Chapter 13
Application of Microbial Enzymes in Food Industry

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Introduction

The utilization of enzymes in food processing is practiced since ancient times. Technology has enabled the development of new enzymes with a broad range of application and specificity (Raveendran et al., 2018). Moreover stable to plant and animal derived enzymes make them appropriate for food industry. They can modified and optimized as per the product need (Gupta et al., 2017). The enzymes derived from microorganism even used to enhance the flavour and texture eventually reduce the coast of outsourcing. Microbial Enzymes applications known across the world in the field of medicine, energy sector and agriculture in recent trends these enzymes are getting attention to researchers due to ecofriendly nature (Choi et al., 2015).

The global market for microbial enzymes was estimated to be worth roughly \$4.2 billion USD in 2014, and by 2020, it is anticipated to grow by 7% (Abada, 2019). Consumers choose enzymes over chemical food processing aids because they are seen as natural, non-toxic food components that come from plants, animals, or microorganisms. Although the outlined criteria are far from universal, regulatory bodies evaluate the safety of these industrially manufactured enzymes due to the specific nature of their usage in the food business, which have evident implications for public health. Man has relied on microbes and enzymes to produce food for thousands of years. Common uses for enzymes over many years include the manufacturing of beer, bread and wine.

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Traditional Uses of Enzymes Derived from Microorganism

Enzymes derived from microorganism, have been used in the food processing for a variety of applications, including improving the texture, flavor, and nutritional properties of food products. Few of the traditional uses of microbial enzymes in the food industry include cheese production, brewing, and baking. Cheese production is one of the oldest and most well-known use of microbial enzymes in the food industry. Enzymes such as rennet, which is derived from the stomachs of young ruminants, are used to coagulate milk proteins and form curds, which are then processed into cheese (Harboe et al., 2010). Microbial enzymes such as chymosin, which is produced by the fungus Aspergillus niger, have been developed as alternatives to animal-derived rennet and are now widely used in the cheese industry (Olempska-Beer et al., 2006). Moreover, an important application of enzymes in food industry is brewing. Enzymes such as amylases, which are produced by bacteria and fungi, are used to break down starch into simple sugars during the malting process. This is essential for the production of beer, as yeast requires simple sugars to ferment and produce alcohol (Gurung et al., 2013). Baking is a third traditional use of microbial enzymes in the food industry. Enzymes such as alpha-amylase and protease, application in enhancing the texture and shelf life of bread by breaking down complex carbohydrates and proteins (Dahiya et al., 2020). In addition to these traditional uses, microbial enzymes are also being developed for a range of new applications in the food industry. For example, enzymes such as transglutaminase, which is produced by bacteria, are being used to improve the texture and appearance of meat products (Kieliszek & Misiewicz, 2014). Overall, microbial enzymes have played an important role in the food industry for centuries and continue to be an essential tool for food scientists and manufacturers. As new enzymes are discovered and developed, they offer the potential to improve the quality and sustainability of food products.

Role of Enzyme Derived from Microorganism in Food Biotechnology

Genetic alteration and modification of DNA technology has made it possible for industrial microbes to manufacture enzymes that were initially taken from pathogenic or toxin-producing microorganisms are difficult to culture or even uncultivable. The use of different microbial enzymes in the food processing sector is increasing at an alarming rate. Furthermore Extremophiles are the source of a growing number of these enzymes, however the productivity and yield enhanced, by gene copies and sequences (Dalmaso et al., 2015; Neifar et al., 2015). As a result, a thorough understanding of the metabolism of two important industrial-grade microorganisms, *Aspergilius niger* and *Bacillus subtilis*, makes it possible to produce large quantities of enzymes in a way that is both rational and economical. Enzymes with increased activity, selectivity, or stability have been designed and produced in large part thanks to advances in protein engineering (Singh et al., 2013). However the solid state fermentation (SSF) has become more important now days, submerged fermentation has historically been the primary method for producing enzymes (Thomas et al., 2013). Industrial bioprocess needs microbial enzymes and a wide spread industrial commercial applications. It is estimated around 500 products manufactured from enzymes. Due to diverse nature of microorganisms, industrial enzymes demand increased as less harmful to environment due to its manufacturing process (Patel et al., 2016).

