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MICROBIAL ENZYMES AS VERSATILE TOOLS FOR THEIR POTENTIAL APPLICATIONS IN FOODSTUFF INDUSTRY

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ABSTRACT

The use of enzymes or microorganisms in food preparations is a very old process. Food preparations have long utilized enzymes or microorganisms. Microorganisms and enzymes are used in food preparations for centuries. The use of microbial enzymes in the dairy sector is of great importance as they can increase the yield of dairy products and improve their organoleptic properties such as taste, color and odor. Various microbial enzymes are used in the dairy industry. Transglutaminase, catalase, aminopeptidase, protease, lactose, peroxidase, etc. They are highly regarded in the industry and have a longer shelf life than coagulants. A combination of pepsin and chymosin (rennet) is used to coagulate milk for cheese and whey. It is a long-standing tradition for people to prepare food using enzymes or microorganisms. To increase the quality of various dairy products such yogurt, cheese, syrup, and bread, microbial enzymes have been used in the business. Proteolysis, an insensitive side effect of enzyme activity during food manufacturing, was also a part of early milk production procedures. Enzyme-based processes are safer than chemical processes because they do not produce harmful byproducts.

Key-words; Microbial Enzymes; Dairy Products; Pectic; Fermentation; Hydrolysis; Food applications

INTRODUCTION

Many types of Foods have been fermented for ages using microorganisms, and this practice is still prevalent in many food preparations today**.** Because they can be produced inexpensively by fermentation and are more stable than plant and animal enzymes, microbial enzymes are crucial in the food industry**.** Due to their great consistency, they are simple to adjust and improve in terms of the process**.** (Raveendran *et al.,* 2018). And today they are used in a variety of food industries to improve the quality, stability, and efficiency of food production**.** Microbial enzymes are also environmentally friendly, as they reduce energy consumption and waste (Ray and Rosell, n.d.). Traditionally, enzymes have been used in dairy, baking, brewing, and winemaking. In baking, enzymes are used to keep bread soft and fresh, increase dough volume, and give a crispy crust (Abada, 2018)**.** In beer and winemaking, enzymes are used to lower the calorie and alcoholic content, improve clarity, and enhance flavor. The use of microbial enzymes in food production has revolutionized the industry. The food business uses a variety of microbial enzymes, such as amylases, proteases, lipases, and xylanases (Blanco *et al*., 2014). Amylases are enzymes that convert starch into sugars that are then utilized to create a variety of products, including glucose syrup, crystalline glucose, high fructose corn syrup, maltose syrup, and others Proteins are broken down by proteases into amino acids, which are then used to create a range of food products, as cheese, bread and meat tenderizers (Monteiro De Souza, 2010)**.** With the help of lipases, fats can be converted into glycerol and fatty acids, which are then utilized to create a number of food products like margarine, shortening, and salad dressings. Xylan, a kind of carbohydrate present in plant cell walls, is broken down by xylanases. Xylanases are used to make it simpler to extract the juice from fruits and vegetables as well as to enhance the texture of bread and other baked food (Collins *et al*., 2005).

1. Alpha-amylase

Alpha-amylase (EC 3.2.1.1) is an enzyme that breaks down starch into smaller molecules, such as maltose and maltriose. It is found in many different organisms, including humans, plants, and bacteria. Alpha-amylase is used in various industries, including food, brewing, and laundry. In the food industry, alpha-amylase is used to make bread, beer, and other products. It is also used to remove starch from fabrics, such as cotton and linen (Monteiro De Souza, 2010). They are widely used in the bakery industry as flavor enhancers and antioxidants to enhance the bread quality. Alpha-amylase is an enzyme that breaks down starch into smaller molecules, called dextrins. Dextrins are more easily fermented by yeast, which produces carbon dioxide gas. Starch is converted into fermentable sugars that can be used to produce ethanol by the action of -amylase. The fermentation process is then continued by Saccharomyces cerevisiae, converting the carbohydrates to alcohols.(Raveendran *et al.,* 2018) provides explanation. This is done in the presence of cellulases and pectinases to improve yields and lower the cost of the process (Raveendran *et al.,* 2018)**.**

Table 1. Applications of microbial enzymes in food industry (Raveendran *et al*., 2018).

S. No.	Microbial enzyme	Method of production	Applications	References
1	α-amylase	SmF	Food preparation, brewing, and liquefaction of starch, Enhancing the quality of bread Cakes of Rice and clearing up fruit juice	(Monteiro De Souza, 2010)
2	Glucoamylase	SmF	Production of beer Enhancing the quality of bread Syrups high in fructose and glucose	(Kumar and Satyanarayana, 2009)
3	Protease	SmF	Tenderising meat while brewing Milk coagulation Enhancing the quality of bread	(Sanghi *et al*., 2010)
4	Lactase (β-galactosidase)	SmF	Lowering of human lactose intolerance Ingredients in prebiotic foods	(Raveendran *et al*., 2018)
5	Lipase	SmF	Development of cheese flavor Production of cheddar cheese	(Aravindan *et al*., 2007)
6	Phospholipase	SSF	Development of a cheese texture Lipolyzed milk fat creation	(Patel *et al*., 2017)
7	Esterase	SmF	An increase in flavor and fragrance in fruit Juice short chain texture esters are developed	(Raveendran *et al*., 2018)
8	Cellulase	SSF and SmF	Production of Animal feed Clearing agent for fruit juice	(Sanghi *et al*., 2010)
9	Xylanase	SSF and SmF	Clearing agent for fruit juice Production of improved Beer	(Collins *et al*., 2005)
10	Pectinase	SSF and SmF	Clearing agent for fruit juice	(N. Sharma et al., 2013)
11	Glucose oxidase	SSF and SmF	Enhancement of food Shelf life	(Kumar and Satyanarayana, 2009)
12	Laccase	SSF	Poly phenol removal during wine Baking	(Blanco *et al*., 2014)
13	Catalase	SSF	Preserve the food items (along with glucose oxidase)	(Raveendran *et al*., 2018)
14	Peroxidase	SSF	In fruits and vegetables, peroxidase can catalyze the oxidation of phenolic compounds to flavor and aromas	(Sanghi *et al*., 2010)
15	α-Acetolactate dehydrogenase	SmF	Shortening maturation of beer	(Ray and Rosell, n.d.)
16	Asparaginase	SmF	Reduction of acrylamide production during baking	(Raveendran *et al*., 2018)
17	Naringinase	SmF	Naringinase reduce the bitter taste also improves the overall flavor profile of citrus-based foods, particularly grapefruits and oranges.	(Abada, 2018)

1.1. Metabolic Pathway of alpha-amylase

The metabolic pathway of alpha-amylase involves the breakdown of starch, a complex carbohydrate, into simpler sugars that your body can readily utilize for energy. Here is a detailed overview of the metabolic pathway of alpha-amylase:

Starch ingestion: Starch is a complex carbohydrate found in foods like grains, potatoes, and legumes. It consists of long chains of glucose molecules (Abada, 2018)

Salivary alpha-amylase: The process begins in the mouth, where salivary alpha-amylase is secreted. Salivary alpha-amylase breaks down starch into smaller polysaccharides, such as maltose and maltotriose.(Monteiro De Souza, 2010)

Stomach: The partially digested starch enters the stomach, where the acidic environment halts the activity of salivary alpha-amylase. However, the enzyme continues to act until it gets deactivated by the stomach acid.

