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Use of enzymes in the food industry: a review

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Abstract

Enzymes are biological catalysts that play a key role in the food industry, responsible for desirable and undesirable chemical reactions. These compounds can occur spontaneously in food products or even be incorporated on purpose. The main enzyme classes (oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase) and their subclasses can be obtained from several sources, presenting numerous applications in foods such as inhibition of microorganisms, insertion of aromas, improvement of physico-chemical properties, decreased candy crystallization, meat tenderization, antioxidants, indicators of heat treatment, and so on. Each enzyme acts effectively under specific conditions of pH, temperature, concentration, and water activity. Enzymatic immobilization techniques have been studied to minimize adverse conditions that enzymes are subjected to during food processing. Among the immobilization technique, the enzymes can be immobilized to the packages, being known as enzymatic active packages, the packages allow the enzymes to be migrated to the product or trapped, increasing the shelf life of the products, this technique being innovative both for the packaging market and for the enzyme market. Thus, the main enzymes and their applications in food were briefly discussed in this review article, as well as the main techniques of immobilization and insertion of these compounds in active packaging.

Keywords: biological catalysts; enzymatic immobilization; enzymatic active packaging.

Practical Application: Use of enzymes in the food industry and their incorporation into active packaging.

1 Introduction

Enzymes are defined as organic molecules of protein origin, designed to catalyze biochemical reactions, and do not effectively participate in the reactions as a reagent (Fennema et al., 2010). They are basically composed of two parts (Figure 1): the protein part called apoenzyme, and the non-protein part known as the cofactor (maybe a metallic ion) or coenzyme (organic molecule), which together give rise to the complete functional enzyme, called the holoenzyme (Ahmad & Sardar, 2015). The enzymatic catalysis process results from its specificity, which is considered one of the main characteristics of the enzymes. In the absence of enzymes, some chemical reactions would probably not be possible. However, its presence alone is insufficient to catalyze reactions since reactions have variable rates (Ordoñez et al., 2005; Lajolo & Mercadante, 2017).

The application of enzymes covers many industries as food industries which basically use three sources: microorganisms, plants or from animal tissues (Ray et al., 2016; Ahmad et al., 2018), with enzymes mainly being used in dairy products, baking, and beverages such as fruit juices, wine, and beer (Guerrand, 2018; Taheri-Kafrani et al., 2021).

The interest of the food industry concerning enzymes stems from the constant search for foods with a long shelf life, in addition to the quest to reduce waste and increase the quality of products, transforming raw materials into the main product or acting as additives to modify desired properties such as flavor, texture, or machinability, among others (Singh et al., 2016; Sanromán & Deive, 2017; Yushkova et al., 2019; Bilal & Iqbal, 2020).

Thus, research has been carried out to explore enzymes which contribute to the quality and stability of food products. Moreover, several studies have explored the inactivation of enzymes that can accelerate undesirable processes, such as food degradation (Ray & Rosell, 2017; Adesegun Kehinde et al., 2020)

Furthermore, enzymes can be affected by factors such as pH, water activity, temperature (Bisswanger, 2014). Therefore, alternatives have been studied to stabilize them due to unfortunate changes which occur due to these factors. Enzyme immobilization is one of these alternatives, which offers stability, allowing to reuse these catalysts and incorporate them into packaging. These techniques enable enzymes to be released into food in a controlled manner, improving the desired properties in certain food products (Almasi et al., 2021; Wahab et al., 2020).

Although the application of enzymes is addressed in several studies, reviews focusing on the classes and subclasses of enzymes used in food combined with the potential for application in packaging have rarely been found. As such, this review aims to briefly present the main enzymes related to food, whether they are

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exogenous and endogenous, as well as their classes and subclasses, in addition to immobilization of these enzymes in packaging.

2 Enzymes: exogenous and endogenous

Enzymes applied to food products are divided into two groups: exogenous and endogenous (Gomes et al., 2018). Exogenous enzymes are those purposely added to foods or raw materials, causing desired changes. In this case, knowledge about the factors that influence enzyme activity is essential to achieve greater effectiveness and cost reduction (Damodaran & Parkin, 2017; Lajolo & Mercadante, 2017). According to Ordoñez et al. (2005) and Fernandes (2016), some reasons explain the incorporation of exogenous enzymes in foods. The first refers to the possibility of enzymes analyzing unique characteristics of foods, replacing severely complex purification techniques with the chance of detecting very small amounts of components using, for example, biosensors for the detection of multiple analytes. In addition, with advanced studies of nanotechnologies, there is the possibility of using nanoenzymatic biosensors, as example, nanozyme-based biosensors for detecting biological contaminants, such as pathogens and biotoxins, that can compromise food quality and safety. Aflatoxin B1 (AFB1) is a product of secondary metabolism from Aspergillus species, which usually contaminates cereal crops, particularly rice, nuts and corn and is responsible to multiple fetal aflatoxicosis outbreaks worldwide. This way, a MnO2 nanoflake-TMB system is applied for AFB1 colorimetric determination, which could accurately detect AFB1 concentration as low as 6.5 pg/mL with linear range of 0.05-150 ng/mL at room temperature (Wang & Gunasekaran, 2020; Alvarado-Ramírez et al., 2021). The second reason is the role of enzymes as indicators. A very common example in the food industry is the use of catalase in determining the quality of milk (Kaushal et al., 2018). The last and most important reason for the use of exogenous enzymes is attributed to a final product that fulfills the intended role and displays adequate features since the enzymes play the role of improvising sensory attributes and other factors such as digestibility, viscosity and tenderness. Being a much-seen example in the meat products industry, the incorporation of controlled conditions of proteolytic exogenous enzymes with meat products result in reducing their toughness and enhancing the eating quality (Madhusankha & Thilakarathna, 2021).