Microbial enzymes play a crucial role in food biotechnology. These enzymes are used in a wide range of food processing and production applications. Here are some of the ways in which microbial enzymes are used in food biotechnology. Microbial enzymes are used in the production of cheese. Rennet, which is derived from the stomach lining of young ruminants, is traditionally used to curdle milk during cheese production. However, microbial enzymes like chymosin and pepsin can also be used as an alternative (Ogel, 2018). Microbial enzymes are used in the production of bread and other baked goods. Amylase, protease, and lipase are commonly used enzymes that help break down starches, proteins, and fats, respectively, to improve the texture and flavor of baked goods. Microbial enzymes are used in the production of beer and other alcoholic beverages. Enzymes like amylase and maltase help convert starches into sugars that can be fermented by yeast to produce alcohol. Microbial enzymes are used in meat processing to tenderize meat and improve its flavor. Proteases like papain and bromelain are commonly used enzymes in meat processing (Bekhit et al., 2014). Microbial enzymes like pectinase are used in the production of fruit juices to break down pectin, a complex carbohydrate that causes cloudiness and viscosity in juice (Danalache et al., 2018). Overall, microbial enzymes are essential in food biotechnology as they help improve the quality, texture, and flavor of food products, while also making the production process more efficient and cost-effective.

Applications of Different Microbial Enzymes in the Food Industry

Enzymes helps in reducing the energy required to catalyse the reaction, In living organisms. However, enzymes in plants and animals are present in small quantities and cannot be used for industrial applications. Microbial enzymes, on the other hand, offer many advantages, including easy handling, rapid multiplication, genetic manipulation, high production yield, and cost-effectiveness. They are eco-friendly, stable, and can convert hazardous compounds into useful products, making them ideal for use in several industries. Microbial enzymes are well-known biocatalysts for producing various products from a wide range of substrates. Therefore, their production at an industrial scale under varied physical and chemical conditions is essential. Microbial enzymes produced by microorganisms that act as catalysts to accelerate various biochemical reactions in food. These enzymes are widely used in the food industry for various applications, including fermentation, baking, brewing, cheese making, meat processing, and many more (Singh et al., 2016). There are several types of microbial enzymes, each with a unique role in the food industry. Here are some of the most commonly used types of microbial enzymes and their roles:

Proteases

Enzymes have become highly significant in global industry due to their ability to act as biocatalysts and decreased the minimum energy to catalyse reactions. Lactic acid bacteria are a well-studied a set of microorganisms, and their proteolytic system is particularly important for the utilization of casein and supply of essential amino acids.

The proteolytic system also plays a role in regulate the grade of polypeptides and regulating protein levels. These functions are important for the sensorial characterises of fermented milk products (Kieliszek et al., 2021). Proteolytic enzymes found in microbial cultures are essential in the dairy industry for producing cheese, yogurt, kefir, and other fermented dairy products. These enzymes not only break down proteins but also coagulate milk proteins during the cheese-making process. The resulting protein hydrolysates can be used to make easily digestible dairy products for sick individuals and children. Exogenous proteolytic enzymes are crucial for cheese production (Abada, 2018; Ozturkoglu-Budak et al., 2016). In meat industry proteases break down muscle protein macromolecules, disulfide bonds, and increase the reactivity of certain chemical groups in meat. This leads to the hydrolysis of sarcoplasmic proteins and partial hydrolysis of myofibrillar and connective tissue proteins. The result is tenderized meat, increased hydration, and improved protein digestibility. The content of soluble amino acids and small peptides increases, improving taste and texture while shortening maturation time and protecting against unfavorable microorganisms (Ahmad et al., 2020). Proteolytic enzymes enhance the flavor, texture, aroma, and color of meat products, improving sausage quality. The addition of proteases to meat has been found to have various positive effects, one of which is the ability to delay lipid oxidation. Lipid oxidation is a complex chemical reaction that occurs in the presence of oxygen, resulting in the degradation of fats in meat products. This process can lead to the formation of undesirable flavors, odors, and colors, as well as the production of harmful compounds that are detrimental to human health. By delaying lipid oxidation, proteases can help to preserve the quality and safety of meat products (Lorenzo et al., 2018).