Pancreatic alpha-amylase: In the small intestine, pancreatic alpha-amylase, secreted by the pancreas, takes over the starch digestion process. It further breaks down the remaining starch into smaller maltose, maltotriose, and oligosaccharides.

Brush border enzymes: The brush border enzymes present in the small intestine's lining, including maltase, isomaltose, and glucoamylase, break down the maltose, maltotriose, and oligosaccharides into individual glucose molecules.

Glucose absorption: The individual glucose molecules are then absorbed through the small intestine's lining into the bloodstream.

Glycolysis and cellular respiration: Once in the bloodstream, glucose can be utilized by cells for energy through glycolysis and cellular respiration. In glycolysis, glucose is further broken down into pyruvate, generating ATP (adenosine triphosphate) as a source of cellular energy.

Storage: Excess glucose can be stored as glycogen in the liver and muscles for later use or converted into fatty acids and stored as fat in adipose tissue (Patel *et al*., 2017)**.**

1.2. Applications

Baking: Alpha-amylase is added to dough to help it rise. It does this by breaking down starches into smaller molecules, which can then be fermented by yeast. This results in a lighter, fluffier loaf of bread.

Brewing: Alpha-amylase is used to convert starches in barley into sugars that can be fermented by yeast. This process is called mashing. The sugars produced during mashing are then fermented by yeast to produce beer (Blanco *et al*., 2014).

Starch liquefaction: Alpha-amylase is used to convert starch into glucose and fructose syrups. This process is used to make a variety of products, such as high-fructose corn syrup, malt syrup, and glucose syrup (Monteiro De Souza, 2010).

Digestive aids: Alpha-amylase can be used as a digestive aid to help break down starches in the digestive tract. This can be helpful for people with digestive problems, such as celiac disease or pancreatitis. The following structure is showing the applications of alpha-amylase in various industries (Mehta and Satyanarayana, 2016)**.**

Fig. 1. Applications of α-Amylase in different industries (Mehta and Satyanarayana, 2016).

2. Glucoamylase

Glucoamylases (EC 3.2.1.3) breaks down starch into glucose with a theoretical yield of 100%. As the dextrin substrate's chain length gets shorter, the reaction rate gets slower. Maltose and isomaltose are the primary products of traditional hydrolysis, which can be catalyzed by GA to generate. This reaction is crucial in industrial processes because it helps some maltose develop when sugar concentrations of up to 40% are present. For the saccharification, grinding, distillation, etc. of starches, GA is used in the food and fermentation sectors. In order to make glucose and fructose syrups as well as sweeteners, mushroom GA is frequently employed (Kumar and Satyanarayana, 2009). Instead of using the enzymatic method to make high glucose syrup, which is extensively employed in the food sector, glucose can be a crucial substrate for fermentation to create other products such ethanol, acids amino acids, or organic acids Additionally, GA aids in lowering dough viscosity to enhance bread look and texture. They are also employed in the creation of pharmacologically effective digestive aids. (Raveendran *et al*., 2018)**.**

2.1. Metabolic Pathway

Glucoamylase is an enzyme produced by microorganisms that breaks down starch into glucose. The process begins with the binding of glucoamylase to starch, followed by the hydrolysis of alpha-1,4-glycosidic linkages between glucose molecules. This releases individual glucose molecules, which can then be further metabolized by the microorganism for energy production or biosynthesis processes (Kumar and Satyanarayana, 2009).

Starch degradation: Microorganisms, such as bacteria or fungi, secrete glucoamylase enzymes into their surrounding environment or within their cells. The process begins with the binding of glucoamylase to starch (Kumar and Satyanarayana, 2009).

Hydrolysis of alpha-1,4-glycosidic linkages: Glucoamylase acts on the starch substrate, specifically targeting the alpha-1,4-glycosidic linkages between glucose molecules. It breaks these linkages, releasing glucose units from the starch molecule (Kumar and Satyanarayana, 2009).

Release of glucose: The hydrolysis of starch by glucoamylase results in the release of individual glucose molecules. (Raveendran *et al*., 2018).

Glucose utilization: The released glucose molecules can be further metabolized by the microorganism for energy production or biosynthesis processes.

Glycolysis and cellular respiration: Once inside the microorganism, glucose can undergo glycolysis, a series of enzymatic reactions that break down glucose into pyruvate, producing ATP as a source of energy.

Biosynthesis: Glucose can also be used as a building block for various biosynthetic processes within the microorganism. It can be converted into other molecules, such as amino acids, nucleotides, or lipids, as required by the organism (Ray and Rosell, n.d.).

It's worth noting that the exact details of the metabolic pathway may vary depending on the specific microorganism producing glucoamylase. Different microorganisms may have variations in the enzymes involved, regulatory mechanisms, and other factors that contribute to carbohydrate metabolism. However, the general process of starch degradation and glucose release by glucoamylase remains consistent across microbial species. The following figure is showing the the diverse applications of Glucoamylase in food industry.

2.2. Applications

Production of high glucose syrup and high fructose syrup: Glucoamylase is used to convert starch into glucose, which is then used to make high glucose syrup and high fructose syrup. These syrups are used in a variety of food products, such as soft drinks, candy, and baked goods.

Baking: Glucoamylase plays an important role in the baking industry to improve the flour quality and improve crust color and the quality of high-fiber baked goods. Glucoamylase converts starch in flour into maltose, which can be fermented by yeast. The fermentation of yeast leads to the rise of the dough.(Ray and Rosell, n.d.).

Production of ethanol: Glucoamylase also used for the production of glucose, which is then fermented with Saccharomyces cerevisiae to produce ethanol. Ethanol is used in a variety of products, including alcoholic beverages, fuel, and solvents (Blanco *et al*., 2014).

Production of sake and soy sauce: Glucoamylase is used to produce the sake and soy sauce. In sake production, glucoamylase is used to convert starch in rice into glucose, which is then fermented with koji mold and yeast to produce sake. In soy sauce production, glucoamylase is used to convert starch in soybeans into glucose, which is then fermented with koji mold and *Aspergillus oryzae* to produce soy sauce.

Production of light beer: Glucoamylase is used to produce the light beer to reduce the calorific value and alcohol content of the beer. Glucoamylase metabolizes the dextrins in the beer and converts them into fermentable sugars that are then fermented by yeast. This process produces a beer that is lower in calories and alcohol content than regular beer (Blanco *et al*., 2014).