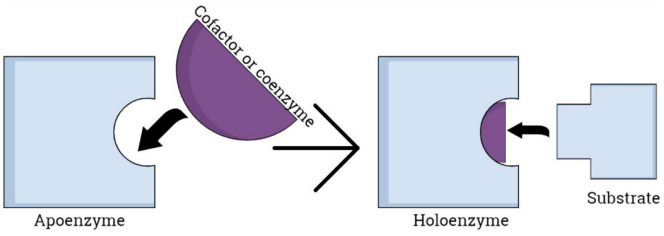

Figure 1. Apoenzyme and holoenzyme presentation scheme.

On the other hand, endogenous enzymes are naturally present in food (cells/tissues) and can have desirable and undesirable effects. This type of enzyme can be used to change the properties of post-harvest plant products and modify animal tissues, color, aroma, texture, and nutritional value of a range of food products. However, endogenous enzymes can cause a nutritional and sensory decrease in food (Ordoñez et al., 2005; Okafor et al., 2019).

Both exogenous and endogenous enzymes can be classified into six main classes according to the catalyzed chemical reaction: oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase (Figure 2) (Rigoldi et al., 2018).

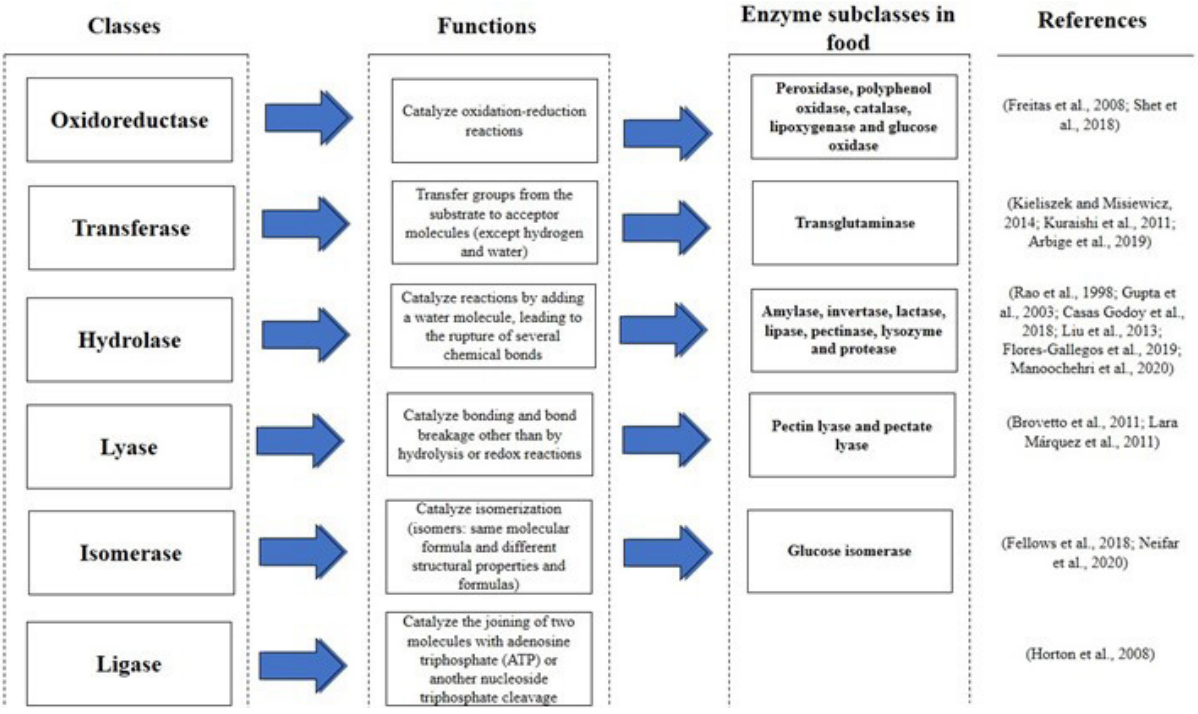

Figure 2. Enzyme classes according to catalyzed chemical reactions and their subclasses found in food.

3 Enzyme applications in the food industry

3.1 Oxidoreductases

Peroxidase (POD), polyphenol oxidase (PPO), catalase, lipoxygenase, and glucose oxidase are among the enzymes of the food-related oxidoreductase class (Table 1). POD and PPO are considered endogenous enzymes in fruits and vegetables and are seen as the main causes of browning in these products. This browning only occurs in the presence of oxygen and when the products are subjected to cuts, slices, or when they suffer mechanical damage during transport or thawing (Singh et al., 2018).

The mechanism of action of the PPO enzyme can occur through hydroxylation of the phenolic substrate in the *ortho*-position, in addition to a hydroxyl group present (monopherol oxidase activity) or by oxidizing diphenol to *ortho*-benzoquinone (diphenol oxidase activity). Oxygen is used as a co-substrate in these two modes. POD aims to catalyze oxidative reactions using peroxide as a substrate or oxygen as the final hydrogen acceptor (He & Luo, 2007; Moon et al., 2020).

