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Microbial Enzymes and Their Applications in Food Industry: A Mini-Review

Michael O. Okpara

Department of Biochemistry, Federal University of Technology, Akure, Nigeria
Email: okpara87michael@yahoo.com

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Abstract

The use of enzymes is replacing chemicals in many industrial production processes because of the eco-friendly nature of enzymes which do not generate greenhouse gases and have reduced the demand for energy in industries. To meet the ever-increasing demand for enzymes in many industries and survive the harsh production conditions, microbial sources of enzyme production are the most preferred source for industrial enzyme production because the microbes are readily available, they grow at a very fast rate, and they can be genetically manipulated to produce enzymes which can perform optimally at different industrial production conditions. Microbial enzymes have found so many applications in various industries (textiles, leather, paper and pulp, research and development, pharmaceutical, agriculture, detergent, waste, biorefineries, photography and food industries), thus making them very essential in several industrial production processes. Here in this review, the application of some important microbial enzymes in food industry and the microbial sources for the enzymes are discussed.

Keywords

Bacteria, Food Industry, Fungi, Microbes, Microbial Enzymes

1. Background

1.1. Enzyme Classification

Enzymes are highly specific biomolecular catalysts that speed up the rate of conversion of substrate(s) to product(s). These biomolecular catalysts can either be proteinaceous macromolecules or catalytic RNA also known as ribozymes. For this review, enzymes will refer to only proteinaceous macromolecules. Enzymes are very large macromolecules with molecular weights ranging from 10 kDa to 2000 kDa and they are made up of amino acids (as monomeric units)

bonded together by peptide bonds [1]. Enzymes possess different identifiable sites when they assume their tertiary or quaternary structures. Most important for their enzymatic activity in their tertiary or quaternary structures are their active sites which are usually located deep within a hydrophobic pocket in the enzyme. The active sites define the specificity of an enzyme for its substrate thus making a particular enzyme unique from other enzymes [2]. Consequently, this formed the basis upon which the over 3000 identified enzymes are classified, named, and numbered by the Enzymes Commission (EC) formed by the International Union of Biochemistry and Molecular Biology (IUBMB) [3].

As of today, there are seven classes of enzymes based on the kind of reactions they catalyse [4].

Oxidoreductases are a group of enzymes that catalyse the oxidation or reduction of substrate(s). Examples of oxidoreductases are reductases, oxidases, peroxidases, oxygenases, dehydrogenases etc.

Transferases are a group of enzymes that catalyse the transfer of a specific functional group from a molecule to another molecule. Examples of transferases are peptidyl transferase, transaminase, acetyltransferase, methyltransferase, transaldolase, glycosyltransferase, transketolase, formyltransferase, transaminase, kinase, sulfotransferase etc.

Hydrolases are a group of enzymes that utilize water to catalyse the cleavage of bonds in large molecules; thus, breaking down the large molecules into relatively smaller molecules. Examples of hydrolases are proteases/peptidases, amylases, glycosidase, lipase, phospholipases, lactase, acylase etc.

Lyases are a group of enzymes that do not require hydrolysis or oxidation to catalyse elimination or addition reactions. Examples of lyases are decarboxylases, pectolyase, aldehyde lyase, dehydratases, adenylyl cyclase, hydratases, etc.

Isomerases are a group of enzymes that catalyse the intramolecular breakage and formation of bonds to convert a parent molecule to its isomeric form. Examples of isomerases are racemases, epimerases, cis-trans isomerases, etc.

Ligases are a group of enzymes that catalyse the joining of two or more molecules to form a new chemical bond and new molecule(s) by utilizing the energy derived from the hydrolysis of ATP or any other energy-rich phosphate bond. Examples of ligases are synthetases, chelatases, DNA ligase, carboxylases, etc.

Translocases are the newest class of enzymes that were classified in 2018 by the EC. They are enzymes that catalyse the translocation of ions and/or molecules across membranes. Examples of translocases are carnitine-acylcarnitine translocase, ADP/ATP translocase, translocase of the inner membrane (TIM) and translocase of the outer membrane (TOM) [1] [4].

