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REVIEW ARTICLE
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Enzyme Applications in the Food Industry- A Clean Food Processing Update

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ABSTRACT

Enzymes are important bio-catalysts that are essential for normal physiological functions in living organisms. In addition to their biofunctional properties, various enzymes find applications in industry. These enzymes are highly specific and efficient, accelerating reactions and reducing harmful compounds. This review article examined recent advances in the use of enzymes in the food industry. Among enzyme classes, hydrolases (amylases, cellulases, esterases, lipases, pectinases, proteases, xylanases, etc.) are widely used in the food industry. The applications and functions of various enzymes across food industries, including dairy, beverages, bakery, starch, meat, and others, have been comprehensively discussed. Furthermore, the stability of enzymes across all food processes has been revisited, and emerging technologies for enzyme stabilization have been presented. Lastly, the future direction for the efficient utilization of enzyme technologies has been outlined.

Keywords: Enzymes, Enzyme activity, Enzyme stabilization, Food industries, Applications.

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INTRODUCTION

The food industry is looking for more environmentally friendly ways to produce food in response to rising public concerns about food and agricultural sustainability, environmental resilience, and food safety. The usage of enzymes is an old practice. Pasteur was the first to perform an enzymatic reaction—the fermentation of sugar into alcohol using yeast as a catalyst—and Kunhe was the first to use the name enzyme, which in Greek means "in yeast" in the early nineteenth century. Later in 1926, Sumner purified the first known enzyme, urease, from jack beans. Later, in the 1950s, a protease enzyme was produced from the bacterium Bacillus licheniformis using a method developed by Northrop and Stanley, which enabled mass-scale enzyme production and further facilitated its industrial applications. Enzymology has never been the same since the chemical synthesis of enzymes was made possible in 1969. Various synthesis and characterization techniques advance the development of enzymes and their applications (Li & Gilbert, 2016; Hau et al., 2024). This has led to the invention of different synthetic catalysts to enhance applications and processes across numerous sectors. Given the aforementioned assertion, enzymes are in high demand across numerous industries because they catalyze specific reactions (Singh, 2018; Okpara & Harding, 2024).

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Enzymes are unique proteins with catalytic activity that are essential for various physiological processes. The majority of them fall under the category of globular proteins. Also, enzymes are found in our foods in both their active and inactive states. Enzymes are excellent biological catalysts that speed up processes by providing an alternate, lower-activation-energy reaction pathway. Enzymes have the additional benefit of accelerating reactions without changing their equilibrium (Ray et al., 2016; Siddikey et al., 2025). Enzymes are frequently categorized and given names based on the reactions they catalyze. For example, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases catalyze oxidation-reduction reactions, transfer functional groups, hydrolyze bonds, cleave bonds, isomerize molecules, and connect molecules, respectively. These enzymes can be readily extracted from microbes, animal tissues, and plants. The production and use of enzymes are growing rapidly in the domains of food, feed, and medicine, fine chemicals, health care, and environmental sustainability. The use of enzymes in food has garnered significant interest, as consumers pay closer attention to food safety and quality (Choi et al., 2015; Okpara & Harding, 2024). Enzymes have been employed in various food product development, such as brewing, bakery, dairy and fruit juice industries. The majority of enzymes used in food processing originate from microorganisms (Ladics & Sewalt, 2018; Mao et al., 2024). Hydrolases (such as esterases and carbohydrases) are widely used in the food industry and add or remove water molecules during various reactions. Most importantly, hydrolases play a crucial role in degrading natural polymers like starch, fibers, proteins, lipids and other complex structures into simpler forms. For instance, casein or chymosin are enzymes used in the milk industry and lipases, proteases, xylanases, amylases, cellulases, and asparaginases are used in other food processing industries (Binod et al., 2019). This process is analogous to enzymatic digestion in mammals, where catabolic enzymes break down complex macromolecules into simpler, absorbable compounds (Arbige et al., 2019).

Enzymes are increasingly used in commercial food processing, and their range of potential applications will continue to grow (Peterson et al., 2007). Industrially, they also help lower manufacturing costs; increase and improve product yield; improve safety; and reduce chemical and toxic by-products. Additionally, they can help improve nutritional value, flavor, color and texture. Moreover, because enzymes are used to process foods for both humans and animals, they are considered safe and are classified as Generally Recognized as Safe (GRAS). As a result, more modern enzymes are being engineered for use under specific conditions, thereby increasing their maximum production. For example, pectinase and lysozyme for low-temperature operations; lactase for low-pH operations; glucose oxidase for oxidative stability requirements; and decarboxylase for substrate-specific operations (Choi et al., 2015; Sewalt et al., 2016; Arbige et al., 2019; Yang et al., 2023).

Enzyme Classification

The Enzyme Classification (EC) number is a numerical system used to classify enzymes based on their catalytic activity. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology suggested this classification, and it has been generally accepted (Concu & Cordeiro, 2019). The Enzyme Commission established a numerical classification system for enzymes in 1961. This scheme divides enzymes into 6 main classes, i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. These six classifications remained unchanged until August 2018, when translocase enzymes were recognized and subsequently added as the 7th classification. The EC nomenclature is divided into four parts that define the enzyme's main class, subclass, sub-subclass, and substrate class (Tao et al., 2020). The listing of an enzyme's main class and subclasses is presented in Table 1. These EC classes are further divided into sub-subclasses based on parameters such as the chemical bond cleaved or created, the reaction center, the transferred chemical group and the catalytic cofactor (Martínez Cuesta et al., 2015).

Applications in Food Industries

Enzymes have been a crucial part of food processing even before they were identified as biological catalysts (Gomes et al., 2018). Enzymes are used in various procedures, including meat tenderization (via papaya leaves), soy sauce production, curd or cheese manufacturing, baking, brewing, and more (Fig. 1). Additionally, their role in optimizing food digestion in the human body is recognized. Microbial enzymes have been widely employed in the food industry to enhance product quality, range and diversity (Singh et al., 2019). The various applications of enzymes representative of common food-industry usage are shown in Table 2.