The POD enzyme is not only linked to the deterioration of vegetables. Due to their sensitivity to heat in these products, their inactivation indicates that the bleaching process was used correctly. It is also possible to observe the indicator function of a type of POD enzyme, lactoperoxidase, in dairy products in general (Sheikh et al., 2018). When milk is subjected to a pasteurization process, it is ideal that the enzyme is not inactivated (Temperature of inactivation: 70-80 °C) to prove that the pasteurization process has not exceeded the appropriate temperature. Also, lactoperoxidase is an enzyme activated by hydrogen peroxide (H_2O_2) present in raw milk with antimicrobial activity, so it is used to inhibit the development of microorganisms when refrigeration is scarce (Freitas et al., 2008; Lara-Aguilar & Alcaine, 2019).

Catalase is a tetrameric protein located in aerobic organisms. The mechanism of action of this enzyme consists of decomposing hydrogen peroxide (H_2O_2) in oxygen and water, easing the oxidative stress caused by this substrate (H_2O_2) (Barynin et al., 2001). Catalase can be used in cheese production in the food industry (Raveendran et al., 2018). It is responsible for removing unwanted H_2O_2 residues in cheese. H_2O_2 has the function of replacing heat treatment processes, such as pasteurization, to protect and maintain the naturally present enzymes in cheese and can be used in milk. Catalase can be found in bovine liver or microorganisms (Kaushal et al., 2018).

In addition, catalase, together with other enzymes, may have its effect enhanced. Combining catalase with the glucose-oxidase enzyme aims to help preserve some foods (Raveendran et al., 2018). Thus, Botezatu et al. (2021) investigated the potential of the enzymatic management of high pH in white juice and wine using a combination of enzymes-glucose oxidase coupled with catalase, once the wine industry in warmclimate regions suffering with the problem of high pH. High pH wines are problematic as they can often be microbiologically unstable, have issues with color stability, and result in organoleptically unbalanced wines. The authors used Catazyme® 25 L (glucose oxidase with catalase) to metabolize glucose into gluconic acid, leading to an increase in total acidity and in conclusion glucose oxidase coupled with catalase was shown to be effective at significantly reducing juice and wine pH in a short amount of time and with a positive impact on the organoleptic profiles of the treated wines.

Röcker et al. (2016) also investigated the combination of catalase and glucose-oxidase enzymes intending to reduce the alcohol content in wines. The authors studied the combined use of glucose-oxidase and catalase enzymes to evaluate the rapid conversion of glucose to non-fermentable gluconic acid. The H_2O_2 hydrolysis activity of the purified catalase is necessary to stabilize the glucose oxidase activity; as a result, it was observed

Table 1. Examples of exogenous and endogenous oxidoreductases in food.

Enzyme	Source	Type of application	Main effects of application/use	Reference
Endogenous oxidoreductases				
Peroxidase	Fruits and vegetables	-	Browning of fruits and vegetables/ Adequate bleaching process indicator	Singh et al. (2018)
Polyphenol oxidase	Fruits and vegetables	-	Browning of fruits and vegetables	Singh et al. (2018)
Lactoperoxidase	Milk	-	Pasteurization process indicator	Lara-Aguilar & Alcaine (2019)
Exogenous oxidoreductases				
Catalase	Animals and microorganisms	Cheese production	Conversion of hydrogen peroxide to water and oxygen, removing excess hydrogen peroxide	Kaushal et al. (2018)
Glucose oxidase	Microorganisms: *Aspergillus* and *Penicillium*	Food preservation	Removal of free oxygen, inhibition of microorganisms, and maintenance of flavor and color	Li et al. (2019); Kiesenhofer et al. (2017)
Lipoxygenase	Soy plants	Food preservation	Protection of flour in bakery products, ensuring colorless products	Gava et al. (2009); Tu et al. (2018)

that an alcohol reduction by 2% was achieved after 30 h of aeration with the enzymatic treatment.

The glucose-oxidase enzyme acts by catalyzing the oxidation of β-d-glucose to gluconic acid, using molecular oxygen as an electron acceptor in concurrently supplying H_2O_2 (Bankar et al., 2009). This enzyme is produced by the fungi *Aspergillus* and *Penicillium*. It is used in the food industry for food preservation, acting in removing free oxygen, inhibiting microorganisms, and maintaining flavor and color, in addition to making desirable changes in some food products, such as in bakery products or modulating ethanol fermentation (Kiesenhofer et al., 2017; Li et al., 2019).

Lipoxygenase is important in the synthesis of fatty acids in plants and animals and is especially obtained from soybean plants. Lipoxygenase catalyzes the deoxygenation of polyunsaturated fatty acids into one or more cis, cis-1,4 pentadiene fractions to create lipid hydroperoxides (Tu et al., 2018). Studies with lipoxygenase have proven the effectiveness of these catalysts, many related to bakery products and aroma production (Baysal & Demirdöven 2007). Zhang et al. (2013) observed that this enzyme improved wheat flour whiteness in bakery products. The treated bread had its properties improved, mainly crumb color, specific volume, resilience, chewing, and hardness.