1.2. Need for Microbial Enzymes in Industries

Among the over 3000 enzymes that have been identified, only about 5% are exploited industrially [3]. The industrial application of enzymes has substantially reduced the demands for energy in many industries and the wastes generated from the application of enzymes in industries are biodegradable and non-toxic

wastes that are friendly to the environment. Also, the use of industrial enzymes is more cost-effective and the possibility of genetically engineering microbes to produce more stable and improved enzymes at an industrial scale can be achieved [5]. These have made many industries embrace the use of enzymes in place of chemical-based production technologies. Further affirming the need for the production of enzymes at an industrial scale for various industrial applications. A drawback for the use of enzymes in industries is that certain production conditions can be quite harsh for the optimal performance of enzymes. The production pH, pressure, temperature, and the presence of potential inhibitors could affect the enzymatic activity of enzymes [6] [7]. For instance, most enzymes (from animal, plant, and microbial sources) perform optimally at a temperature of about 37˚C, a neutral pH and in the absence of inhibitors. To ameliorate this challenge, microbial sources of enzymes are preferred to plant and animal sources for the production of industrial enzymes. The microbes are readily available and easy to culture in cheap growth media. Their growth rate is very high, and more importantly, recombinant DNA technology can be used to manipulate the genome of the microbes to produce enzymes that can perform optimally at different industrial production conditions. Consequently, thermoactive and thermostable enzymes have been produced by thermophilic and hyperthermophilic micro-organisms. Acid and alkaline enzymes can also be produced by genetically engineered microbes cultured in selective growth media to produce extracellular enzymes that can perform optimally in an acidic or alkaline environment [3]. Enzymes that perform optimally under harsh industrial conditions are currently in high demand because they have found so many applications in food, animal feed, dairy, beverage, agriculture, leather, paper and pulp, research and development, pharmaceutical, textiles, detergent, waste, biorefineries, and photography industries.

The rise in the demand for industrial enzymes is largely attributed to the increasing demand for enzymes as an alternative for both traditional and synthetic chemicals in many industrial processes because of the eco-friendly nature of enzymes application in industries unlike the use of chemicals which generates so much greenhouse gases. The use of enzymes as an alternative for chemicals in industrial processes prevents the release of approximately 700 million kg of CO_2 into the atmosphere per year [8]. Consequently, the industrial enzymes market has been growing steadily for the past 6 decades from a net worth of about USD 0.31 billion in 1960 to a net worth of USD 6 billion in 2020 (**Figure 1**) [2] [8]. Also, the increasing demand for enzymes by many industries has led to the growth of numerous enzyme-producing companies. Global production of industrial enzymes is dominated by companies like DSM, DuPont™ Danisco®, Novozymes, Advanced Enzyme Technologies, Amano Enzyme, Associated British Foods Plc. (ABF), Chr. Hansen Holding A/S, Enzyme Development Corporation (EDC), Aumgene Biosciences, Creative Enzymes, Adisseo, Megazyme, Enzyme Supplies Ltd., Enzyme Solutions, Biocatalysts Ltd., MetGen, Tex Biosciences, Sunson Industry Group, Enzymatic Deinking Technologies (EDT), AB

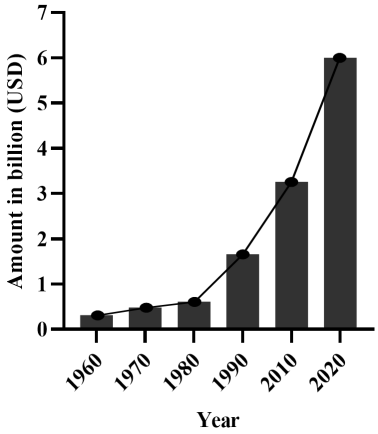

Figure 1. Graphical representation of the net worth of industrial enzymes market from 1960 to 2020.

enzymes, Soufflet Biotechnologies, and many others. According to a recent report by BlueWeave Consulting, the industrial enzymes market was valued at approximately USD 6 billion in 2020 and it is expected to be valued at over USD 9 billion by 2027 at a compound annual growth rate of 6.8% [8].

Amongst the many industries where enzymes have found numerous applications, the food industry provides the biggest market for enzymes with approximately 55% of industrial enzymes finding applications in food industry [9]. The food industry includes baking, beverages, brewing, dairy, oil refinery, food packaging, fruit and vegetable juice industries. As of 2020, the global food enzymes market was valued at about USD 2.3 billion which accounted for approximately 40% of the value of the entire industrial enzymes market. And by 2026, the global food enzymes market is expected to be valued at over USD 3.3 billion [10]. Thus, suggesting that microbial enzymes with applications in food industry will continue to be in demand. In this review article, the applications of some important microbial enzymes in food industry and the microbial sources for the enzymes are summarized.

2. Applications of Enzymes in Food Industry

Microbial enzymes have found several applications in the various sectors of food industry including dairy, baking, food processing and packaging, animal feed, fruit and vegetable juice, beverage, oil refinery, and confectionery. The applications of microbial enzymes in different sectors of food industry are summarized in **Figure 2** and the following sub-sections.