Starch Industry

Starch is the major plant polysaccharide composed of amylose, a linear α-1,4-linked glucan, and amylopectin, a branched glucan containing α-1,4 and α-1,6 linkages (Kumar et al., 2023). Native starches' physicochemical

Table 1: Enzymes' classes, reactions, and functions

Enzyme classes	Reaction catalyzed	Subclasses (based on functional parameters)	References
Oxidoreductases	$^{a}AH_2 + B = A + BH_2$	Catalyze oxidation/reduction reactions	(Martínez Cuesta et al., 2015)
Transferases	AX + B = BX +A	Transfer a chemical group	
Hydrolases	A-B +H_2O = AH + BOH	Hydrolysis of chemical bonds	(Tao et al., 2020)
Lyases	A=B + X - Y = A -B Y X	Chemical bond cleavage using a method other than oxidation or hydrolysis	(Strauss, 2010)
Isomerases	A=B	Change the isomers' geometry and structure	
Ligases	A + B + ^{b}NTP = A-B +NDP + P	Connect two compounds by the hydrolysis of a nucleoside triphosphate molecule	
Translocases	AX + $B_{(side\ 1)}$ II= A+X + II B $_{(side\ 2)}$	Translocation of anion and cation	

Table 2: Application of enzymes in different systems

Industry	Enzyme	Mechanism	Application	References
Dairy	Rennet	Cleavage of peptide bonds and a series of reactions to produce cheese	Cheese production	(Abada, 2019; Sutay Kocabaş et al., 2022)
	Lipase	Hydrolysis related to triglycerol, diacylglycerol, and monoglycerol	Development of Cheddar cheese and other cheese flavors. Proteinases combined with lipases or peptidases generated cheeses with mild to sharp flavor (age-dependent) and minimal bitterness.	(Li et al., 2023)
	Protease	Hydrolyze the peptide bonds that connect amino acids to proteins	In cheese making, proteases are added to milk to hydrolyze caseins.	(Gurumallesh et al., 2019)
	Lactase	Increases lactose breakdown into galactose and glucose	Increase the sweetness, solubility, and ease of digestion for milk products. Decreasing and eliminating lactose in milk products.	(Khan & Selamoglu, 2020)
	Transglutaminase	Catalyzes posttranslational protein modification via transamidation of glutamine residues and the formation of glutamine-lysine crosslinks.	Improves milk gel strength by cross-linking caseins and whey proteins	(Akbari et al., 2021)
Beverage	Pectinase	Hydrolysis of pectic substances	The immobilized pectinase enzymes in grape juice enhance the clarification (compared to untreated juice)	(Azimi et al., 2021)
			The immobilized pectinase enzymes in barberry juice enhance the clarification, physiochemical properties, color, and antioxidant properties (compared to untreated juice)	(Hosseini et al., 2021)
	Xylanase	Hydrolyze the β-1,4 glycosidic linkage of the xylan chain	Beer viscosity and filtration time are reduced by utilizing novel thermostable xylanase	(Amel et al., 2016; Wang et al., 2016)
	Cellulase	hydrolyze the β-1,4-glucosidic bonds of cellulose	Clarification of fruit juice	(Ozyilmaz & Gunay, 2023)
Baking	α-amylase	Cleavage enzyme cleaving α-1, 4-glycosidic bonds in the starch's inner region. Rapid reduction in substrate molecular weight and viscosity	Degradation of starch in flour Sugar release boosts bread volume Regulation of bread's volume and crumb structure Act as an anti-staling property	(Gomes et al., 2018; Miguel et al., 2013)
	β-amylases	The cleavage enzyme breaks down to bind glucose and produce maltose	Intensify bread color and crust Responsible for Maillard's reaction	(Miguel et al., 2013)
	Protease	Cleave the peptide bond nearest to the substrate's amino or carboxy terminus	Decrease the consistency (fluidity?) of the dough and the mixing time required Regulate gluten strength in the bread Reduce protein in flour	(Miguel et al., 2013)
	Lipoxygenase	Catalyze the addition of oxygen to polyunsaturated fatty acids, generating fatty acid hydroperoxides		(Patel et al., 2016)
	Transglutaminase	Catalyze the wheat proteins, and crosslink glutamine with lysine	Improved dough strength	(Gomes et al., 2018)
	Xylanase	Hydrolyze the β-1,4 glycosidic linkage of the xylan chain	Pentosan degradation promotes water redistribution and gluten network formation.	(Gomes et al., 2018)
	Asparaginase		Reduction of acrylamide production during baking	(Covino et al., 2023)
Starch	α-amylase	Cleavage enzyme cleave α-1, 4-glycosidic bonds in the starch's inner region. Rapid reduction in substrate molecular weight and viscosity	Fermentation of beverages such as beer	(Disharoon et al., 2021; Letsididi et al., 2008)
	β-amylases	The cleavage enzyme breaks down to bind glucose and produce maltose	Fermentation of beverages such as beer	(Letsididi et al., 2008)
	Pullulanase	Hydrolyze the α-1-6 glycosidic bond in pullulan and amylopectin	Starch modification and enhancement of SDS and RS. Enhance the SDS and RS compared to the native variety.	(Sahoo & Roy, 2023)
	Glucoamylase	Breakdown α-1, 4-linkages and α-1, 6-linkages from the non-reducing ends to release β-d-glucose.	Starch saccharification. Release of glucose as an end product.	(Parashar & Satyanarayana, 2017)
	Amylosucrase (AS)	Catalyze the transglycosylation process utilizing sucrose as a substrate while releasing fructose, and elongating starch chains by adding glucose molecules to their non-reducing ends	AS-modified corn starch increases SDS and RS content compared to native corn starch	(Shin et al., 2010)
			AS-modified waxy corn starch was found to be better than native corn starch in terms of forming starch-fatty acid complexes.	(Lim et al., 2019)
			Expression of AS in potatoes resulted in improved physiochemical properties, freeze-thaw cycle, and digestibility	(Huang et al., 2014)
	Isoamylases	Hydrolysis of α-1, 6-linkages to amylopectin		(Gous & Fox, 2017)
Meat	Protease Papin Bromolein Ficin Actinidin Zingibain		Proteins found in myofibrils, namely desmin, titin, troponin, and nebulin. Insignificant effects on actin and myosin action on both myofibrillar and collagen proteins	(Bekhit et al., 2014; Gomes et al., 2018)