3.2 Transferase

One of the types of enzyme transferase that the food industry has used is transglutaminase, whose principle consists of catalyzing the transfer reaction of acyl groups of the ɣ-carboxamide group from glutamine residues to different acceptors. When this enzyme acts on protein molecules, they undergo cross-linking and polymerization reactions through ε- (ɣ -glutamyl) lysyl peptide bonds. In the absence of appropriate primary amines or the event that chemical reagents block the lysine ε-amine, it is possible to make the water act as an acceptor, and the glutamyl residue changes to a glutaminyl residue by deamidation through the transglutaminase reaction (Kuraishi et al., 2000; Soares et al., 2004).

Transglutaminase can be obtained from an animal or microbiological source; however, this enzyme can have a lower cost and a greater application when synthesized from microorganisms. It has mainly been isolated from *Streptoverticillium* spp. bacteria since 1989. Transglutaminase can be used to improve the gelling properties of various products in the food industry. Having the capacity of post translation modification of proteins, it can deamidate or cross-link substrates. The crosslinking forms high molecular weight proteins that can modify functional properties such as viscosity, gelation, solubility, and water holding capacity. Due to its ability, this enzyme being known as "meat glue". In this way, transglutaminase can be used to improve the appearance, texture, preservation and toughness of meat, as well as to improve the texture and quality of milk and dairy products. In fish-based products, it increases the hardness of the product, improves the appearance and stability of the protein film and even decreases the caloric content. In addition to being able to be used in the sweets and confectionery industries to increase the texture and elasticity of the product and in plant-based, such as soy and wheat, to make tofu, bread, bakery products and pasta (Kuraishi et al., 2001; Kieliszek & Misiewicz, 2014; Moreno et al., 2020; Lerner & Benzvi, 2021; Schlangen et al., 2023).

3.3 Hydrolase

According to Schmidt and Salas-Mellado (2009) and Patel et al. (2016), all six enzyme classes play a fundamental role in food, but hydrolases can be considered the most influential and important due to your subclasses, such as amylase, invertase, lactase, lysozyme, lipase, pectinase, and protease that are directly used in the food industry as mentioned in each topic (Table 2 and 3).

Amylase

Amylase is obtained from fungi and bacteria, especially from the *Bacillus*, *Pseudomonas,* and *Clostridium* families. It is known as one of the most important enzymes in the food industry, involved in the starch, beverage (beer), bread, and sugar sectors, and is described as the first starch degrading enzyme. It was discovered and isolated in 1811 by Kirchloff (Gupta et al., 2003; Abada, 2019).

Amylase hydrolyzes starch act on the α-1,4 and α-1,6 glycosidic bonds of starch and glycogen. It is classified into endo and exoamylase and normally divided into α- and β-amylase depending on the type of anomeric sugar produced by the reaction (Gupta et al., 2003; Kumar & Chakravarty 2018; Shukla, 2019).

α-amylase has been applied in bakery products when it is extracted from fungi or malted cereals because this enzyme can be used in both flour and in preparing dough to complement the fermentation rate and to reduce the dough's viscosity, which consequently leads to improvement in the volume and texture of the product. It also contributes to forming extra fermentable sugars which improve some characteristics of the bread, such as color, flavor, quality, and crust. The brewing industry can also use α-amylase during the brewing process (mixing ground malt with hot water at rest to degrade proteins and starch, and to produce soluble malt extract, the wort) to hydrolyze the starch when it gelatinizes, making the must viscosity not high and making it difficult to retrograde starch. On the other hand, β amylase is used in the manufacture of maltose syrups, which can be used by the food, beer, and pharmaceutical industries (Mishra et al., 2017; Zhang et al., 2018; Duan et al., 2019).

Invertase

Invertase is among the enzymes classified as hydrolases. It has the function of catalyzing the hydrolysis of sucrose into fructose and glucose, and sucrose synthase has the function of converting sucrose and uridine diphosphate (UDP) into fructose and UDP-glucose, thus becoming charged enzymes with the cleavage of sucrose in plants. Sucrose is a disaccharide produced from joining a glucose molecule and a fructose molecule; it is produced by plants in photosynthesis, being a valuable form of carbohydrate transport and an essential carbon and energy source in plants. However, the evolution or continuity of cellular activities comes from the breakdown of the glycosidic bond of

Table 2. Examples of exogenous and endogenous hydrolases in food.

Enzyme	Source	Type of application	Main effects of application/use	Reference
Endogenous hydrolases				
Pectinase	Fruits and vegetables	-	Alteration of the texture of fruits and vegetables during the stages of ripening, storage, and processing	Ordoñez et al. (2005)
Exogenous hydrolases				
α-Amylase	Microorganisms and malted cereals	Bakery and brewing products	Improvement in volume and texture, color, flavor, quality of bakery products; beer brewing process	Mishra et al. (2017); Zhang et al. (2018)
β- Amylase	Microorganisms and malted cereals	Syrup manufacturing	Releases carbohydrates from smaller chains needed for fermentation process by brewer's yeast, providing sugars that will serve to form alcohol in beer	Duan et al. (2019)
Cellulase	Microorganims	Fruit juices and winemaking	Improve the texture, quality, yield of various food products	
Invertase	Microorganisms, plants and animals	Sweets in general, artificial honey, jams, confectionery, drinks, and so on	Prevents crystallization of sugary products	Manoochehri et al. (2020); Trujillo Toledo et al. (2019)
Lactase (β–galactosidade)	Microorganisms	Dairy products	Improvement in the taste and color of milk and dairy products, incorporation of greater creaminess in the products, reduction of the maturation time of cheeses	Gava et al. (2009)
Lipase	Stomach and pancreas of man and animals and filamentous fungi, yeasts, and bacteria	Cheese	Improvement of texturing and flavor and development of cheese flavor and cheddar cheese production	Aravindan et al. (2007); Guerrand (2017); Raveendran et al. (2018); Trbojević Ivić et al. (2016)
Pectinase	Microorganisms	Beverage	Extraction of juice and aroma, removal of mist caused by pectin, whitening, and reduction of turbidity, extraction of natural pigments in wines and manufacture of sparkling wines, extraction of oil, and fermentation of coffee and tea	Dal Magro et al. (2018); Khan et al. (2013)
Lysozyme	Eggs, plants, bacteria, and animal secretion	Foods prone to microbial contamination	Food preservative against gram-positive, gram-negative bacteria and fungi	Liu et al. (2013)