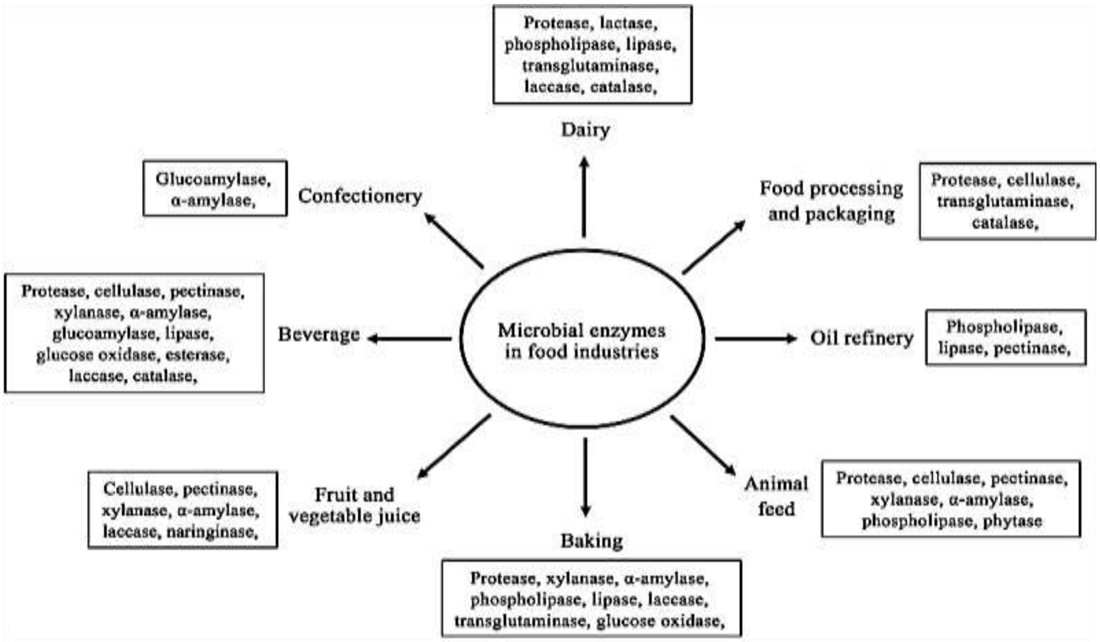

Fig. 2. Summary of enzymes that have found applications in food industry (Okpara, 2022).

3. Protease

Proteins and polypeptides include peptide bonds, which are hydrolyzed by proteases, an enzyme. They are extensively utilized in the food industry, medicines, and detergents. On the market, they account for 60% of industrial enzymes. Animals, plants, and microbes (bacteria and fungi) are responsible for the production of protease enzymes. Proteases fall into one of two categories:

Exopeptidase and Endopeptidase, based on the polypeptide chain's location of action. The extremities of polypeptide chains are affected by exopeptidases, and the interior of the polypeptide chain is randomly affected by endopeptidases. Depending on the catalytic residues located in the active site, endopeptidases are divided into six classes (Razzaq *et al*., 2019).

Serine endopeptidases: These enzymes use a serine residue as the catalytic amino acid. They are the most common type of endopeptidase and are found in both prokaryotic and eukaryotic cells. Examples of serine endopeptidases include trypsin, chymotrypsin, and elastases of cysteine endopeptidases includes papain and cathepsin B.

Aspartic endopeptidases: These enzymes use an aspartic acid residue as the catalytic amino acid. They are found in animals and bacteria. Examples of aspartic endopeptidases include pepsin and renin. (Raveendran *et al*., 2018)

Metalloendopeptidases: These enzymes use a metal ion, such as zinc, as the catalytic amino acid. They are found in animals, plants, and bacteria. Examples of metalloendopeptidases include thermolysin and collagenase.

Glutamyl endopeptidases: These enzymes use a glutamic acid residue as the catalytic amino acid. They are found in animals and bacteria. Examples of glutamyl endopeptidases include leucine aminopeptidase and dipeptidyl peptidase IV. (Razzaq *et al*., 2019)

3.1. Metabolic Pathway

Gene expression and protein synthesis: Microorganisms produce proteases by transcribing and translating genes that encode for these enzymes. The expression of these genes is regulated by environmental factors, substrate availability, and microbial growth phase (Patel *et al*., 2017)

Protein secretion: Once synthesized, proteases are typically transported across the cell membrane and released into the extracellular space. This process can occur via various mechanisms, including the use of specific secretion signals or secretion systems present in the microorganism. (Abada, 2018)

Proteolytic activity: Proteases hydrolyze peptide bonds in proteins, leading to the degradation of complex protein structures into smaller peptides or amino acids. This proteolytic activity contributes to the desired functional.

Regulation and feedback: The production and activity of proteases are tightly regulated by feedback mechanisms. As the concentration of peptides and amino acids generated by protease activity increases, they can act as feedback signals that modulate the expression of protease genes, thereby regulating the production of proteases by the microorganisms (Contesini *et al*., 2018)

Product utilization: The breakdown of proteins by proteases generates smaller peptides and amino acids, which can be further utilized by microorganisms as a nutrient source. In some cases, the end products of proteolysis can contribute to the development of desired flavors, textures, and nutritional characteristics in food products. (Razzaq *et al*., 2019)

It is important to note that the specific metabolic pathway of microbial proteases can vary depending on the microorganism involved. Additionally, advancements in genetic engineering and biotechnology have enabled the modification and optimization of microbial proteases for specific applications in the food industry, further expanding their utility and potential**.** (Patel *et al*., 2017)

3.2. Applications

Nutritional value: Proteases can be used to break down proteins into smaller peptides and amino acids, which are more easily absorbed by the body. This can be beneficial for people who have difficulty digesting protein, such as infants, the elderly, and people with digestive disorders (Contesini *et al*., 2018).

Taste: Proteases can be used to modify the taste of food. For example, they can be used to make meat more tender and flavorful.

Digestibility: Proteases can be used to make food more digestible. This can be beneficial for people with digestive disorders, such as lactose intolerance.

Functional properties: Proteases can be used to change the functional properties of food, such as its ability to coagulate, emulsify, foam, or gel. This can be used to improve the texture, appearance, and shelf life of food products. The applications of Protease enzyme are mentioned in the following figure representing various industries (Anbu *et al.,* 2017).

Fig. 3. Applications of Protease in different industries (Liburdi and Esti, 2022).

4. Lactase (β-galactosidase)

β-galactosidase, also known as lactase (EC 3.2.1.23), plays a crucial role in hydrolyzing lactose, the sugar found in milk. The enzyme finds numerous applications in the food industry, where its use is particularly significant. Excessive lactose in the intestines can lead to adverse effects, such as tissue dehydration and reduced calcium absorption due to low acidity, resulting in symptoms like diarrhea, bloating, and cramps. Lactose absorption hinges on the action of lactase, primarily located in the small intestine, breaking the bonds between the two sugars (monosaccharides) in lactose. A deficiency in this enzyme results in lactose intolerance, rendering individuals unable to consume milk and dairy products.

Beyond its role in addressing lactose intolerance, β-galactosidase has gained importance in molecular biology due to its ability to produce colored products in chemical reactions. This unique property has made the enzyme a valuable tool in various molecular biology techniques. The enzyme's capacity to cleave specific glycosidic bonds in synthetic substrates generates detectable color changes, aiding researchers in visualizing and quantifying biological processes (Saqib *et al*., 2017).

4.1. Metabolic Pathway

Lactase is an enzyme that breaks down lactose, a sugar found in milk and dairy products, into glucose and galactose. This process happens in the small intestine. Glucose and galactose are then absorbed into the bloodstream and used for energy (Raveendran *et al*., 2018).

Here are the steps involved in the metabolic pathway of lactase:

Lactose ingestion: Lactose is consumed through the diet in foods like milk, cheese, and yogurt.

Lactase production: The small intestine cells produce and secrete lactase enzyme.(Patel *et al*., 2017)

Hydrolysis of lactose: Lactase acts on lactose, breaking the beta-1,4-glycosidic bond between glucose and galactose.

Absorption: The glucose and galactose resulting from lactose hydrolysis are then absorbed across the brush border membrane of the small intestine cells.

Transport into bloodstream: The absorbed glucose and galactose are transported into the bloodstream via specific transport proteins present in the cell membrane.

Utilization and metabolism: Once in the bloodstream, glucose and galactose can be utilized by various tissues and organs for energy production through glycolysis and other metabolic processes (Saqib *et al*., 2017).