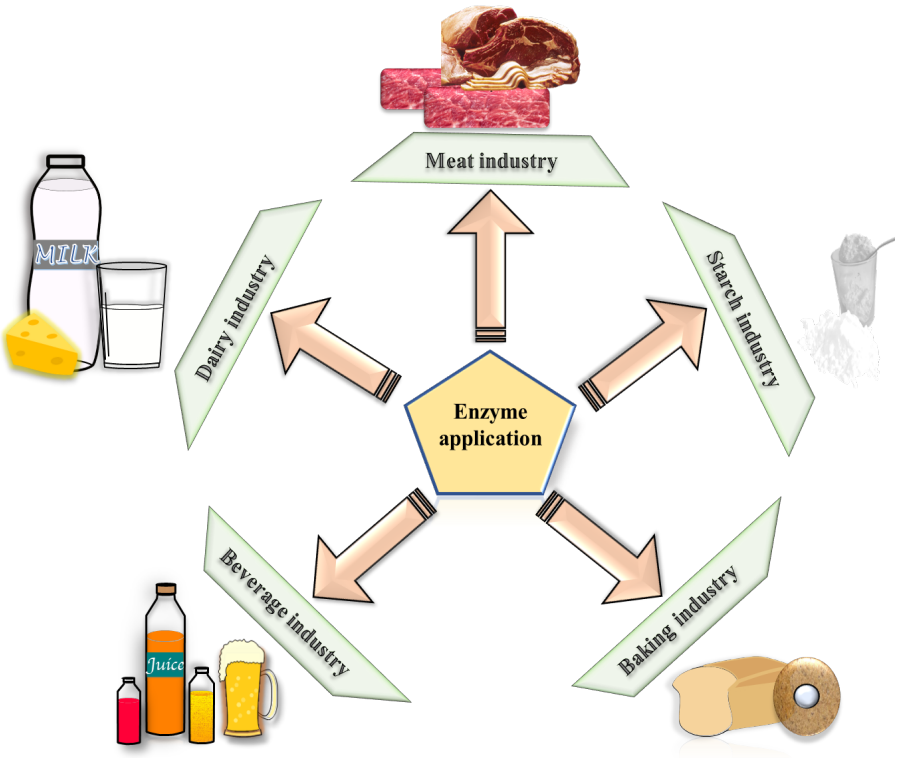

Fig. 1: Enzyme applications in different food industries.

properties do not meet the requirements of nutritional and industrial applications due to susceptibility to pH, high temperatures and freezing conditions (Park et al., 2018). Thus, enzymatic approaches to modify such starches have not only been found to be efficient but are also considered environmentally friendly (Zhang & Bao, 2021). Enzymatically-modified starches are termed "Clean Label Ingredients" because these approaches are free from synthetic and artificial chemicals (Park et al., 2018). Two major types of enzymes normally used to modify starches are glycosyl hydrolases and transglycosylases. While functionalization of starch can be achieved using β-amylase, α-amylase, amylosucrase, cyclodextrin glycosyltransferase, debranching enzyme (pullulanase, isoamylase), and branching enzyme (glucan transferase), among others (Punia Bangar et al., 2022). Commercially, cyclodextrins are produced from starch by utilizing a synergistic combination of α-amylase and bacterial cyclodextrin glycosyltransferase. Cyclodextrins can be used to incorporate hydrophobic molecules into their hydrophilic exterior surface and a moderately hydrophobic core chamber (Honda, 2017).

Recently, amylosucrase (AS) from *Neisseria polysaccharea* has attracted considerable attention because it can produce starches rich in low-glycemic-index starch fractions, slowly digestible starch (SDS), and resistant starch (RS). When incubated with sucrose at 37°C, AS utilizes sucrose as a substrate to increase the length of the non-reducing end of amylopectin and amylose external chains by 13–19 glucose units. This creates more stable double helices, which are further held together by hydrogen bonds and also changes the crystalline region. This makes it harder to digest by increasing the amount of SDS and RS (Lee & Park, 2020). Apart from this, glucan branching enzymes also promote the α-1,6-linked branches in starch, which are essential for starch modification with favorable characteristics (Chengyao et al., 2021). The debranching enzyme reaction involves three exclusive mechanisms- intra-chain transfer, inter-chain transfer, and intra-chain cyclization. Debranching enzymes such as pullulanase and isoamylase catalyze the hydrolysis of α-1, 6 glycosidic bonds, resulting in linear glucan chains (Li & Gilbert, 2016). Hence, enzymes are important for the starch industry because they help break down starch into simpler sugars, which can then be used in various products.

Bakery Industry

Baked goods are gaining popularity due to their taste and nutritional benefits, as well as ease in large-scale feeding programs and during global emergencies such as natural disasters (Arepally et al., 2020). Baking is a traditional method of cereal-based cooking that occupies a prominent place in the modern food processing industry. Consumer preference for baked food stuffs is based on personal perceptions of flavor, color, texture, and aroma. Baking involves coupled heat and mass transfer phenomena, including non-enzymatic browning, starch gelatinization, and protein denaturation, which together determine the final crumb structure (Devu et al., 2022). In addition, enzymes play a crucial role in most baked goods, particularly bread and other fermented products, by improving dough handling, fermentation, and product quality (Kumar et al., 2024). Enzymes used in baking come from three different sources: namely, endogenous enzymes found in grains, enzymes from microorganisms, and exogenous enzymes added to the dough (Miguel et al., 2013). Enzymes such as α- and β-

amylases play distinct yet complementary roles in bread-making. During dough preparation, α-amylase hydrolyzes starch into low-molecular-weight dextrins, which are subsequently converted into maltose by endogenous β-amylase. This maltose serves as a fermentable sugar for yeast or sourdough microorganisms, supporting gas production and dough development (Kim & Yoo, 2020). As in the starch industry, most bakery products are derived from starchy raw materials, highlighting the crucial role of enzymes in degrading complex polymers into simpler molecules that can participate in biochemical reactions. In addition, enzymes significantly enhance dough handling properties and improve the texture, flavor, volume, and overall appearance of baked goods. Moreover, with growing consumer demand for clean-label and natural products, modern enzyme formulations are increasingly used to replace chemical additives and simplify ingredient lists in bakery products (Liu et al., 2024).