2.1. Proteases (EC 3.4)

Proteases (EC 3.4) are enzymes that catalyse the hydrolytic cleavage of peptide bonds in proteins to yield smaller polypeptides or amino acids. Proteases are produced by a wide variety of living organisms including plants, Archaea, fungi, bacteria, and animals. They have found application in many sectors under food industry including brewing, dairy, baking, food processing, and animal feed industries.

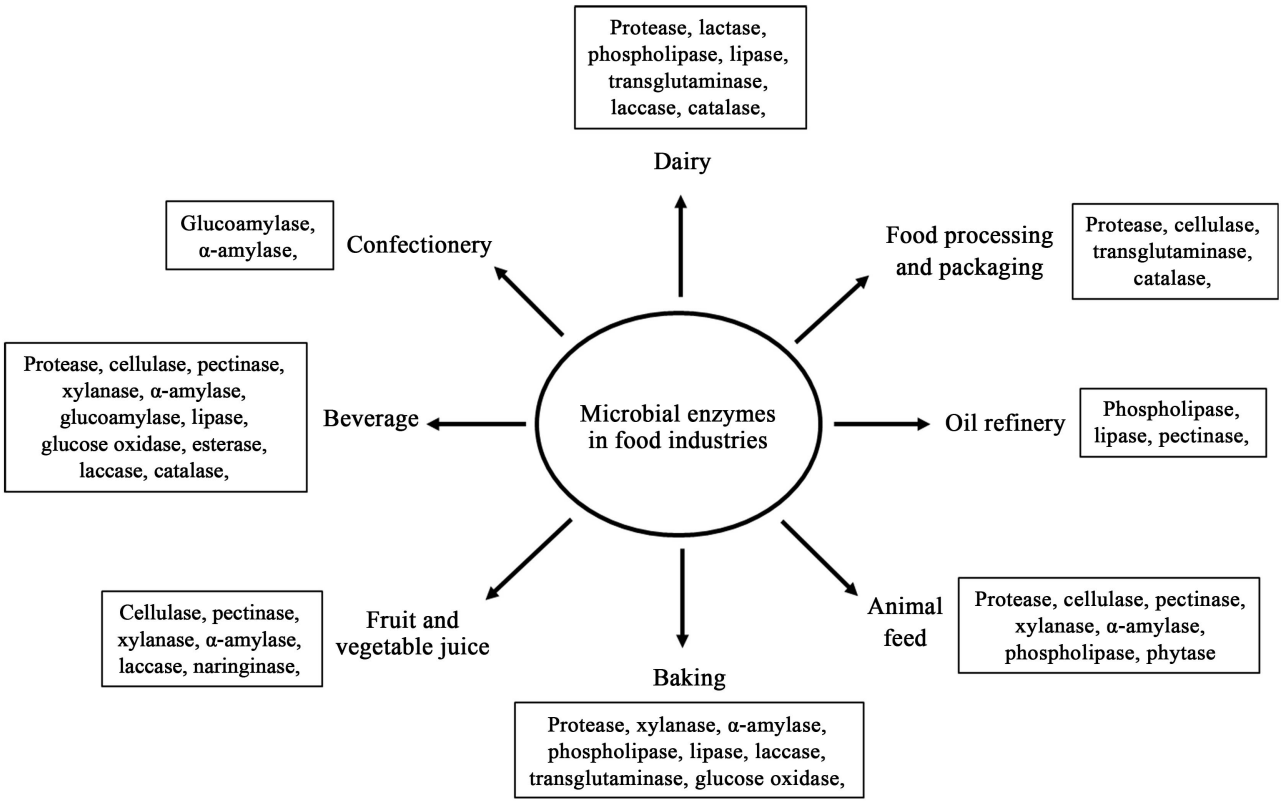

Figure 2. Summary of enzymes that have found applications in food industry.

Proteases are important in brewing industry. During the production of beer or whisky, proteases are added to wort to break down proteins and release more peptides and/or amino acids in wort thus improving the fermentation process and the overall quality of the beer or whisky.

In dairy industry, proteases are added to milk to hydrolyse a peptide bond in kappa-casein, thereby destabilizing casein micelles during the production of curds for cheese-making. Proteases also improve the organoleptic and rheological characteristics of cheese and also reduce the allergenic properties of some fermented milk products. Recently, milk-clotting protease was produced by Mamo *et al.* (2020) from *Aspergillus oryzae* DRDFS13MN726447 and they showed that the protease from the *Aspergillus oryzae* strain improved the overall organoleptic properties of cheese [11].