4.2. Applications

- Make dairy products more digestible for people with lactose intolerance
- Produce lactose-free milk and dairy products
- Improve the taste and texture of dairy products
- Prevent lactose crystallization in frozen dairy products
- Reduce the lactose content of whey, a byproduct of cheese production
- Treat lactose intolerance in infants and young children
- Improve the absorption of calcium and other nutrients from dairy products (Al-Manhel, 2018

The following figure is showing the different applications of Lactase (β-galactosidase) in Food industry.

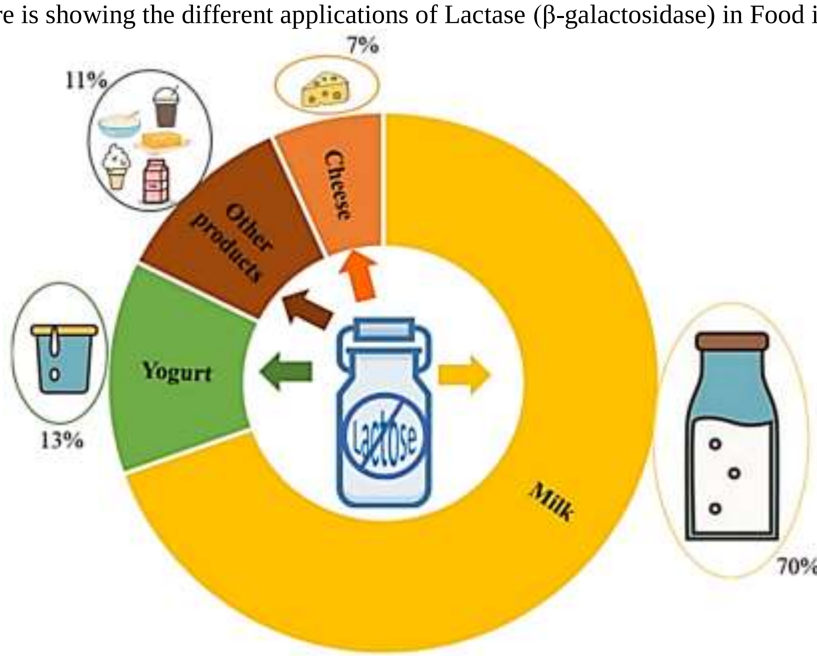

Fig. 4. Applications of Lactase (β-galactosidase) in Food industry.

5. Lipase

Lipases (EC 3.1. 1.3), particularly microbial lipases, are significant important in food industry, hence efforts to produce and purify lipase enzymes from microbial strains are ongoing to meet demand from the food and pharmaceutical industries. Increased lipase synthesis in microbial strains has recently been attempted using a variety of efficient and affordable approaches. In light of this, this work makes an effort to bridge the gap between the production of lipase utilizing several strains of microorganisms, including yeast, fungus, and bacteria. These strains have been highlighted and discussed to help researchers choose the best one for the production of lipase. The discussion that followed focused on the screening of lipase and lipase producers in many fermentation systems (Aravindan et al., 2007) The effects of variables, including PH, temperature, carbon and nitrogen sources, and yield-increasing microbial strains, were then assessed and presented. The latest research on the extraction of lipases from microbial stains is reviewed and summarized, and lipase enzyme purification methods have been briefly discussed**.** (Abada, 2018).

5.1. Metabolic Pathway

Lipases are enzymes that break down triglycerides into free fatty acids and glycerol. Triglycerides are fats that are found in foods like meat, dairy products, and oils. Lipase enzymes are produced by the pancreas, small intestine, and adipose tissue.

Here are the steps involved in the metabolic pathway of lipase:

Triglyceride ingestion: Triglycerides are consumed through the diet in foods containing fats and oils (Abada, 2018)

Lipase production: Lipase enzymes are produced and secreted by various tissues and organs, including the pancreas, small intestine, and adipose tissue.

Emulsification: In the small intestine, bile salts produced by the liver emulsify the ingested fats, forming smaller droplets that have a larger surface area for lipase to act upon (Ray and Rosell, n.d.).

Lipase action: Lipase acts on the emulsified triglycerides, breaking the ester bonds between glycerol and fatty acids. This hydrolysis reaction releases free fatty acids and glycerol.

Absorption: The released free fatty acids and glycerol are absorbed through the lining of the small intestine into the intestinal cells (Patel *et al.*, 2017).

Formation of chylomicrons: Within the intestinal cells, the absorbed free fatty acids and glycerol are reassembled into triglycerides. These triglycerides, along with cholesterol and other lipids, combine with proteins to form chylomicrons, which are lipoprotein particles (Raveendran *et al*., 2018)

Transport via lymphatic system: Chylomicrons are released into the lymphatic system and transported through lymphatic vessels to enter the bloodstream.

Utilization and storage: Once in the bloodstream, the chylomicrons are transported to various tissues, where lipoprotein lipase breaks down the triglycerides within them. The released fatty acids can be utilized by cells for energy through beta-oxidation, or they can be stored as triglycerides in adipose tissue for later energy use. The metabolic pathway of lipase is given in the following figure (Alekos *et al*., 2020).

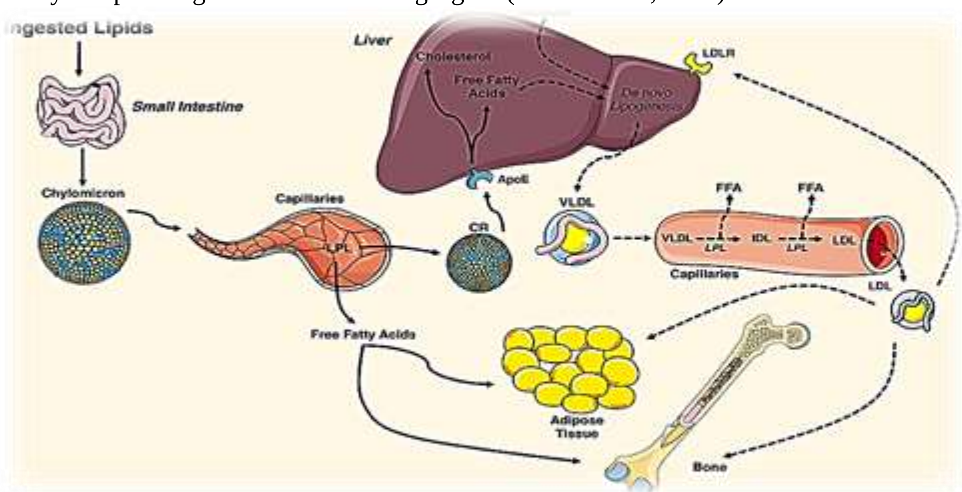

Fig. 5. Metabolic pathway of Lipase (Alekos *et al.*, 2020).

5.2. Applications

Enhancing cheese flavor and maturation time: Lipase can be used to break down milk fat into free fatty acids, which contribute to the flavor of cheese. Lipase can also be used to accelerate the maturation of cheese, resulting in a more flavorful product in a shorter amount of time. (Raveendran *et al*., 2018)

Lipolysis of milk fat and cream: Lipase can be used to break down milk fat and cream into smaller molecules, which can be used in a variety of dairy products. For example, lipase can be used to make butter, yogurt, and ice cream (Chandra *et al*., 2020).

Coffee mate: Lipase can be used to add a creamy taste to coffee mate. Lipase breaks down the milk fat in coffee mate into smaller molecules, which are more easily absorbed by the body. This results in a creamier and more flavorful coffee mate (Raveendran *et al*., 2018).