Dairy Industry

The dairy industry is one of the most important industrial sectors for enzyme applications because it produces a wide variety of foods, including cheese, butter, and yogurt. (Roohi et al., 2019). The enzymes used in these applications range widely, from coagulants used in cheese production to bioprotective enzymes that increase shelf life. Esterase, protease, catalase, rennets/chymosin, lipases, and lactases are widely employed in dairy, and their mechanisms are well discussed (Khan & Selamoglu, 2020; Sutay Kocabaş et al., 2022). As shown in Table 2, these enzymes and their specific roles as biocatalysts are critical to the conversion of milk into other products. The manufacture of a great majority of dairy items, now commonplace in our modern world, would not be feasible without the chemical reactions that are set in motion, regulated, and accelerated by enzymes.

Protease

Proteases are a group of large, complex enzyme molecules that perform highly specialized proteolytic functions. Proteases hydrolyze the peptide bonds that connect amino acids to proteins (Gurumallesh et al., 2019). Some important properties of proteases include substrate specificity, active site property, catalytic action, optimal pH and temperature and stability characteristics (Martínez-Medina et al., 2019). Proteases can be extracted from various sources, including plants (e.g., bromelain from pineapple, ficin from fig, actinidin from kiwi, and papain from papaya), animals (e.g., chymosin, chymotrypsin, pepsin, and pancreatic trypsin), and microbes (Gurumallesh et al., 2019; Sutay Kocabaş et al., 2022). Proteases are divided into exopeptidases and endopeptidases, depending on their mechanism of action. Plasmin is an extensively studied indigenous protease in bovine milk. Researchers' interest in plasmin-induced proteolysis has grown due to its complexity and implications for milk and dairy products quality (Sutay Kocabaş et al., 2022). In the cheese-making process, proteases are added to milk to hydrolyze caseins, a critical step in curd formation, texture development, and flavor generation. In addition, hydrolytic proteases are widely preferred in the production of infant milk formula, as controlled protein hydrolysis improves digestibility and reduces allergenicity (Fox et al., 2022; Li & Zhu, 2024).

Rennet

Rennet is one of the earliest known exogenous enzymes used in the preparation of dairy foodstuffs, particularly cheese. Traditionally, the dairy industry has employed animal-derived rennet as a milk-coagulating agent in the manufacture of high-quality cheeses, where it contributes to milk coagulation, texture development, and flavor formation (Abada, 2019; Niero et al., 2024). Traditionally, rennet is obtained from the fourth stomach of calves less than 30 days old, as rennet is a combination of renin and pepsin (Sutay Kocabaş et al., 2022). Theoretically, there are three phases to enzymatic coagulation utilizing calf rennet. In the first 10min, calf-rennet cleaves approximately 80% of the casein micelles, leaving hydrophobic para κ-caseins on the surface. This then leads to a second stage, in which the partially stripped casein micelles join to form clusters. Due to electrostatic and hydrogen-bonding interactions, the contact region between casein micelles can expand over time. Depending on the degree of proteolysis and the specificity of the enzymatic activity, this second stage may lead to a partial or, possibly, complete fusion of the casein micelles. In the third stage, the casein micelle clusters grow further, and an extended gel network of strands, crosslinks, and junction nodes is produced in this last phase (Callaghan-Patrachar et al., 2021). Currently, thistle flower stigmas (often from the cardoon thistle) and lady's bedstraw (*Galium verum*) offer plant-based alternatives to calf-sourced rennet for producing a variety of cheeses. Additionally, wild-type microbial chymosins have also been recognized as a plant-based alternative to rennet. These commercially produced enzyme preparations can be sourced from genetically modified micro-organisms or wild-type varieties such as *Mucor species* (Sutay Kocabaş et al., 2022). Moreover, alternative micro-organisms like *Aspergillus oryzae*, *Rhizomucor miehei*, *Rhizomucor pusillus*, *Endothia parasitica*, and *Irpex lactis* are commonly used to produce rennet for the cheese-making industry (Abada, 2019).

Lipase

Lipase belongs in the hydrolase enzyme classification. These hydrolyzed ester linkages in triglycerides produce fatty acids, partial glycerides (such as mono- and di-glycerides), and glycerol at the oil-water interface (Javed et al., 2018). Most lipases used in industry are microbial in origin and produced by bacteria, fungi, and yeast (Vivek et al., 2022). Microorganisms produce microbial lipases either alone or in combination with esterases. *Serratia marcescens*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are a few examples of microbes that produce lipase (Abada, 2019). Lipases play an important role in cheese ripening, texture, and flavor development, mostly by indigenous and endogenous

lipases (Sutay Kocabaş et al., 2022). For instance, lipases from *Mucor miehei* or *Aspergillus niger* have been employed to enhance the richer taste of Italian cheeses by increasing free butyric acid (Patel et al., 2016). Animal or microbe-derived lipases made clear cheese with low bitterness, enhanced taste, and a robust aroma. However, cheeses made with proteinases, lipases, or peptidases have a highly desirable taste and a characteristic absence of bitterness (Sharma & Sharma, 2018). Lipases exhibit substrate selectivity in terms of chemo-specificity, regio-specificity, and enantioselectivity, which underlie their broad biotechnological utility. Chemo-specificity includes fatty-acid and lipid-class specificity, enabling lipases to hydrolyze triacylglycerols as well as di- and monoacylglycerols. Based on regio-specificity, lipases are classified as non-specific or 1,3-specific enzymes; the latter preferentially hydrolyze ester bonds at the *sn-1* and *sn-3* positions of triacylglycerols, producing free fatty acids and diacylglycerol intermediates that may undergo acyl migration. Enantioselectivity allows lipases to discriminate between enantiomers in racemic substrates, a property widely exploited in asymmetric synthesis (Patel et al., 2022).