Amylases

Amylases are capable of breaking down starch into reducing sugars. Microbial amylases could be derived from bacteria, fungi, and yeast. Amylases able to catalyse carbohydrates especially starch to simple units. Microbial amylases are widely used in the food processing for their ability to enhance texture, flavour, as well as nutritional value of various food products. They have application in manufacturing of glucose syrup, sweeteners, and alcoholic drink. The sources of microbial amylases include Bacillus, Aspergillus, and Rhizopus species, among others. These enzymes are more selective compare to other sources of amylases due to their high yield, stability, and ease of production. Amylases are widely used food product manufacturing, and they represent 25% of the global enzyme sell (John, 2017).

Pectinases

These enzymes are derived from microorganisms, including bacteria, yeasts, and actinomycetes. However, filamentous fungi are known to be particularly effective in producing pectinases. These enzymes are responsible for breaking down pectin, a complex carbohydrate found in plant cell walls, and to enhance the functional properties of food, juices and extracts. Fungal pectinases are considered to be among the most effective in terms of their ability to break down pectin, and they are often used in large-scale production processes (de Souza & Kawaguti, 2021). The use of pectinolytic enzymes is an important part of food processing, as it allows manufacturers to create a wide range of products with improved texture, flavor, and consistency (Jahan et al., 2017). Pectinases are used to clarify fruit juices by breaking down the pectin molecules that can cause cloudiness in the juice. This process helps to produce clear and smooth juice (Grassin & Coutel, 2010). These are used in the wine industry to improve the extraction of juice from grapes and to clarify the wine during the production process (Tapre & Jain, 2014). These enzymes are also used to soften fruit and to facilitate the extraction of juice during fruit processing. They are also used in the production of jams, jellies, and other fruit-based products. Pectinases are used to improve the quality of baked goods such as bread and cakes. They help to improve the texture and increase the volume of the products (Ozatay, 2020). Overall, pectinases are helpful to maintain the quality, texture, and consistency of various food products.

Cellulases

Microbial cellulases are derived from microorganism are responsible for breaking down cellulose, to glucose and fructose. Cellulases are widely used in the food industry for their ability to improve the texture and flavour of various products. One of the most important applications of microbial cellulases in the food industry is in the production of fruit juices. Cellulases used in the disintegration of cell wall of fruits, releasing the juice and making it easier to extract (Shariq & Sohail, 2019). This process improves the yield and quality of the juice, resulting in a product with a better taste and texture. Another important application of microbial cellulases in the food processing sector in the production of dairy products. These enzymes are used to break down the cellulose in the plant material used to feed cows, resulting in a better-quality milk. Cellulases can also be used to enhance functional characteristics of cheese, making it more palatable for consumers (Ejaz et al., 2021).

Transglutaminases

These enzymes crosslink proteins to form a stronger bond. Transglutaminases are used in the meat industry to create meat products with improved texture and juiciness. They are also used in the production of cheese, where they improve the texture and melting properties of the cheese (Akbari et al., 2021). The enzyme transglutaminase can be applied to fruits and vegetables as a coating to help maintain their freshness. In a particular study, applying transglutaminase to cut celery not only preserved its freshness, but also decreased bacterial growth (Manessis et al., 2020). Transglutaminases have applications in dairy products, such as cheese and yogurt. They can help to increase the firmness of cheese and improve its sliceability. In yogurt, they can help to improve the viscosity and stability of the product (de Góes-Favoni & Bueno, 2014). Transglutaminases are used in the manufacturing of bakery products, such as rusk and cakes. They can help to enhance the texture and volume of the food items. Furthermore, they are used to improve the texture and functionality of seafood food items, such as fish fillets and surimi. They can help to increase the firmness and gelation properties of these products. Transglutaminases are also used in the production of plant-based products, such as meat substitutes and dairy alternatives. They can help to improve the texture and functionality of these products, making them more similar to their animal-based counterparts.

Lactase

Lactases are a type of enzyme that has the ability to decompose lactose, which is the natural sugar present in dairy products and milk, into simpler forms of sugar such as glucose and galactose. In food processing industry, lactases are mainly utilized for the production of dairy products that are either lactose-free or have lower lactose content. This has gained popularity because many people are lactose intolerant (Sutay Kocabaş et al., 2022). Lactase is used in the dairy industry to improve the quality of dairy products. It can help to reduce the crystallization of lactose, improve the texture, and enhance the flavor of dairy products. Lactase is also used in the production of infant formula to make it easier to digest for babies who are lactose intolerant or have difficulty digesting lactose (Oak & Jha, 2019). Lactase is used in the baking industry to improve the texture and flavor of baked goods, such as bread, cakes, and cookies, synthesis of lactose. Lactase is also used in sports nutrition products to improve the digestion and absorption of lactose in athletes who consume large amounts of dairy products as part of their training diet (Odell & Wallis, 2021). Overall, the application of lactase in the food processing has several applications, in the production of lactose-free products, improving the quality of dairy products, and enhancing the digestibility of lactose in various food products.