Table 3. Subclasses of the protease enzyme and its applications in the food industry.

Enzyme	Source	Main effects of application/use	Reference
Alkaline protease	Microbian (*Aspergillus oryzae*)	Production of fermented foods, such as soy sauce, with tolerance to high concentrations of salt	Gao et al. (2019)
Bromelain	Vegetable - Pineapple (*Ananas comosus*)	Therapeutic, meat tenderizer, and beer clarification	Banerjee et al. (2018); Gurumallesh et al. (2019)
Ficin	Vegetable – Fig (*Ficus carica*)	Meat tenderizer	Nishimura et al. (2020)
Papain	Vegetable -Papaya (*Carica papaya*)	Meat tenderizer	Philipps-Wiemann (2018)
Pepsin	Animal (stomach)	Milk coagulation	Grumezescu & Holban (2018)
Renin	Animal (calf stomach)	Cheesemaking (rennet)	Gava et al. (2009)
Trypsin	Animal (Swine or bovine pancreas)	Manufacture of food aromas	Chew et al. (2019)

sucrose that is carried out by invertase (Koch 2004; Stanhope & Havel, 2008; Chen et al., 2009).

Invertase is an enzyme which can be obtained from microorganisms, plants, and animals. In addition, the invertase enzyme can be obtained by autochthonous fruit microorganisms, such as some Amazonian fruits. This means that these fruits can host microorganisms due to their important micro-habitat for a wide variety of microorganisms such as filamentous fungi, yeasts and bacteria due to the high concentration of sugars, the low pH and the intense appearance of insect vectors. These microorganisms are then isolated and selected, and invertase can be obtained from fruits that inhabit the microorganisms: *Aspergillus niger*, yeasts and bacteria (Lima et al., 2020; Souza et al., 2020). When this

enzyme hydrolyzes sucrose, an equal combination of fructose and glucose, called inverted sugar, is generated. Inverted sugar syrup is sweeter than sucrose and is better incorporated into food, as it does not allow the product to crystallize. Thus, its application is broad in sweets in general, artificial honey, jams, confectionery products, and drinks, among others (Trujillo Toledo et al., 2019; Manoochehri et al., 2020).

Lactase

Just as invertase can hydrolyze a disaccharide into two monosaccharides, β-galactosidase (lactase) also plays this role with the lactose disaccharide, converting it into glucose and galactose. As lactose is a sugar commonly found in milk and consequently in dairy products, there is an abundant consumption of this carbohydrate. However, some consumers may have lactose intolerance due to the enzyme lactase deficiency in the organism (Schaafsma, 2008; Mir Khan & Selamoglu, 2020).

These consumers are mostly non-Caucasians, of indigenous and Asian origin, and may experience unpleasant symptoms such as flatulence, severe abdominal pain, and intestinal breakdown when consuming dairy products. Many consumers affected by this dysfunction reject lactose-free dairy products due to the sensory changes that these products have, especially the potentiated sweetness flavor. It is then a possible alternative to incorporating lactase in dairy products, directly attracting such consumers (Charles, 2019; Zhang & Zhong, 2017).

Lipase

Lipases are described as enzymes capable of hydrolyzing long-chain acylglycerol carboxylic esters (with ten carbon atoms) (Casas-Godoy et al., 2018). They are easily found in the stomach and pancreas of humans and monogastric animals with the function of digesting fats and lipids. However, they can be obtained from animals or microorganisms for different industrial applications, including filamentous fungi, yeasts, and bacteria, with the main producing microorganisms being: *Candida* sp., *Aspergillus* sp., *Rhizomucor* sp., *Rhizopus* sp., *Humicola* sp., *Yarrowia lipolytica,* and *Pseudomonas* sp. (Guerrand, 2017).

Several factors such as eminent catalytic multifunctionality, the possibility of innovation through different strategies, and greater stability make the lipase enzyme obtained from microorganisms to be among those preferred by the food industry and uses it to improve some characteristics like texturing and flavor, such as in the development of the cheese flavor and in cheddar cheese production (Aravindan et al., 2007; Trbojević Ivić et al., 2016; Raveendran et al., 2018).

Pectinase

Pectinase is an enzyme of plant or microbial origin responsible for hydrolyzing pectin components, a polysaccharide consisting of α-1,4-linked d-galacturonic acid and generally present in plant cell walls (John et al., 2020; Shet et al., 2018). The food industry has used this enzyme in fruit juices to extract juice and aroma, remove the mist caused by pectin, whiten and decrease turbidity, extract natural pigments in wines and manufacture sparkling wines, in addition to extracting oil, and in coffee and tea fermentation (Dal Magro et al., 2018; Khan et al., 2013).

