcome by the use of rational designing. Zhang et al. (2018) modified the galactose-binding motif, Asp258-Ser-Tyr-Pro-Leu-Gly-Phe264 of *Aspergillus niger β*-galactosidase to Asp258-Phe-Tyr-Thr-Ser-Ser-Phe264 to reduce enzyme's binding to galactose.

Conclusions

The global need for food-related products is anticipated to rise even further as the world population rises. As a consequence, it is expected that both the demand for enzymes with food applications and the size of the global market for food enzymes will continue to expand. To meet the need for industrial enzymes, new enzymes with greater efficiency and diversity of utilization in the food industry must be developed.

Microbes can be genetically changed to create large numbers of enzymes with specified molecular characteristics and higher enzymatic activity using modern biotechnological methods. It is also feasible to promote the development of chimeric enzymes with multiple catalytic activities by genetically combining the genes for each enzyme from various microorganisms. By overexpressing their genes, microbes can produce high-specific activity enzymes in excess amounts that meet commercial needs. There are still untapped potential uses for several microbially derived enzymes, and there is potential to expand the range of commercial uses for these biological catalysts, particularly in the food processing industry.

Future Perspectives

Microbial enzymes offer several benefits in the food industry over traditional catalysts in terms of specificity as well as their safety for use in food items. Various microorganisms have been employed for the extraction of industrially important enzymes because of their easy availability and cost-effective bulk production. However, these natural catalysts have limitations including adaptation to industrial processing conditions, like high temperature and pH, and the amount required at the industrial level. Therefore, there is a need to improve the basic properties of these enzymes for their utilization in industrial applications. Enzyme engineering techniques are highly effective technique to improve the properties of the enzyme for commercial applications. These techniques not only help to design enzymes with desirable properties but also contribute to a better understanding of the structure of industrially important enzymes, their substrate binding sites as well their catalytic mechanisms. Therefore, enzyme engineering has opened the door for the remodelling and prediction of sequences with novel functions and properties. Despite several advances in enzyme engineering techniques, many challenges still exist to improve the efficiency of these bio-catalysts. There is a lack of screening techniques for genetic variants resulting from random mutagenesis. More advancements in high-throughput screening methods and genetic engineering tools will be helpful in the generation of improved enzymes. Further updated algorithms are needed to expand the enzyme databases having structural and functional information in the field of food processing. Advanced bioinformatics tools can be helpful in the improvement of these databases. Furthermore, CRISPR-based gene modulations could be of great help in improving the catalytic, substrate-binding, and stability properties of enzymes.Such advanced techniques can be utilized to improve microbial performance at the gene level and might be helpful for fulfilling consumer's demands at commercial and industrial levels. Therefore, in the future, the combination of advanced experimental and computational tools will lead to improvement in existing ones or design the of new enzymes with broad substrate specificity, thermostable structures, and multifunctional properties for the food industry.

All the efforts made to develop the highly specific and efficient enzymes are in vein due to the difficulties in approval of these enzyme. Modifications of enzymes involve genetic engineering which is highly questionable. Therefore, it is important to note to fill the gap of limited knowledge about these technologies among non-professional people.

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