Application of Enzymes as Food Antioxidants

Enzymes are not only essential for biological functions like digestion and metabolism, but they also have effective role in food preservation and antioxidant applications. Acting as natural antioxidants, enzymes can prevent food spoilage and enhance the shelf life of products by breaking down harmful substances. Certain enzymes, like catalase and superoxide dismutase, can specifically break down hydrogen peroxide and superoxide radicals, respectively, to prevent oxidative damage to food (Zeb, 2020). In food preservation, enzymes like lactase and peroxidase can be used in meat products to prevent lipid oxidation, which leads to spoilage (Bensid et al., 2022). Additionally, enzymes such as ascorbate oxidase and polyphenol oxidase can be utilized in fruits and vegetables to prevent browning and increase shelf life. Enzymes also have a place in food processing, where they can improve texture and flavor, such as tenderizing meat with papain and bromelain, or improving cheese and yogurt quality (Carocho et al., 2014). Overall, enzymes have a broad range of uses in food preservation, and quality improvement due to their natural antioxidant properties. With further research, the food industry can effectively use enzymes to produce healthier, higher-quality food products.

Enzymes Applications Dairy Industry

Enzymes have a vital role in the dairy industry, serving various stages of production. Cheese making heavily relies on enzymes, with rennet being a commonly used mixture that curdles milk to form curds (Mir Khan & Selamoglu, 2020). Proteases are also used during the aging process of cheese to break down proteins and fats, creating different flavors and textures for each type of cheese. Milk processing also benefits from enzymes, enhancing the quality and taste of dairy products. For example, lactase is a widely used enzyme that synthesis lactose in milk, making it tolerable for lactose intolerant individuals (Facioni et al., 2020). Proteases are also employed to enhance the flavor of certain dairy products. Enzymes are also essential in yogurt production, where lactase is the primary enzyme used to convert lactose in milk into glucose and galactose, creating a smoother and thicker texture. Other enzymes are also used to improve the texture and taste of yogurt (Sutay Kocabaş et al., 2022). Whey, a by-product of cheese making, can also be utilized through the use of enzymes. Enzymes can break down lactose and protein in whey to produce whey protein concentrate, which is high in protein and used in various food products, including nutrition bars and sports drinks. Overall, enzymes are essential in the dairy industry, with applications in cheese making, milk processing, yogurt production, and whey processing. These enzymes can help create high-quality dairy products with unique flavors and textures while also improving their nutritional value, ultimately satisfying the demands of consumers (Rocha & Guerra, 2020).

Enzymes Application in Meat Industries

Enzymes play a crucial role in the meat industry as they can significantly improve the quality, taste, and shelf life of meat products. In particular, enzymes like papain, bromelain, and ficin are used to tenderize meat by breaking down collagen and connective tissues present in muscle fibers. By doing so, the texture and tenderness of the meat are enhanced, resulting in a more enjoyable eating experience (Singh et al., 2018). Moreover, proteases, another type of enzyme, are used to enhance the flavor of meat. These enzymes break down proteins into smaller peptides and amino acids, contributing to the savory taste and aroma of cooked meat. Preservation is also an essential application of enzymes in the meat industry. Lactoperoxidase and glucose oxidase are examples of enzymes used for this purpose as they stop further growth of bacteria and maintain the color, organoleptic properties of meat products (Raveendran et al., 2018). In meat processing, enzymes like transglutaminase are utilized to bind meat pieces together, resulting in a uniform product such as sausages, meatballs, and burgers. Transglutaminase is particularly useful in creating meat products that have a consistent texture and shape, making it easier for manufacturers to produce a high volume of identical products (Kieliszek & Misiewicz, 2014). Lastly, enzymes like lipases are used to decrease the fat content in meat products by breaking down fat molecules into smaller components. This is particularly useful for consumers who are health-conscious and want to reduce their fat intake. By using enzymes, manufacturers can create leaner meat products without compromising their flavor or texture (Chandra et al., 2020).