Bread production: Lipase can be used to extend the shelf life of bread, control non-enzymatic browning, increase the volume of bread, and improve the structure of bread. Lipase breaks down the starch in bread into smaller molecules, which makes the bread softer and tenderer. Lipase also prevents the bread from browning too quickly, which helps to extend its shelf life. The following figure is showing the different applications of lipase in various industries (Chandra *et al*., 2020).

Fig. 6. Applications of Lipase in food industry (Vishnoi *et al*., 2020).

6. Phospholipase

Phospholipases (EC 3.1. 1.32), All living cells contain phospholipids, a form of lipid, and phospholipases are a group of enzymes that break down phospholipids. A glycerol molecule, two fatty acids, and a phosphate group make up phospholipids. Phospholipids' special characteristics, such as their capacity to form membranes, are due to the presence of the phosphate group. A developing trend in the food industry is the usage of phospholipases. Phospholipases are anticipated to be used much more frequently as further study on their advantages is undertaken (De Maria *et al*., 2007).

6.1. Applications

Degumming of vegetable oils: Vegetable oils contain phospholipids, which can give the oil a cloudy appearance and a soapy taste. Phospholipases can be used to remove the phospholipids from the oil, resulting in a clear, tasteless oil (Ray and Rosell, n.d.).

Improvement of the emulsifying properties of food: Phospholipases can be used to improve the emulsifying properties of food, such as mayonnaise, salad dressings, and ice cream.
Production of functional foods: Phospholipases can be used to produce functional foods, such as infant formula and dietary supplements. Functional foods are foods that have health benefits beyond basic nutrition (Uraz and Özer, 2014).

7. Esterase

Esterases (EC 3.1) are enzymes that catalyze the hydrolysis of ester groups from a variety of substrates to release esterified acids. The main group of esterases used in industries are lipases. A lipase is an enzyme that can catalyze a reaction which can be tailored to produce a de-esterification or transesterification, a free acid or another ester of an acid, usually an alkyl ester. Lipase is also used as part of the enzymes used in detergents. Lipase is also used to degrease leather. New applications include the use of lipases and proteases in lens cleaning solutions to clean contact lenses. Immobilized lipases are also used to produce biodiesel from vegetable oils or non-edible oils such as jatropha. Esterases are used to synthesize the antibiotic penicillin and to degrade the pesticide DDT (Bohnenkamp, 2021).

7.1. Applications

Food and beverage industry

Esterases are used in the food and beverage industry to modify the flavor and texture of food products. For example, esterases are used to hydrolyze fats and oils in fruit juices to produce aromas and flavors. toxins. For example, esterases are used to synthesize the antibiotic penicillin and to degrade the pesticide DDT.

Pharmaceutical industry

Esterases are used in the pharmaceutical industry to synthesize drugs and to degrade (D. Sharma *et al*., 2022).

8. Cellulase

A complex family of enzymes known as cellulases (EC 3.2.1.4) is secreted by a variety of microorganisms, including fungus, bacteria, and actinomycetes. The hydrolysis of lignocellulosic polymeric materials occurs naturally as a result of synergistic interactions between cellulolytic microbes. The effectiveness of this process is actually determined by the combined action of three important enzymes Exoglucanases, endoglucanases, and -glucosidases are what they are. These enzymes are produced by microorganisms in a variety of ways, which affects how effectively cellulose is hydrolyzed. Enzymes target -1,4 bonds in the polymer structure during cellulolytic processes. This is a crucial ecological procedure because the biosphere recycles cellulose. This scenario's industrial use has been recognized as a new topic for research. manufacturing of biofuels, finishing and polishing of textiles, and paper and pulp Industries (Gusakov, 2013) .

8.1. Metabolic Pathway

The production of cellulase enzymes is often regulated by the presence of cellulose, and that the enzymes can be produced by a variety of organisms, including fungi, bacteria, and algae. Cellulase enzymes have a wide range of potential applications, including the production of biofuels, bioproducts, and animal feed (Zhong *et al*., 2019).

Here are some additional details about the different types of cellulase enzymes:

Endoglucanases (EC 3.2.1.4), are the most abundant type of cellulase enzyme, and they are responsible for breaking down the internal glycosidic bonds in cellulose. This process creates shorter cellulose chains, which can then be further broken down by other cellulase enzymes.

- Exoglucanases (EC 3.2.1.91, also known as cellobiohydrolases, cleave the terminal glycosidic bonds of cellulose, releasing cellobiose, a disaccharide consisting of two glucose units, from the ends of the cellulose chains (Gusakov, 2013).
- β-Glucosidases are the final step in the cellulase pathway, and they break down cellobiose and other cello-oligosaccharides into individual glucose molecules.

The combined activity of these three types of cellulase enzymes is necessary for the complete hydrolysis of cellulose into glucose (Ejaz *et al*., 2021).

8.2. Applications

Food and beverage: Cellulases are used to clarify fruit and vegetable juices, improve the texture of bread and other baked goods, and extract tea polyphenols and essential oils from olives (de Souza and Kawaguti, 2021a)

Pulp and paper: Cellulases are used to break down wood pulp into cellulose fibers, which are then used to make paper.

Biofuel production: Cellulases are used to break down biomass, such as wood chips and agricultural waste, into sugars that can be fermented to produce biofuels such as ethanol and biodiesel (Raveendran *et al*., 2018).

9. Xylanase

Juices from fruits and vegetables frequently have turbidity because to suspended polysaccharide materials like cellulose, hemicellulose, lignin, pectin, starch, metals, proteins, and tannins. Juice quality and consumer demand will decline if these compounds are present. A commercial enzyme product called "Rapidase pomaliq" contains cellulases, hemicellulases, and pectinases that are derived from Trichoderma reesei and *Aspergillus niger*. It is particularly advantageous to use this product in the clarifying of fruit juice. There have also been reports of hemicellulases and pectinases combining with cellulases produced by bacteria like Bacillus and Paenibacillus to clarify fruit and vegetable liquids. In addition, this enzymatic method has successfully been used to treat nectars and purees (Collins *et al*., 2005).

9.1 Applications

Breadmaking: Xylanases can be used to improve the quality of bread by increasing its volume, crumb softness, and shelf life. They do this by breaking down the xylans in wheat flour, which allows the gluten to form more easily and gives the bread a better texture (Polizeli *et al.,* 2005).

Papermaking: Xylanases can be used to improve the strength and brightness of paper by breaking down the xylans in wood pulp. This makes the paper more resistant to tearing and makes it easier to bleach.

Animal feed: Xylanases can be added to animal feed to improve the digestibility of the feed and to increase the growth rate of the animals. They do this by breaking down the xylans in the feed, which makes the nutrients more available to the animals. Diverse applications of Xylanase in various industries are shown in the following figure (Ray and Rosell, n.d.).