Lactase

Lactase is an enzyme that hydrolyzes lactose into glucose and galactose. It is widely used to enhance the sweetness and solubility of milk and dairy products and to improve lactose digestibility, particularly for lactose-intolerant consumers (Facioni et al., 2020). These play a prominent role in the diets of individuals with lactase insufficiency in their digestive tracts (Sutay Kocabaş et al., 2022), which leads to a widespread hereditary disorder of lactose intolerance (Churakova et al., 2019). Thus, mass-produced lactose-free or low-lactose foodstuffs are currently predigested using exogenous lactase and β-galactosidase (Zhang et al., 2020). Lactases are usually categorized as neutral or acid, with neutral lactases used in dairy products and acid lactases used as nutritional supplements. These enzymes are from various glycosyl hydrolase families and have highly varied sequences, structures, and biochemical characteristics (Dekker et al, 2019). Lactase may be obtained from a variety of sources, including plants, animal organs, bacteria, yeasts (intracellular enzymes), and molds. These are then used to manufacture additive enzyme preparations for commercial applications, designed to achieve specific results in the end product. A safe-for-consumption profile has been granted to lactase production derived from *Aspergillus niger*, *Aspergillus oryzae*, and *Kluyveromyce slactis*. This is based on a long record of harmlessness, as well as research from available sources establishing its safe use history. These sources and the enzymes acquired from them have undergone systematic toxicological safety tests. This is in stark contrast to a common lactase derived from *Escherichia coli*, which is unsuitable for enzyme production due to its high cost and causes of food poisoning (Abada, 2019).

Transglutaminase

Transglutaminase is an enzyme that catalyzes the formation of covalent cross-links between glutamine and lysine residues in various dietary proteins, including milk casein (Domagała et al., 2016). Transglutaminase modifies proteins through mechanisms such as amine insertion, protein crosslinking, and deamination. These reactions result in the formation of high-molecular-weight protein polymers with altered functional properties, improving the rheological behavior and sensory attributes of food products (Çelik & Sarıçoban, 2023; Akbari et al., 2021). Transglutaminase cross-links αS-, β-, and κ-caseins in milk by connecting between and within casein micelles. Unfolded or denatured forms of whey proteins like α-lactalbumin and β-lactoglobulin are the most suitable substrates for transglutaminase (Aaltonen et al., 2014). Further, it has been established that increasing transglutaminase levels up to 0.5% enhances the functional qualities of yogurt produced from goat milk (Khan & Selamoglu, 2020). Apart from this, transglutaminase is also used in conjunction with rennet to modify cheese's nutritional and textural characteristics and to increase yield. It is advised to add both rennet and transglutaminase simultaneously to achieve these results (Domagała et al., 2016). Additionally, transglutaminase has been shown to arrest the increase in moisture content, which reduces the cheese's hardness and condensed mass dimensions. As seen in Edam cheese production, the use of transglutaminase enhances yield by 4% (Aaltonen et al., 2014). The one that relates to all of the enzymes discussed in this section, including lactase, transglutaminase, lipases, rennet, and protease, is the breakdown of complex molecules to create a distinct texture or flavor in various dairy products, such as cheese and yogurt, as well as increasing their rheological property, sensory quality, and consumer acceptance.

Beverage Industry

Beverage processing industries utilize raw materials through various key procedures, such as extraction and maceration. Pectin, cellulose, starch, proteins, tannins, and lignin are responsible for the cloudiness of fruit juice and cause fouling problems during juice filtration. To address these issues, pectinases, amylases, and cellulases were used in the beverage industry to improve extraction, clarity, and aroma, among other desired manufacturing and product objectives (Ramadan, 2019; Uzuner & Cekmecelioglu, 2019).

Juice Industry

Plant cell walls are composed of complex polysaccharides, predominantly hemicellulose, cellulose, and pectin. These compounds are primarily composed of dietary fibers and are the major building blocks of cell walls for fruits and vegetables (Danalache et al., 2018; Toushik et al., 2017). The initial turbidity in freshly pressed fruit juices occurs due to cell debris and small insoluble pectin, whereas haze formation can be caused by prior polymerization or condensation between sugars, polysaccharides, proteins, and metal ions (Danalache et al., 2018; Kashyap et al., 2001). Several enzymes can degrade complex polysaccharides in cell walls during the

processing of vegetables and fruits into their final beverage products. Cellulases, hemicellulases, and pectinases are the enzymes that are most often used to break down the native carbohydrate matrix (Danalache et al., 2018; Toushik et al., 2017; Bennett, 2025).

Pectinase, one of the most commercially important enzymes, plays a crucial role in the production of fruit juices, soft drinks and alcoholic beverages by improving juice yield, clarity, and processing efficiency (Jayani et al., 2022; Kashyap et al., 2001). Pectinase is considered essential in juice purification because it degrades the pectin and lowers turbidity (Hosseini et al., 2021). Pectinases are broadly classified into three main groups: pectinesterases, depolymerizing enzymes (including polygalacturonases and pectate lyases), and proto-pectinases (Jayani et al., 2022). About 25% of all food enzymes sold worldwide are microbial pectinases. They are primarily derived from the filamentous fungus known as *A. niger* (Ahmed & Sohail, 2020). Pectinase is produced by only a small number of bacterial taxa, including *Bacillus*, *Pseudomonas*, *Chryseobacterium*, and *Erwinia* (Prajapati et al., 2021). Most microbial pectinases are used to extract and clarify juice by hydrolyzing fruit-pulp polysaccharides, thereby decreasing turbidity and viscosity and imparting consistency. Pectinase also improves the pulp-press process, dissolves the jelly structure, increases fruit juice yields, stabilizes juice shelf life, and retains end-product quality (Prajapati et al., 2021). Additionally, a concentrated combination of hemicellulases and cellulases is applied as an enzymatic treatment for peeling apricots, nectarines, and stone fruits (such as peaches) (Toushik et al., 2017).

Cellulases are another class of enzymes that have gained substantial industry attention for their potential to process previously discarded cellulosic biomass and convert it into marketable products. The synergistic impact of cellulases (such as exoglucanases, endoglucanases, and β-glucosidases) on cellulosic biomass is essential for cellulose depolymerization (Ramadan, 2019). Tannin acyl hydrolase, known as tannase, is an intracellular or extracellular enzyme. It catalyzes the hydrolysis of gallotannins, epigallocatechin-3-gallate, and other gallic acid-containing ester and depside linkages, releasing glucose, gallic acid, and related phenolic acids (Biswas et al., 2022). Additionally, tannase contributes to fruit ripening and the production of acorn wine, instant tea, and coffee-flavored soft drinks (Aracri et al., 2019).