Lysozyme

One of the most important properties sought by the food industry is its antimicrobial capacity, once the food industry has made use of various additives to inhibit the growth of microorganisms that trigger food spoilage, the spoilage microorganisms and those that cause illness to consumers, pathogenic microorganisms (El-Saber Batiha et al., 2021). In this contex, some enzymes have been explored due to this important feature, such as lysozyme. Lysozyme, also referred to muramidase or N-acetylmuramic hydrolase, is a small, monomeric protein stabilized by four disulfide linkages among the eight cysteine residues of its polypeptide chain and is obtained from several natural sources, including eggs, plants, bacteria, and animal secretion. This enzyme has great potential in the food preservation industry due to the strong bacteriostatic activities against gram-positive and gram–negative bacteria (they are more sensitive to gram-positive), and that's whay is considered to be a safe food additive in some parts of the world (Wu et al., 2019). Your antimicrobial capacity is associated, according to Anastas et al. (2021), to hydrolyzes the peptidoglycan chains found in the cell walls of gram-positive and gram-negative bacteria. In this way, the enzyme hydrolyzes the β-(1,4)-glycosidic bonds between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues. Hydrolysis occurs at the active site of lysozyme which involves the carboxylic acid moieties of glutamate-35 (Glu-35) and aspartate-52. Glu-35 donates a proton to the glycosidic ether linkage between NAG and NAM creating an oxonium ion which is followed by nucleophilic displacement of the hydroxy NAG and concurrent formation of an ester linkage of NAM to Asp-52. The ester is then hydrolyzed to provide a terminal hydroxy NAM, completing the scission of the glucosidic bond.

Protease

Protease is considered an enzyme capable of catalyzing the hydrolysis of peptide bonds, being divided into exopeptidases and endopeptidase. Exopeptidase acts near the end of a polypeptide chain, while endopeptidase acts within the polypeptide chains (Tavano et al., 2018; Chew et al., 2019).

It is obtained from several sources such as plants, animals, and microbial organisms because it is an important and indispensable enzyme for living organisms (Gurumallesh et al., 2019). There are essential subclasses for each source which are commonly used by the food industry. Bromelain, papain, and ficin can be mentioned among the proteases from plant matrices; animal sources are pepsin, renin, and trypsin, while those of microbial origin have alkaline protease. The protease enzyme subclasses and its applications are found in Table 3.

3.4 Lyase

Pectin lyase and pectate lyase (Table 4) related to pectin decomposition are among the lyases. Both catalyze the breakdown of polygalacturonate and esterified pectin through β-removal, which consists in removing a proton and producing

Table 4. Examples of exogenous lyases in food.

Enzyme	Source	Type of application	Main effects of application/use	Reference
		Exogenous lyases		
Pectin lyase	Microorganisms	Preservation of various vegetable foods	Clarification of fruit juice, degumming, and crushing of natural fibers	Dal Magro et al. (2020); Pili et al. (2018); Poturcu et al. (2017); Saharan & Sharma (2019)
Pectate lyase	Microorganisms	Preservation of various vegetable foods	Fruit firmness gives chromaticity and durability of red wines, extraction of fruit juice and vegetable oils, fermentation of teas and coffees, and wastewater treatment	Kamijo et al. (2019); Yang et al. (2017)

an unsaturated bond between the C-4 and C-5 carbons present at the non-reducing end of the pectin. While pectate lyases are typical for unesterified pectins and require Ca^{2+}, pectin lyases break down methylesterified pectin and do not require Ca^{2+} (Lara-Márquez et al., 2011).

Pectin lyases are usually obtained from fungi and eventually through bacteria and yeasts. They have wide application in the field of biotechnology, including food, contributing to clarify fruit juices, degumming, and crush natural fibers (Poturcu et al., 2017; Pili et al., 2018; Saharan & Sharma, 2019; Dal Magro et al., 2020).

Pectate lyases are also obtained through bacteria, fungi, and yeasts; however, the pectate lyases which have shown greater stability or thermostability are those from bacteria. The use of these enzymes covers a series of food applications such as improving the firmness of fruits, improving the chromaticity and durability of red wines, extracting fruit juice, extracting vegetable oils, aiding in the fermentation of teas and coffees, and even in the treatment of wastewater (Yang et al., 2017; Kamijo et al., 2019).

3.5 Isomerase

Glucose isomerase has stood out among the isomerases in the food industry due to its function of converting D-glucose into D-fructose. Thus, glucose isomerase is used in producing high fructose corn syrup (High Fructose Corn Syrup - HFCS), a liquid sweetener that can replace sucrose due to its stability in foods and beverages, including foods and acidic drinks, in addition to prolonging the flavor, acting as a humectant, and preventing crystal formation. The glucose isomerase is found in microorganisms such as fungi and prokaryotes, mainly *Streptomyces* and *Bacillus* species (White, 2008; Neifar et al., 2020; Rengasamy et al., 2020).

3.6 Ligase

Due to its function explained in Figure 2, the ligase enzyme is more related to studies with molecular biology in the literature. Therefore, the closest enzymes to food ligases are associated with their use in transgenic plants, with DNA ligase being responsible for binding the DNA strands that were suspended to the original strand of the organism to be modified (Chellegatti et al., 2018).