Enzymes have various applications in the meat industry, and their use has become increasingly important for manufacturers who want to improve the quality, taste, and longevity of their meat products. The various enzymes used in the industry serve specific purposes, such as tenderization, flavor enhancement, preservation, meat processing, and fat reduction. By using enzymes, the meat industry can continue to innovate and provide consumers with high-quality products that meet their changing needs and preferences.

Application of Immobilized Enzymes in Food Industry

Immobilized enzymes have various applications in the food industry. They are used for producing high fructose corn syrup, wine, cheese, bread, beer, and fruit juices. These enzymes help in breaking down complex carbohydrates, coagulating milk, and breaking down pectin (Homaei, 2015). The use of immobilized enzymes improves the efficiency and quality of food production while reducing the cost and environmental impact of the processes (Yushkova et al., 2019). Immobilized enzymes have become increasingly popular in the food industry due to their advantages over free enzymes. Immobilization is a process in which enzymes are bound or trapped onto a solid support, such as a matrix or a carrier material, to create a stable and reusable enzyme system. One of the main benefits of immobilized enzymes is their increased stability, which allows for better performance and longer lifespan. They also offer improved control over enzyme activity, which is critical for precise reaction conditions and product quality. Additionally, making product recovery and purification more efficient. In the food industry, immobilized enzymes have broad range of use, such as improving food quality, enhancing process output, and reducing costs. For instance, immobilized enzymes can be used for the manufacturing of high-fructose corn syrup, which is a sweetener commonly used in the food industry. These Enzymes can also be used for the production of wine, beer, and cheese, as well as for the hydrolysis of proteins to produce bioactive peptides. Immobilized enzymes are also used in the production of fruit juices, where they can improve the yield and quality of the juice, as well as reduce processing time and costs. In the baking industry, immobilized enzymes are used to modify flour characteristics, resulting in improved dough handling properties and better texture of baked goods (Swaisgood, 2002).

Application Enzymes in Food Industry

The food industry encompasses a broad range of dairy products that includes various items such as milk (Fischer et al., 2011). Coagulants derived from acid proteases specifically chymosin (EC 3.4.23.4), are utilized in cheese production along with other enzymes. Chymosin is responsible for approximately 20–30% of milk coagulants that are utilized globally. Cheese maturation is facilitated by proteinases which expedite the process of protein breakdown, a crucial biochemical event that greatly influences the texture and flavor of cheese. Moreover, peptidases are employed to eliminate the bitter taste resulting from protein breakdown during cheese maturation (Hati et al., 2013). The types of cheese estimated by the process of maturation, which results in unique textures and aromas. During the maturation process, Lipase synthesis the triglycerides to simple glycerol lead to development of flavour in cheese. The use of lipases in the maturation process can accelerate the process by two to five times, but it is crucial to carefully regulate the amount and activity of the enzyme, as excessive lipases can cause rancidity and a decrease in cheese yield (Chandra et al., 2020). The meat industry faces a significant challenge in improving the sensory qualities of meat products. Proteolytic enzymes can be used to tenderize meat without the use of chemicals and brines, and while papain and ficin have been used, transglutaminase is the most widely utilized enzyme. This enzyme, first produced by *Streptoverticillium mobarence* in the early 1980s, offers advantages over other enzymes due to its low cost and ability to enhance nutritional value by adding essential amino acids to protein matrices. Transglutaminase also improves the texture, firmness, elasticity, and emulsification of meat products like sausages, resulting in higher quality and greater variety. In combination with proteases, thermolysin is employed to produce food protein and hasten the ripening of dry sausages (Cobos & Díaz, 2015). When it comes to making fermented beverages like wine and beer, the main priorities are optimizing the process, improving yield, and maintaining or enhancing colors and flavors. Enzymes can help achieve these goals while also reducing the calorie and sulfur content of beer and improving wine clarity. For example, sulfhydryl proteases can enhance clarity and remove butter odor in beer caused by diacetyl. β-glucanases are used to reduce beer viscosity through β-glucan hydrolysis. Pectinases play a critical role in wine and beer production by facilitating extraction and filtration, improving juice yield, flavor, odor, and clarification. Although fruits and vegetables contain pectinases with low activity, the industry typically uses microbial enzymes because of their stability and resistance to fermentation conditions. For flavored beverages, juice producers must address issues related to stability, quality, clarification, viscosity, and yield during production to ensure consumer acceptance. The nutritional value of food products has become increasingly important to consumers. Enzymes such as α-galactosidase and phytases are commonly used to improve food products quality, particularly those made from legumes and cereals. Phytate is known to reduce the bioavailability of nutrients by binding with positively charged proteins and chelating divalent and multi-valent cations. Phytase efficiently dephosphorylates phytate, releasing minerals and increasing their bioavailability. The development of hypoallergenic foods is another potential market area. Antigenic proteins found in wheat, peanuts, soybeans, chickpeas, milk, and eggs can cause allergic reactions. Enzymes such as proteases, actinase, alcalase, flavourzyme, and neutrase can help reduce allergens, providing a health benefit. Enzymes are also used in the food industry to monitor process quality parameters. Biosensors that use enzymes can tightly combine biorecognition elements and physical transducers to monitor and detect target compounds (Vashishth et al., 2017).