Beer Industry

Beer is an extremely complex beverage comprising approximately 3,000 distinct constituents, including carbohydrates, proteins, ions, microorganisms, organic acids, and polyphenols. It is recognized as one of the most consumed alcoholic beverages worldwide, following water and tea, and represents a significant economic segment of the global beverage market (Anderson et al., 2019). Beer production fundamentally relies on microbiological and enzymatic activities occurring during both the malting process and the alcoholic fermentation of wort, which play a critical role in determining product quality, flavor development, and processing efficiency (Bokulich & Bamforth, 2013). Broadly speaking, the phases of brewing are: barley drying, malting, milling, wort generation, hopping, fermentation, maturation, and bottling (Romero-Rodríguez et al., 2022). A variety of enzymes, such as α-amylase, β-glucanase, lipase, protease, pullulanase, and xylanase, are used during the beer production phases (van Donkelaar et al., 2016). Pullulanase is added to beer during the mashing process. Pullulanase enzymes promote the hydrolysis of the α-1, 6 branch of starch, resulting in maximal fermentability of the wort. Consequently, this increased fermentability improves the yield of low-calorie beer. Furthermore, adding pullulanase can reduce dextrin content during beer production, thereby more accurately controlling the body, taste, and color of the end product (Prakash et al., 2012). The main starch-degrading enzymes utilized in the beer industry are α-amylase, β-amylase, and α-glucosidase. The enzyme α-amylase induces the breakdown of native starch granules by hydrolyzing α-1,4-linked glucose polymers. This enzyme degrades amylose and amylopectin chains randomly, generating maltose, maltotriose, and other dextrins. The maltose units at the non-reducing ends of the branches are removed by β -β-amylase. Both enzymes are quite active during mashing, and their combined activity can effectively break down amylose and amylopectin in starches (Ledley, & Cockburn, 2024). Therefore, enzymes play a crucial role in the beverage industry by enabling the production of high-quality products with consistent flavor, texture, and appearance, as well as improving the shelf life and stability of beverages.

Meat Industry

The palatability of meat is affected by several factors, such as tenderness. This is believed to be the most significant factor in determining consumer preference. Physical, chemical, and enzymatic treatments may increase meat quality and tenderness (Arshad et al., 2016). In meat tenderization, enzymes (most notably protease, papain, bromelain, and ficin) play an important role. The toughness of meat is due to the presence of collagen, actin, and myosin, and to a lesser extent, worker handling during meat processing (Chaudhary et al., 2015). To address the biological determinants of toughness, the papain enzyme (derived from papaya latex) has been shown to hydrolyze large protein molecules into amino acids and peptides. Given this ability, papain is commonly used as a meat tenderizer in the meat industry. Moreover, it has been determined that papain is an effective defense against unwanted microbes, such as *Proteus vulgaris, Bacillus subtilis, S. aureus, Enterobacter cloacae,* and *E.coli* (Eshamah et al., 2014). Furthermore, actinidin, a cysteine protease derived from kiwifruit (*Actinidia deliciosa*), belongs to the same enzyme class as papain, ficin, and bromelain. Actinidin may serve as an alternative to these enzymes when lower inactivation temperatures, antimicrobial activity, and mild tenderization effects are desired. Recent studies have highlighted its effectiveness in improving meat tenderness while preserving sensory quality, making it a promising enzyme for clean-label and controlled meat

processing applications (Niamah et al., 2024). Bromelain, derived from the pineapple, breaks down the myofibrillar proteins and collagen and is thus suitable for meat tenderization. Bromelain also helps ensure meat's microbiological quality and purity in controlled environments (Arshad et al., 2016). Zingibain is also a meat tenderizing enzyme and is derived from ginger. It contains two cysteine proteases that are highly specific for collagen breakdown (Bekhit et al., 2014; Gomes et al., 2018). Enzymes play a crucial role in the meat industry by degrading complex structural components, such as collagen and muscle fibers, thereby enhancing meat tenderness, palatability and digestibility. In addition to their tenderizing effects, enzymes are also employed in certain preservation strategies during meat storage and processing, where they contribute to quality maintenance and shelf-life extension (Bekhit et al., 2014; Toldrá et al., 2021). Recent advances highlight the growing application of proteolytic enzymes and enzyme-assisted technologies to improve meat texture, processing efficiency, and product consistency while meeting clean-label and sustainability demands (Toldrá et al., 2022).

Other Industries

The use of enzymes is steadily expanding in a wide range of industries due to their rapid processing times, minimal labor input, cost efficiency, nontoxicity, and environmentally friendly properties, as well as their established and novel functional capabilities. Industrial applications for enzymes include baking, brewing, detergents, fermented products, medicines, textiles, and leather processing (Singh et al., 2016). In the food and beverage sector, microbial enzymes play pivotal roles in improving dough handling, fermentation efficiency, product quality, and nutritional outcome, and their demand continues to grow with technological advancements and sustainability initiatives (Hau et al., 2024). Additionally, enzymes have several critical roles in the pharmaceutical and diagnostic sectors. They are widely used as therapeutic medications for enzyme deficiencies and gastrointestinal disorders, as diagnostic techniques (e.g., Enzyme-Linked Immunosorbent Assay (ELISA) and antibody detection), and in diabetes testing kits (Mane & Tale, 2015). More recent medical applications of microbial enzymes include wound debridement, burn treatment using proteolytic enzymes, and thrombolytic therapy employing fibrinolytic enzymes. Given their versatility, specificity, and biocompatibility, enzymes have become indispensable in the drug and pharmaceutical industries, with applications spanning drug synthesis, formulation, detection and clinical diagnostics (Singh et al., 2016).

Factors Affecting Enzymes' Stability and Activity

Enzymes are proteins, and these molecules can fold into various three-dimensional structures that can adapt to the specificities of various substrates and react in the presence of other molecules, different ionic environments, pH, temperature, and hydrophobicity. These interactions frequently change enzyme shape, which in turn affects its catalytic efficacy. Also, enzyme concentration plays a vital role in the speed of the reaction process. In general, a higher enzyme concentration results in more enzyme-substrate complexes, leading to a higher reaction rate and product formation. Shu et al. (2016) confirmed that the increasing enzyme concentration increases the reaction rate. Hence, there is a linear relationship between the reaction rate and enzyme concentration available to catalyze it (Zhang et al., 2018). This microenvironment directly affects the enzyme activity rate. Hence, pH and temperature are crucial for stabilizing the enzyme and maintaining its activity (Nadar & Rathod, 2017). Enzyme activity reduces with increasing temperature, which presents a challenge for enzyme function. Further, enzyme activity also affects pH variation. The term "optimum pH or temperature" refers to the pH level or temperature at which the enzyme is most active. Enzymes lose their activity at extremely high or low pH levels or temperatures (Bearne, 2014). Bassi and Tyner (2020) reported that the hyperthermo-stable glucose (xylose) isomerase from *Thermotoga neapolitana* performs well at low pH and has thermal stability as high as 97°C. However, its activity is reduced at neutral pH levels and at lower temperatures than 60°C (Bassi & Tyner, 2020).