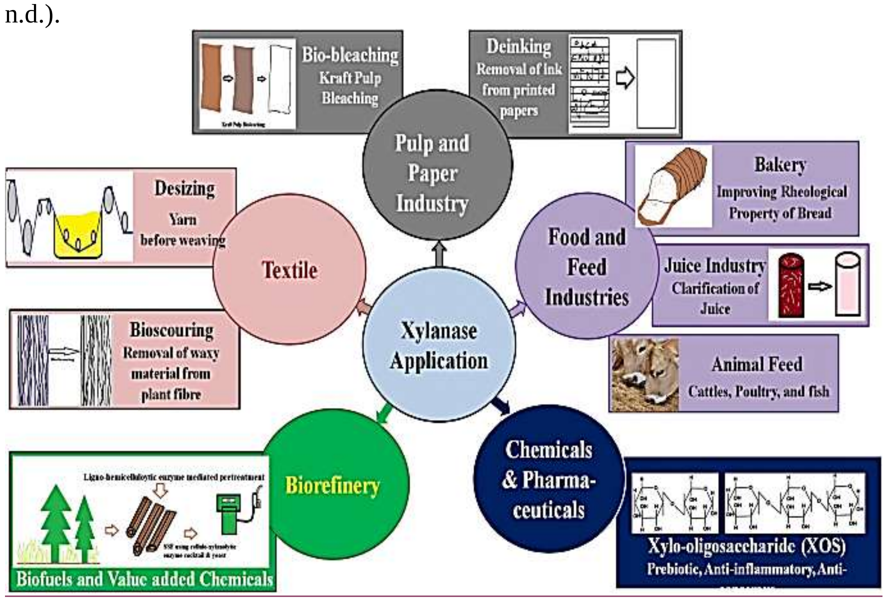

Fig. 7. Applications of Xylanase in different industries (Bhardwaj *et al.,* 2019).

10. Pectinase

Pectinases (EC 3.2.1.15) are proteins that act as catalysts in different reactions. Pectinases are a group of enzymes that break down pectin, a polysaccharide found in plant cell walls, into sugar monomers. Pectinases have a wide variety of applications in a number of industries (Sharma *et al.,* 2013). Pectinases can be classified into three types according to their mode of action on the substrate:

Polygalacturonase: This type of pectinase breaks down pectin into small fragments through the process of hydrolysis.
Pectinesterase: This type of pectinase breaks down the polymer of pectin into monomers through the process of trans-elimination.
Pectin lyase: This type of pectinase breaks down pectin through the process of de-esterification (Sakhuja *et al.*, 2021)
Different types of fungi, yeasts, and bacteria are used to produce pectinases. Pectinases produced by plants are more active and therefore more tolerant to alkalis, acids, and temperatures (Khan *et al.*, 2013).

10.1. Applications

Improve the texture and flavor of fruits and vegetables: Pectinases break down the pectin in fruits and vegetables, making them softer and more flavorful.
Clarify fruit juices: Pectinases break down the pectin in fruit juices, making them clearer and more appealing (Patidar *et al.*, 2018).
Make wine: Pectinases are used to break down the pectin in grapes, which helps to extract the juice and improve the flavor of the wine (Abada 2018).

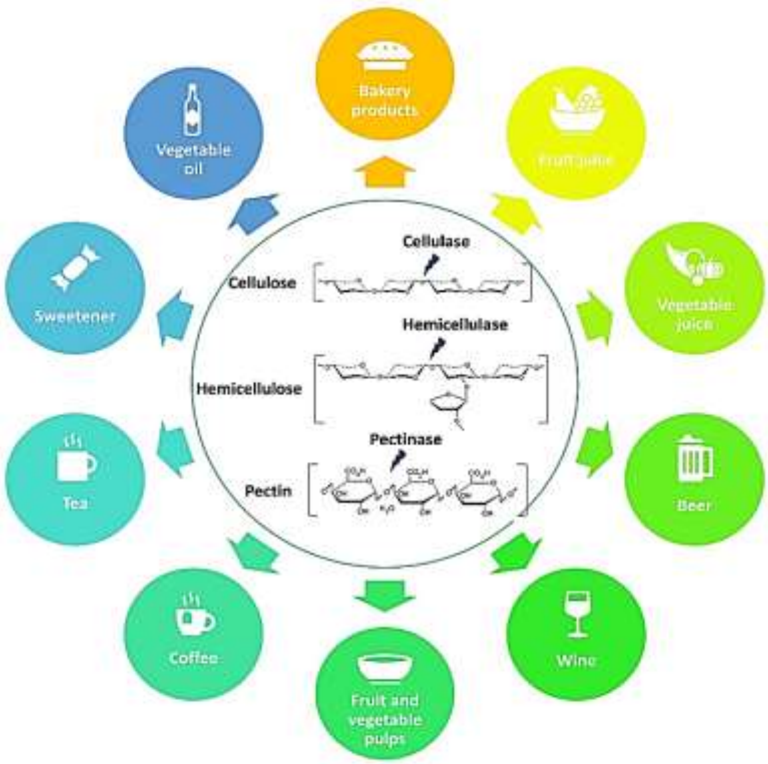

Fig. 8. Applications of Pectinase in various industries (de Souza and Kawaguti, 2021b).

11. Glucose Oxidase

Glucose oxidase (EC 1.1.3.4) in the presence of molecule oxygen, oxidizes glucose to gluconolactone, generating hydrogen peroxide. Because the reaction is catalyzed, GOD is frequently used to extract glucose or molecular oxygen to increase the shelf life of foods, or to produce hydrogen peroxide or gluconic acid under regulated conditions. Building glucose biosensors is one of GOD's most significant application fields. Numerous studies have been done on GOD purification, immobilization, industrial, and analytical applications, therefore it is crucial for these studies to quickly and accurately determine GOD activity (Wong et al., 2008) In this study, four basic methodologies of measuring GOD activity were taken into consideration: measure the change in glucose or oxygen concentration and the change in concentration of Hydrogen peroxide (Wong *et al*., 2008).

11.1. Application

- Remove/deplete oxygen in beverages to extend shelf life
- Removing glucose in egg powder processing
- Flour improver or dough hardener
- Online glycemic control and monitoring in commercial biological processes (fermentation)
- Prevents color loss in food due to enzymatic reactions and the presence of oxygen
- Determination or quantification of glucose (analytical method in food) (Wong *et al.*, 2008).

12. Laccase

Laccases (EC 1.10.3.2) are an important group of multi co-enzymes that have received a great deal of research interest in the past few decades due to their ability to oxidize both phenolic and non-phenolic lignin-binding compounds, as well as contaminants environment is highly regenerative. As a result, these biocatalysts are particularly beneficial for usage in a variety of biotechnological processes, including those in the food sector. As a result, laccase has enormous potential as a food additive in the production of food and beverages. Laccase-based biocatalysts that conserve energy and are biodegradable are ideal for the growth of highly effective, environmentally friendly companies (Osma *et al*., 2010).

13.1. Applications

Various foods and beverages include many laccase substrates, including carbohydrates, unsaturated fatty acids, phenols, and proteins containing thiols.

- Their alteration by laccase may produce novel functions, higher-caliber products, or cheaper products.
- Numerous procedures that enhance or alter the color look of foods or beverages might include laccase.
- One of the principal applications for laccase in the food sector as a replacement for physio-chemical adsorbents is alcohol stabilization.
- Various chemical constituents, including ethanol, organic acids (fragrance), salts, and phenolic compounds are blended together in great detail to create musts and wines (Patel *et al.*, 2017).

13. Catalase

Catalases (EC 1.11.1.6) boasts one of the highest turnover rates among enzymes, capable of efficiently decomposing over a million hydrogen peroxide molecules per enzyme molecule. Expanding beyond its physiological functions, catalase finds widespread application in various biotechnological processes. In the realm of bio-remediation, catalase emerges as a valuable indicator of hydrocarbon degradation in soil during crude oil contamination. Its ability to supply oxygen renders it instrumental in aerobic bioremediation, aiding in the efficient removal of contaminants. Beyond environmental applications, catalase is actively involved in wastewater treatment, particularly in the bleaching industry, where it effectively eliminates hydrogen peroxide, contributing to enhanced water quality. Moreover, the enzyme's versatility extends to potential applications in the food industry, where its role in breaking down hydrogen peroxide could be harnessed for food processing or preservation. This comprehensive review underscores the multifaceted significance of catalase in biotechnology, emphasizing its crucial contributions to environmental remediation, wastewater treatment, and its potential role in ensuring food safety (Abada, 2018).