Role of Microbial Enzymes in Food Analysis

Microbial enzymes play a critical role in food analysis as they can be used for the detection and quantification of various food parts like carbohydrates, proteins, lipids, and enzymes themselves. They can also be used to assess food quality, freshness, and safety. Protease enzymes produced by microorganisms such as bacteria, fungi, and yeast can be used to analyse protein content in food. For example, the enzyme trypsin can be used to hydrolyze proteins in food samples, and the resulting peptides can be analysed using advance techniques to determine protein content. Protease enzymes have a important role in food analysis as they used to determine the protein content of food and to identify specific proteins of interest. Protease enzymes have a range of applications, including food processing, quality control, and safety assurance. In food analysis, protease enzymes disintegrate protein to peptides, which can then be quantified using various analytical techniques such as chromatography or spectroscopy. Protease enzymes such as trypsin, pepsin, and papain are commonly used for this purpose (Panchaud et al., 2012). One application of protease enzymes in food analysis is the determination of protein content in food samples. The most commonly used method for determining protein content in food is the Kjeldahl method, which involves the digestion of proteins with sulfuric acid and the subsequent quantification of the resulting ammonia. However, this method can be time-consuming and requires specialized equipment. Alternatively, protease enzymes such as trypsin can be used to digest proteins in food samples, and the resulting peptides can be quantified using analytical techniques such as HPLC or MS (Arte et al., 2015). Another application of protease enzymes in food analysis is the identification of specific proteins in food samples. Protease enzymes can be used to digest proteins into smaller peptides, which can then be analysed using mass spectrometry or other techniques to identify specific proteins. This approach can be useful for detecting allergens, contaminants, or other specific proteins of interest in food samples. Overall, protease enzymes play a crucial role in food analysis, enabling the determination of protein content and identification of specific proteins of interest in food samples (Schlüter et al., 2008).

Amylase enzymes produced by microorganisms can be used to analyze carbohydrate content in food. For example, the enzyme alpha-amylase can be used to hydrolyze starch in food samples, and the resulting glucose can be quantified using techniques such as colorimetry or HPLC (Hall, 2015). Lipase enzymes produced by microorganisms can be used to analyze lipid content in food. For example, the enzyme lipase can be used to hydrolyze triglycerides in food samples, and the resulting fatty acids can be quantified using techniques such as gas chromatography (GC) or HPLC. ELISA is a technique that uses antibodies to detect and quantify specific food components. Microbial enzymes can be used to produce these antibodies, which can then be used in ELISA assays to detect food allergens, pathogens, or contaminants (Chandra et al., 2020).

Conclusion

The current study, the use of microbial enzymes has brought about significant advancements in the food processing sector by offering several benefits in food production and preservation. These enzymes are essential in the production of various food products such as dairy, meat, bread, and beer, among others. They have proven to be superior to traditional enzymes because of their specificity, cost-effectiveness, and ability to ensure precise control over the final product's quality. Additionally, they are more environmentally friendly and sustainable compared to chemical catalysts, which pose health and environmental risks. The increasing demand for safer, healthier, and more sustainable food products will continue to drive the adoption enzymes derived from microrgansims.

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