4 Enzyme immobilization - active enzymatic packaging

Immobilization is a technique which consists of confining the enzyme inside support that has a different phase of the product or substrate, avoiding contact of the enzyme with the external environment. Immobilization enables controlling enzymatic activities and repeated reuses in a controlled manner (Brena & Batista-Viera, 2006; Mateo et al., 2007; Souza et al., 2017; Wahab et al., 2020).

Economically speaking, the possibility of reusing enzymes makes immobilization an important tool since the use of catalysts such as enzymes is generally a high-cost process; also, there is the possibility of using low-cost supports with a high link capacity. On the other hand, when it comes to the process itself, immobilization allows the enzyme to resist adverse factors such as pH and temperature, so immobilization also improves the enzyme characteristics, such as performance in organic solvents, selectivity, and functional stability; moreover, to prevent enzymatic inactivation. The stability of immobilized enzymes depends on factors such as the enzyme-carrier interaction, bond positioning, as well as the number of bonds, possibility of conformational alteration of the matrix, how the enzyme was immobilized, the environment, the physical-chemical arrangement of the carrier, and finally, the characteristics of the spacer responsible for binding the enzyme molecules to the carrier (Ahmad & Sardar, 2015; Mateo et al., 2007).

One of the forms of enzyme immobilization is the immobilization in the form of a package. According to Lim (2015) with the enzyme constantly immobilized inside the carrier, the matrix must contain a sufficient pore size to allow the enzyme to relate to the substrates and facilitate the products from the reaction to propagate out of the matrix, or the enzymes can be encapsulated. In this case, the matrix expands and releases enzyme molecules at a rate that is moderated by the expansion capacity of the matrix from the moment it comes into contact with the food product. Other possibilities involve the matrix being produced by material from a food product that suffers wear and tear in the food environment with the simultaneous release of the enzyme. The solubility of the matrix determines the release of the enzyme molecules. Thus, the reaction success stems from the global kinetics of the enzyme migration in the food.

Active food packaging interacts with food by adding active substances that can be absorbed or released from the packaged food or its headpspace. This interaction between the packaging and the product takes place through packaging such as films, sachets, and edible coatings which are used to act as vehicles to absorb or release active substances to products. These active substances are usually antimicrobial or antioxidant substances or substances with functions that it wishes to change in the product. Adding these active substances to the packaging material instead of adding them directly to the food aims to reduce the required amount of use of such substances, as well as decreasing the likelihood of losing the active compound through product processing or agent diffusion and when it wishes the substance to be released gradually. In particular, the addition of these active ingredients into polymer matrix packaging materials can provide performance improvement of food packaging material in addition to new functions (Yildirim et al., 2018; Diken et al., 2022). Enzymatic packaging is considered promising for the food industry among the active packaging systems and could meet the demand for new classes of food-packaging systems. The technology for incorporating enzymes into packaging comes from the specific functions of enzymes (Sharma et al., 2022). When incorporating enzymes in polymeric matrices aiming to develop active packaging, it is necessary to use an ideal system for each type of enzyme with compatible physical-chemical properties. As mentioned earlier, enzyme activity depends on factors such as temperature, pH, and water activity. It is also important to check the conditions in which the enzymes will be exposed during the processing of the material, the physical and chemical properties of the food in question, and the storage conditions (Lim 2015).

Thus, the choice of the base to form active enzymatic packaging is a relevant aspect to be considered since processing can inactivate the enzymes. For example, starches are insoluble in water, and therefore it is necessary to subject them to a gelatinization process to make them soluble, in which these polysaccharides are subjected to high temperatures and constant pressure (Teixeira et al., 2018). Chitosan must be solubilized in aqueous acids in pH ranges < 6.5 (Endres & Weichold, 2019). As both temperature and pH are determining factors for enzyme activity, the enzymes chosen to be incorporated must be compatible with the formulation bases of the packaging.

Several studies with films incorporated with enzymes (Baggio et al., 2022; Benucci et al., 2018; Cunha et al., 2007; Hanušová et al., 2013; Mendes de Souza et al., 2010; Santos et al., 2021; Wongphan et al., 2022), edible coatings (Karina & Setiadi, 2020; Wang et al., 2017) from different bases are reported in the literature as active packaging for use by the food industry as shown in Table 5.

According to Table 5, it is observed that the enzymes, in general, are incorporated in order to improve some undesirable aspect of the food product, with the aim of prolonging their quality. Packages with immobilized enzymes, in addition to protecting the enzymes against adverse factors that are susceptible, can be reused and can have a migration control, so that the characteristics and the very formulation of the package allow the way the

Table 5. Examples of research with enzymatic packaging.