13.1. Applications

- Food processing: Catalase can be used to remove hydrogen peroxide from food products, such as milk and cheese. This helps to prevent the growth of harmful bacteria and to improve the shelf life of the products.
- Wastewater treatment: Catalase can be used to break down hydrogen peroxide in wastewater. This helps to reduce the environmental impact of wastewater treatment plants.

Bioremediation: Catalase can be used to clean up contaminated soil and water. It can break down a variety of pollutants, including pesticides, herbicides, and heavy metals (Ray and Rosell, n.d.).

14. Peroxidase

Hydrogen peroxide and other hydroperoxides can be reduced to water by the enzyme peroxidase. In this process, some substrates experience oxidation. Based on the chemistry of the active site's interactions with peroxide, peroxidase is classified as either heme or non-heme peroxidase. While the latter have reactive elenol or thiol at their active site, the former have a heme catalytic centre. Here, peroxidase's distinct enzymatic traits and biochemical attributes are discussed. In instance, peroxidase has oxidative physiological roles in addition to their well-known peroxide-trapping characteristics. The primary function of peroxidase in this subject is also described as hydrogen peroxide emerges as a significant regulatory molecule in redox signaling (Raveendran *et al*., 2018).

14.1. Applications

- Peroxidase catalysis the oxidation of a variety of substrates using hydrogen peroxide or other peroxides. The food industry uses this enzyme to improve meals' nutritional content while also adding flavor, color, and texture.
- Additional applications include environmental pollutant control, polymer synthesis, and biosensors. Industrial effluent containing phenol can be handled with it.
- The food industry measures the effectiveness of the blanching process, which lengthens food shelf life, using thermal inactivation of peroxidase (Abada, 2018).

16. Naringinase

Microbial naringinase (EC 3.2.1.40) is an enzyme that has the potential to significantly improve the quality and appeal of citrus-based products. It can be used to reduce bitterness, enhance flavor, improve product quality, extend shelf life, and optimize processes. This enzyme is a valuable tool for food manufacturers who want to create more appealing and shelf-stable citrus products that meet consumer preferences (Raveendran *et al*., 2018).I am also interested in the potential of naringinase to extend the shelf life of citrus products. As you mentioned, naringin can undergo enzymatic and chemical reactions over time, leading to the formation of off-flavors and loss of product quality. By using naringinase, these reactions can be minimized, thereby preserving the freshness and shelf stability of the citrus-based food items. I think this is an important area of research, as it could help to reduce food waste and improve the availability of fresh citrus products. I am excited to see what future research reveals about the potential of naringinase to extend the shelf life of citrus products. Overall, I think microbial naringinase is a promising enzyme with a wide range of potential applications in the food industry. I am excited to see how it is used in the future to create more appealing and shelf-stable citrus products (Yadav *et al*., 2018).

15.1. Applications

Microorganisms, has various applications in the food industry. It is primarily used in the processing of citrus fruits and related products. Here are some key applications of naringinase in the food industry:

Bitterness reduction in citrus beverages: Naringinase is commonly employed to reduce the bitter taste associated with citrus fruits, particularly grapefruits and oranges. By hydrolyzing naringin, the enzyme breaks down the bitter compounds, resulting in milder and more palatable citrus beverages such as fruit juices, concentrates, and flavored drinks (Yadav *et al*., 2018).

Flavor enhancement in citrus products: Naringinase not only reduces bitterness but also improves the overall flavor profile of citrus-based foods and beverages. By breaking down naringin, the enzyme releases aromatic compounds, contributing to the development of a more vibrant and appealing flavor. This application is particularly useful in the production of citrus juices, jams, jellies, marmalades, and fruit-based desserts (Raveendran *et al*., 2018).

Haze reduction in citrus juices: Naringinase plays a crucial role in reducing the haze formation in citrus juices. Naringin, if not properly hydrolyzed, can cause cloudiness or turbidity in the juice due to its interaction with other components. By using naringinase, the enzyme breaks down naringin, minimizing the formation of haze and enhancing the clarity and visual appeal of citrus juices.

Extraction of flavonoids and other bioactive compounds: Naringinase is employed in the extraction of bioactive compounds, particularly flavonoids, from citrus fruits. Flavonoids possess various health benefits and are widely used as natural food additives and nutraceutical ingredients. Naringinase helps in releasing these compounds from their glycosidic form, facilitating their extraction and utilization in food products and dietary supplements (Yadav *et al*., 2018).

Fermentation processes: Naringinase can be used in fermentation processes to improve the utilization of citrus by-products. Citrus peels, which are rich in naringin, can be enzymatically treated with naringinase to release sugars and other nutrients. These can then be used as substrates for microbial fermentation, resulting in the production of value-added products such as ethanol, organic acids, and bioactive compounds. The applications of Naringin in medicinal industry are given below to treat the different diseases. Click or tap here to enter text.

16. ROLE OF MICROBIAL ENZYMES IN DIARY INDUSTRIES

The dairy industry relies on a variety of microbial enzymes to produce diverse and delicious products like yogurt, cheese, and bread. Lactase, for instance, breaks down lactose in milk, catering to lactose-intolerant individuals. Lactic Acid Bacteria (LAB) contribute to flavor and texture in products like yogurt and cheese through lactose fermentation. Rennet, derived traditionally from young ruminant stomachs, contains chymosin, crucial for coagulating milk proteins in cheese making. Proteases and amylases play roles in cheese maturation and bread fermentation, respectively. The interplay between traditional artisanal practices and modern scientific understanding is evident, with modern techniques refining and controlling microbial processes. This connection prompts speculation about undiscovered enzyme-driven processes in other traditional food practices, hinting at the potential for further exploration and discovery in the world of fermentation and culinary traditions (Abada, 2018).

Table 2. Applications of Microbial Enzymes in the Dairy Industry (Abada, 2018).

Sr. No.	Enzymes	Roles	Microorganisms	References
1	Aminopeptidase	Faster cheese ripening	*Lactobacillus* sp.	(Masoud *et al.*, 2017)
2	Acid proteinase	Milk coagulation	*Aspergillus* sp.	(Abada, 2018)
3	Catalase	Cheese processing	*Aspergillus niger*	(Palomba *et al*., 2017)
4	Lipase	Faster cheese ripening	*Aspergillus niger*	(Sharma and Sharma, 2018)
5	Neutral proteinase	Faster cheese ripening	*Bacillus* sp., *Aspergillus oryzae*	(Abada, 2018)
6	Transglutaminase	Protein cross linking	*Streptomyces* sp.	(Domagała *et al*., 2016)

17. CONCLUSION AND FUTURE PERSPECTIVE

Enzymes find applications in the food, detergent, pharmaceutical and paper industries. Due to its low cost of purification, high yield, effective process control, safety, and environmental friendliness, enzyme-based techniques are now preferred over chemical procedures. Microbial enzymes may be manufactured on a large-scale utilizing fermentation process and are more productive to create than plant and animal enzymes. It is simple to alter microbial enzymes to enhance their characteristics, and there is still a lot of room for the creation of novel microbial enzymes with commercial uses (Raveendran *et al*., 2018).