Cofactors are inorganic substances that are necessary for many enzymes to function actively. Some enzymes can also bind molecules other than cofactors or substrates, and frequently, these are molecules that block the enzyme or restrict the catalytic process (Robinson, 2015). Besides this, enzyme inhibitors affect the catalytic process, either by slowing down or stopping it. Hence, enzyme stability is governed by the extent to which an enzyme retains its structural conformation or activity during various processing conditions and storage. At the industrial level, the operational conditions required to convert substrates are normally extreme and different. Therefore, enzyme stability is an important issue during various operational conditions (Silva et al., 2018). Physical and chemical factors, including high temperature, extremes of pH, extremes of ionic strength, and high water activity, affect enzyme stability because they either promote non-covalent unfolding and/or aggregation/precipitation or enhance the rate of the covalent processes (Ahmad & Sardar, 2015). It is often challenging to retain the stability and activity of enzymes in industrial environments, as they have not evolved naturally for it. Their biological activity depends on their native structure; any significant conformational change may lead to enzyme inactivation (Bommarius & Paye, 2013). The industrial application of enzymes is hampered by their stability and recovery issues. These disadvantages are generally overcome by various immobilization techniques (Stepankova et al., 2013; Ahmad & Sardar, 2015).

Stabilization Techniques

The ability of an enzyme to maintain its active structural shape in the face of disruptive stimuli, such as rising temperature, is what is meant by the term "stability." Enzymes tend to lose activity when exposed to heat, pH, or other conditions; therefore, maintaining their stability is crucial. There are two ways to achieve this task: (i) use of

natural extremozymes, or (ii) stabilization of unstable enzymes. Further, there are two types of stabilities: storage stability and operational stability (Bornscheuer et al., 2024). Various techniques have been investigated by many researchers for improving enzyme stability for industrial applications. These include approaches like the use of additives or stabilizing agents, silk stabilization, chemical modification, (Phosphorillation, Glycosylation), Polyethylene glycol conjugation (PEGylation), immobilization of enzyme by physical adsorption, covalent binding, micro and nano-encapsulation and matrix entrapment, crosslinking, protein engineering, etc. (Fig. 2). Apart from above-stated techniques recently use of extremozymes, single enzyme nanoparticles (SEN), metal-organic frameworks (MOFs), and protein chimerization, hybrid support matrixes for immobilization, etc. are widely applied to obtain enzymes proven to be performed under desired processing conditions (Cao et al., 2025). Extremozymes are obtained from extremophilic microorganisms that survive in harsh conditions such as extreme pH, temperature, and salinity (Akanbi et al., 2020). Navanietha Krishnaraj and Sani (2017) reported that these enzymes can be isolated from thermophilic, hyper-thermophilic, psychrophilic, barophilic, acidophiles, alkaliphiles, xenophiles, halophiles, as well as metal-resistant microorganisms. Extremophilic enzymes showed higher stability under different environmental conditions, compared to commonly used enzymes.

Nanotechnology offers new approaches to enzyme immobilization, in which enzymes are stabilized on nanostructures such as nanoparticles, nanofibers, and nanorods. The intrinsically large surface area of these materials makes them an excellent choice for enzyme immobilization. Compared with conventional supports, their high surface area allows greater enzyme loading, thereby increasing enzymatic activity per unit mass or volume (Abdel-Mageed, 2025). Researchers have explored immobilizing enzymes on carriers, entrapping them in matrices, frameworks, or nanoparticles, to limit their mobility, protect them from hostile environments, and delay their denaturation. These methods balance three conflicting goals: (i) retaining a high level of enzyme activity, (ii) maintaining long-term stability, and (iii) modifying or reversing the activity of enzymes. In SENs, the enzyme is held in place by a thin shell, which is typically composed of a polymer. This shell can either be formed in situ by polymerizing the enzyme surface or be assembled around the enzyme using a preformed polymer (Chapman & Stenzel, 2019). Magnetic nanoparticles (MNP) are nanoscale magnetic particles with higher loading capacity, super para-magnetism under external magnetic fields, easy separation, and reduced diffusion limitation (Xu et al., 2014). Magnetite (Fe_3O_4) and maghemite (ɤ-Fe2O3) are the most commonly used magnetic particles owing to low toxicity and biocompatibility (Xu et al., 2014). Advancements in nano-engineering have expanded the range of materials available for enzyme immobilization. MNP consists of a magnetic core covered with a surface coating and a functionalized outer coating (Singh et al., 2013). Lower resistance, higher loading capacity, and easier recovery, reusability and recyclability are a few prominent features of MNPs compared to traditional immobilization methods (Seenuvasan et al., 2018). Consequently, enzyme immobilization on nanostructured materials has gained considerable attention, with studies demonstrating that nanomaterial-based immobilized enzymes function as efficient and economically viable nano-biocatalysts for industrial applications (Rai et al., 2019; Zhang et al., 2024).