Enzyme	Kind of active packaging	Application Purpose	Main effects of application/use	Reference
Lactase	Films	Films based on cellulose acetate using the casting method incorporated with the enzyme lactase to reduce the lactose present in milk for individuals who have lactose intolerance.	Films containing 1 and 1.5 mL of the enzyme lactase managed to decrease 78 and 85% of lactose, respectively, after 24 hours of contact at 7 °C and 92 and 100% after 25 hours of contact at 25 °C.	Cunha et al. (2007)
Glucose oxidase and lysozyme	Films	Glucose oxidase and lysozyme were immobilized in polyamide films and DuPontTM Surlyn® 8140 ionomer films (ethylene/methacrylic acid copolymer containing 19% methacrylic acid) partially hydrolyzed by hydrochloric acid to create antimicrobial films.	Films immobilized with the enzyme lysozyme did not show a satisfactory result. However, the ionomer films with immobilized glucose oxidase inhibited the growth of *Escherichia coli*, *Pseudomonas fluorescens*, *Lactobacillus helveticus*, *Listeria ivanovii,* and *Listeria innocua*.	Hanušová et al. (2013)
Lysozyme	Films	Antimicrobials films were produced based on sodium caseinate and enzyme lysozyme to obtain a controlled release of lysozyme to release it for a longer period.	A slow release of lysozyme was observed after mixing with glyoxal, reaching prominence in the antimicrobial activity against *Micrococcus lysodeikticus* and *Staphylococcus aureus*. The results showed that active films were efficient in postponing the migration of lysozyme, being a promising way to extend the antimicrobial activity	Mendes de Souza et al. (2010)
	Coating	To evaluate the effect of collagen-based coatings (4%) incorporated with different concentrations of the enzyme lysozyme (0.1, 0.3, 0.5 and 0.7% lysozyme) on the conservation of fresh salmon fillets.	All lysozyme treatments improved the conservation quality of fresh salmon fillets. However, the treatment that contained 0.7% lysozyme reduced the numbers of N-BVT and presented a better antimicrobial effect compared to the other treatments, although it was less sensorially acceptable.	Wang et al. (2017)

Table 5. Continued...

Enzyme	Kind of active packaging	Application Purpose	Main effects of application/use	Reference
Bromelain	Films	Development of films based of chitosan and nano-clay (montmorillonite, sepiolite, and bentonite) that would serve as vehicles for covalent immobilization via crosslinking with glutaraldehyde (GDH) of the proteolytic enzyme bromelain, aiming at an alternative for the winemaking process.	The results showed that although the mechanical properties have been improved, the addition of clays negatively affected the catalytic properties of the immobilized protease, being then better used without the presence of nanoparticles.	Benucci et al. (2018)
Transglutaminase	Coating	Incorporation of different transglutaminase enzyme concentrations to act as a crosslinking agent in coatings based on soy protein to cover Spanish mackerel.	Although transglutaminase has improved the moisture barrier properties of coated fish compared to coated fish without transglutaminase (control), the by-product from the enzymatic reaction was responsible for reducing the quality of fish meat according to the results of pH and Nitrogen of Total Volatile Bases (N-BVT).	Karina & Setiadi (2020)
	Film	Incorporate the transglutaminase enzyme into gelatin-based films in order to improve the mechanical properties, as well as the moisture barrier and solubility once the transglutaminase enzyme (TGase) can reduce the interaction of gelatin films with water.	The higher concentration of TGase made the films more resistant to biodegradation, increasing its application due to its increased shelf life and at the end of the test, the films were degraded, showing potential to replace polymers of chemical origin	Baggio et al. (2022)
Papain	Film	Development of active packaging incorporated with papain (0%, 2%, 6% and 10%, w/w), reinforced with cellulose nanofibers (CNF) (0%, 4% and 8%, w/w) for application in meats for tenderization	CNF impaired the dispersion of papain in the polymer matrix and increased crystallinity, however, the films showed good transparency. The proteolytic activity of the films increased according to the papain concentration. Films made with 2% papain and 4% CNF have the lowest papain diffusion coefficient and, therefore, can be used in products where slow release of the active compound is required.	Santos et al. (2021)
		Films based on pregelatinized cassava starch of high (HD) and low security (LD) with different solubilities in water were incorporated with papain serving as edible packaging for meat tenderization.	Films containing 5% to 15% papain ensured better meat softness. In addition, films provided greater tenderness due to the rapid dissolution and release of enzymes on the surface of the meat, while the increase in papain in the films improved the degree of tenderness and protein conformation in the packaged beef.	Wongphan et al. (2022)

enzyme will go be migrated: gradually or quickly, or depending on the material the enzyme can be trapped in the polymeric matrix, with no migration, in this case, the active packaging acts through the packaging-food contact (Almasi et al., 2021). These mentioned points mean that enzymatic packages have advantages regarding the incorporation of the enzyme directly into the product. In addition, when talking about packaging, there is concern about the negative impacts that these products cause on the environment, especially regarding the waste that takes years and years to be degraded. However, studies with active packaging can be carried out based on natural polymers, which contributes positively to the environment (Sharma et al., 2020).

5 Conclusion

Several enzymes are essential for the food industry due to the multiple functions they play in food products. Among the six enzymatic classes (oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase), hydrolases are considered the most important, but ligase is little studied in food research. Subclasses can be extracted from plant, animal or microbiological sources, or be endogenous, naturally part of a certain food. Each subclass has a specific function due to the characteristic of the enzyme and the food.

The incorporation of food enzymes can be through immobilization in packaging which can enable protecting the enzymes against unfavorable conditions of extrinsic and intrinsic factors. The advantages for enzymatic packaging allow the enzymes to be released in a controlled manner, extending the shelf life of the food for a longer time, in addition to the packaging protecting the enzyme and making its stability more efficient compared to the food in question when compared to directly incorporating enzymes. In addition, studies have demonstrated

this thesis, in which the enzyme immobilized in the packaging is efficient and manages to be protected, with the possibility of being reused, since the cost of applying the enzyme is still high, which would be an alternative for the enzyme industry. However, few studies are still reported on the activation of packages with enzymes, requiring further scientific exploration with this approach. In addition, despite being quite effective, at least in Brazil, active packaging is rarely seen on the market, which requires greater interest from the industry in observing the innovation of these packaging.

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