Environmental friendliness: Enzyme-based processes are more environmentally friendly than chemical processes because they do not produce harmful byproducts.

Efficient process control: Enzyme-based processes are more easily controlled than chemical processes because enzymes are highly specific and can be easily inhibited by certain conditions.

High yield: Enzyme-based processes can produce higher yields than chemical processes.

Low purification costs: Enzyme-based processes require less purification than chemical processes, which can save money.

Safety: Enzyme-based processes are safer than chemical processes because they do not produce harmful byproducts.

There is still great potential for the development of new microbial enzymes with unlimited industrial applications. Microbial enzymes are already used in a various industry such as food, pharmaceutical, and textile industries. As research in this area continues, it is likely that new microbial enzymes will be developed with even more useful properties (Raveendran *et al*., 2018).

18. GLOBAL ENZYME MARKET SCENARIOS

The industrial enzymes market is a large and growing market. It is expected to reach $4.4 billion by 2015, with a compound annual growth rate of 6%. The market is driven by a number of factors, including:

1. The increasing demand for food and beverages, which require enzymes for production.
2. The growing demand for biofuels, which require enzymes for production.
3. The increasing demand for personal care products, which require enzymes for production.
4. The increasing demand for pharmaceuticals, which require enzymes for production.

The market is also being driven by the development of new and innovative enzymes. For example, new enzymes are being developed that can be used to produce biofuels from biomass. These new enzymes are more efficient and cost-effective than traditional methods of producing biofuels. The industrial enzymes market is a global market, with no single region dominating. The United States and Europe are the largest markets, followed by Asia-Pacific. However, Asia-Pacific is expected to be the fastest growing market in the next few years. The industrial enzymes market is a competitive market, with a number of large companies competing for market share (Raveendran *et al*., 2018). The leading companies in the market include:

1. **Novozymes**
2. **BASF**
3. **DuPont**
4. **DSM**
5. **Kerry Group**

These companies are investing heavily in research and development to develop new and innovative enzymes. They are also expanding their production capacity to meet the growing demand for enzymes. The industrial enzymes market is a dynamic market with a number of growth opportunities. The market is expected to continue to grow in the next few years, driven by the increasing demand for food and beverages, biofuels, personal care products, and pharmaceuticals (Patel *et al.*, 2017).

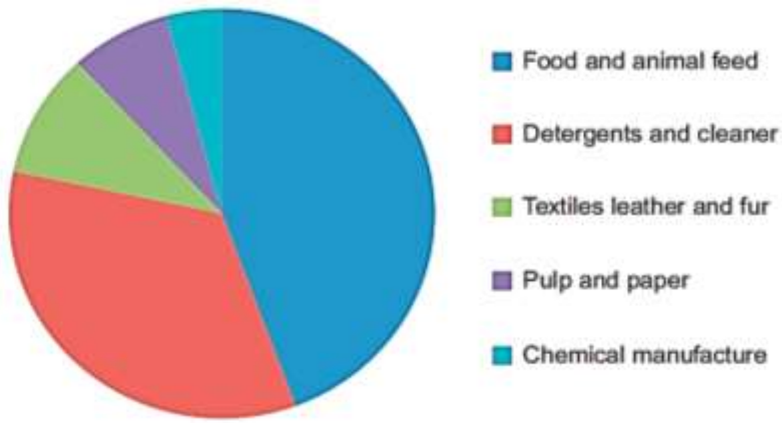

Fig. 9. Industrial enzyme sales by sector (Patel *et al*., 2017).

19. WORLD ENZYME MARKET BY APPLICATION SECTOR

Enzymes are biological catalysts that can speed up chemical reactions. They are used in a variety of industries, including food, pharmaceuticals, and textiles. Enzymes can be used to improve the efficiency of manufacturing processes by reducing the time and energy required to produce products. They can also be used to improve the quality of products by making them purer and more consistent. The enzyme industry is constantly looking for new and innovative ways to use enzymes. One of the latest trends is the use of enzymes in sustainable processes. Sustainable processes are those that minimize the environmental impact of manufacturing. Enzymes can be used to reduce the amount of energy and chemicals required in manufacturing processes. They can also be used to recycle waste products (Patel *et al*., 2017).

Here are some examples of how enzymes are being used in sustainable processes:

In the food industry: enzymes are being used to produce lactose-free dairy products. Lactose is a sugar that is found in milk. Some people are unable to digest lactose, which can cause digestive problems. Enzymes can be used to break down lactose into smaller sugars that can be easily digested.

In the textile industry: enzymes are being used to remove stains from clothing. Enzymes can be used to break down stains caused by food, sweat, and other substances. This can help to reduce the amount of water and chemicals that are used in the textile manufacturing process.

In the biofuel industry: enzymes are being used to convert biomass into fuel. Biomass is organic material that can be used to produce energy. Enzymes can be used to break down biomass into smaller molecules that can be used to produce biofuel.

The use of enzymes in sustainable processes is a growing trend. As the demand for sustainable products continues to grow, the enzyme industry is likely to develop new and innovative ways to use enzymes to reduce the environmental impact of manufacturing (Patel *et al*., 2017).

The global market for industrial enzymes was relatively unaffected by the economic turmoil of 2008-2009. In mature economies, the market was stable, while developing economies in Asia-Pacific, Eastern Europe, and Africa saw the fastest growth. The United States and Europe collectively command a major share of the global market, while Asia-Pacific growth was stagnant at 8% CAGR from 2008-2012 (Patel *et al*., 2017).

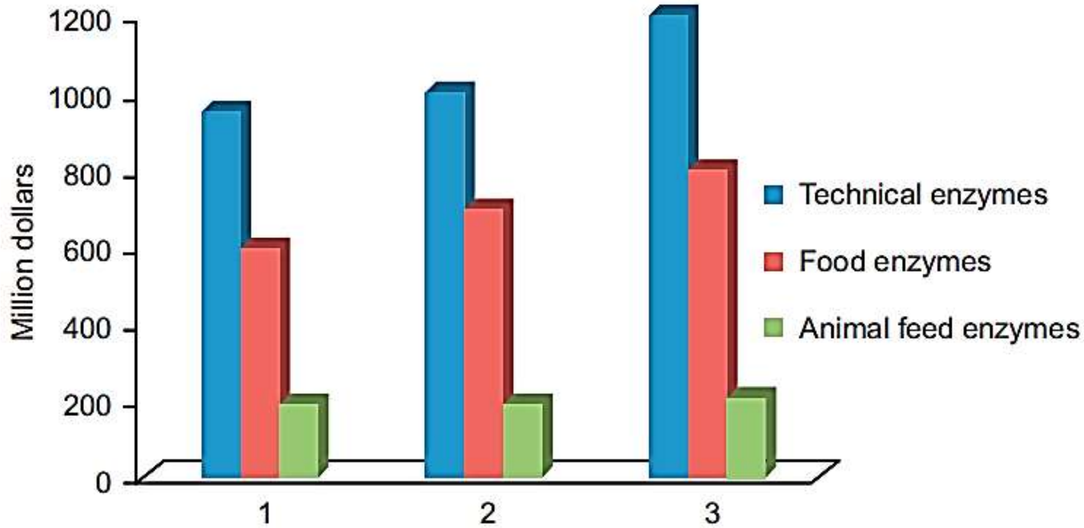

Fig. 10. World enzyme market by application sectors (Patel *et al*., 2017).

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