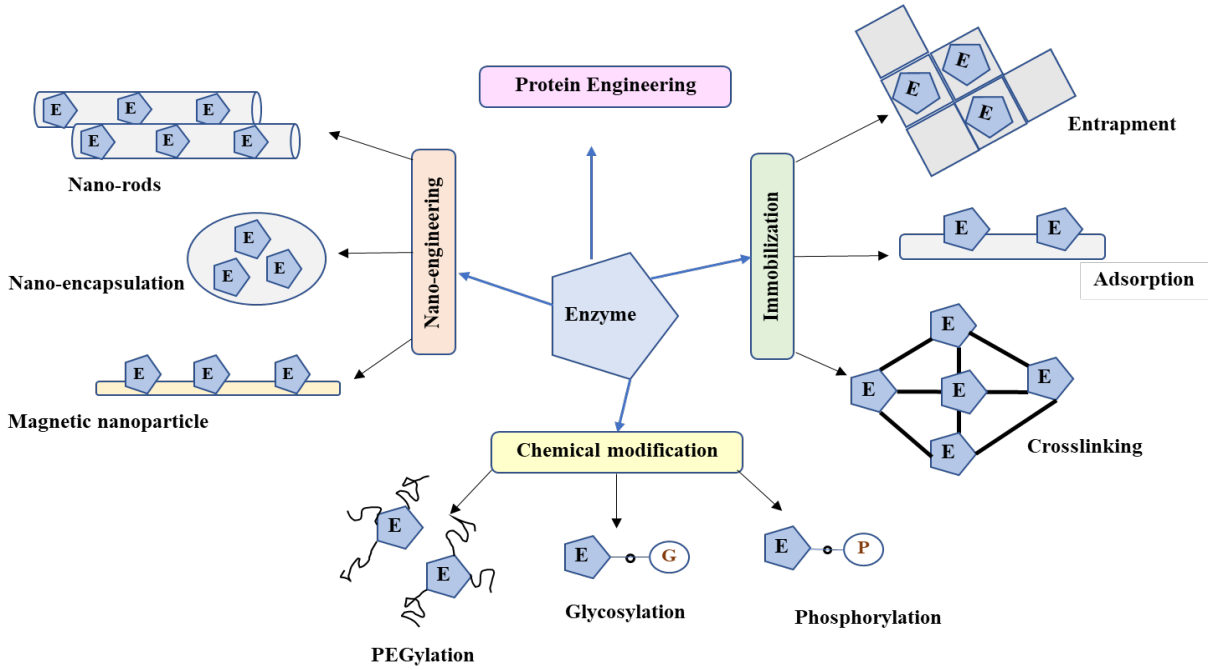

Fig. 2: Schematic representation of different enzyme stabilization techniques.

MOFs are crystalline porous materials consisting of metal ions or organic compounds. These MOFs possess higher surface area, porosity, functionality, and thermal stability, enabling them to be an ideal matrix for enzyme immobilization (Ye et al., 2020). These features allowed MOFs to exhibit significantly higher enzyme-loading capacity compared to traditional porous materials. Moreover, MOFs can be modified as per the given enzyme characteristics. This allows complete protection and stability to the enzyme encapsulated in the MOF. With such tunable functionality and excellent thermal stability, MOFs stand higher in the field of enzyme immobilization (Hu et al., 2020). Souza et al. (2022) reported that MOFs coupled with enzymes as biosensors could be a promising tool for biomedical, food safety, and environmental monitoring areas.

Protein chimerization is a process where a hybrid protein is generated by genetically crosslinking different functional genes to obtain superior and enhanced properties of protein or enzyme (Iyengar et al., 2019). Masakari et al. (2020) constructed chimeric enzymes (Mr144–297) using flavin-dependent glucose dehydrogenase (FAD-GDH) from *Mangifera prainii* (MpGDH), which has higher substrate specificity, and FAD-GDH from Mucor sp. RD056860 (MrdGDH), which has higher thermal stability. The obtained Mr144–297 chimeric enzyme showed higher thermal stability at 55 °C compared to the parent MpGDH enzyme. Additionally, Mr144–297 maintained high affinity and specificity for D-glucose. Therefore, protein chimerization can be used to construct the most thermostable and substrate-specific enzymes.

A hybrid matrix is obtained by combining various materials to enhance adsorption, catalysis, and surface area for enzyme immobilization (Perez et al., 2020). Besides this, the hybrid matrix also showed higher thermal stability and protection to an enzyme (Zdarta et al., 2018). The hybrid supporting matrix enhances the mechanical resistance and stability of the enzyme, performance, reusability and functional stability during storage (Wahab et al., 2020). The zeolite/chitosan hybrid supporting matrix showed higher stabilization of the *Aspergillus fumigatus* α-amylase enzyme reported by Yandri et al. (2022). The authors reported a 4.65-fold increase in the enzyme half-life upon immobilization on the hybrid support compared to the native enzyme. The other hybrid matrices, such as the chitin/bentonite hybrid matrix used for immobilization of *A. fumigatus* α-amylase, showed four-fold higher thermal stability than the free enzyme (Tiarsa et al., 2022). Another possible strategy is to incorporate inorganic materials to produce hybrid biocatalysts with unique properties. Similarly, the conjugation of inorganic materials with organic or inorganic components yields versatile hybrid systems. Such modifications are frequently reported for a range of support, notably mesoporous matrices, ceramics, and nano-engineered frameworks (Cavalcante et al., 2021).

Future Prospects and Conclusion

Enzymes play a vital role in speeding up reactions. Various enzymes are reported to have different applications in the food system, enhancing food processing and food safety. Hence, the prospects of enzyme application in the food industry are increasing. However, these enzymes are more susceptible to the environmental conditions, including temperature, pH, salinity, and other additives. Therefore, the main challenge is their stability in different environmental conditions. Advancements in biotechnology have introduced several sophisticated strategies to enhance catalytic performance, including genomic modification and protein engineering for the production of recombinant enzymes. Further enzyme stability can be improved using various novel technologies, including nano-engineering, protein chimerization, and hybrid technologies.

In conclusion, enzymes or microorganisms have been employed in food preparation for a long time. Consumers have long favored enzymes due to their all-natural characteristics. Their environmentally friendly, efficient process control, high yield, inexpensive refining costs, and process safety, enzymatic hydrolysis, and enzyme-based technologies are preferable over chemical ones. With the advancement in technology, newer enzymes with a vast array of uses and specificities have been developed, and new application areas are continually being explored. Enzymes produced from bacteria, yeast, and fungi are commonly utilized in food production to improve flavor and texture. Due to their limited application, animal rennets are rarely discussed. More research is needed to find strains that manage cheese ripening and minimize by-products. As an alternative to calf rennet, recombinant genetic technology has developed recombinant chymosin, but it hasn't gained traction. There are still many enzymes of microbial origin that have not been investigated, and there are a lot of possibilities for expanding the commercial applications of microbial enzymes, particularly in the food industry.

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