In baking industry, proteases are added to flour during biscuits/crackers production to weaken the gluten protein structure in the flour otherwise the dough will be difficult to handle. The use of protease to weaken the gluten does not affect other nutritional constituents of the dough unlike when a chemical compound, like sodium bisulphite, is used to achieve the same purpose. Consequently, the nutritive value of the biscuits/crackers is improved. Deng *et al.* (2016) reported that acid protease from *Aspergillus usamii* improved the rheological properties of wheat gluten used for baking [12].

Proteases are used in food processing industry to improve the nutritional and

functional properties of food materials that are rich in proteins. For instance, in meat processing companies, proteases are used to enhance meat tenderization thereby improving the rheological properties of the meat.

Proteases can be added to animal feed to break down the protein content of the feed into amino acids thereby reducing the anti-nutrient content of the feed.

Proteases can be produced from plants, animals, and microbes but fungi and bacteria are the best sources for the production of proteases for application in food industry. Fungal sources of proteases used in food industry include *Aspergillus usamii* [12], *Aspergillus niger*, *Aspergillus flavus* [13], *Aspergillus fumigatus*, and *Aspergillus oryzae* DRDFS13MN726447 [11]. Bacterial proteases used in food industry are produced by *Bacillus subtilis* SMDFS 2B MN715837 [11], *Bacillus licheniformis*, *Chryseobacterium* sp. [14].

2.2. Cellulases (EC 3.2.1.4)

Cellulases (EC 3.2.1.4) are a group of enzymes that catalyse cellulolysis. They hydrolyse the β-1,4 glycosidic bonds in polymeric cellulose, hemicellulose, lichenin, and glucans to produce glucose monomers. Depending on which part of the cellulose that the cellulase acts on, cellulases can be classified as either endo-(1,4)-β-d-glucanase, exo-(1,4)-β-d-glucanase or β-glucosidases [15].

Cellulases have found some applications in fruit and vegetable juice industry. During the production of fruit and vegetable juice, floating cellulose and hemicellulose from the fruits and vegetables tend to form some cloudiness in the fruit and vegetable juice. This negatively affects the quality of the fruit and vegetable juice, making the juice less appealing. Consequently, cellulases are used in fruit juice industry as fruit-softening enzymes to hydrolyse cellulose and hemicellulose in raw fruit and vegetable juice thereby enhancing juice extraction, clarification, stabilization, and overall yield [16].

During the processing of fruit and/or vegetable purees, cellulases are used to enhance the yield of purees, minimize damage due to heat and also to reduce the viscosity of purees. Peach, mango, pear, plum, guava, and pawpaw are some of the fruits whose purees have been treated with cellulase during their processing.

Animal feed for ruminant animals is usually rich in complex polysaccharides like cellulose, pectin, lignin, and hemicellulose which must be digested by the animal. To improve the digestibility of these animal feed, cellulases are used in processing ruminant animal feed as they can break down cellulose and hemicellulose in animal feed to a more digestible form.

Although cellulase can be produced by a few metazoans like snails, termites, and earthworms, however bacteria and fungi remain the best sources for the production of cellulases for industrial application. Cellulases for food industry application can be produced from fungi such as *Aspergillus niger* and *Trichoderma reesei* [16]. Bacteria sources for producing industrial cellulases include *Paenibacillus* spp., *Bacillus subtilis* ABDR01) [17], *Bacillus licheniformis* [18] and *Streptomyces* sp. Strain J2 [19].

2.3. Pectinases (EC 3.2.1.15)

These are a group of enzymes that catalyse different reactions in pectic substances found in plant cell walls. These reactions include the hydrolysis of α-1,4-glycosidic bonds catalysed by polygalacturonase, transeliminative cleavage of α-1,4-D-galacturonan methyl ester catalysed by pectolyase, and deacetylation and demethoxylation of pectin by pectin esterase [20].

Pectinases are used in fruit juice industry to degrade pectin (depectinization) in the cell wall of fruits thereby enhancing juice extraction, flavour, clarification, filterability, and overall yield of the fruit juice [20] [21] [22].

In beverage industry, pectinases can be used during the curing stage of wet processing for coffee production as this improves the yield, aroma and flavour of the coffee and also reduces the processing time. Pectinases can also be used in cocoa processing companies to ensure the complete removal of mucilaginous layers of cocoa beans thereby improving the quality of the coffee produced [23]. For tea production, treatment of tea leaves with pectinases reduce the pectin content in the leaves thereby ensuring a relatively quicker fermentation process [24].

Pectinases are used in animal feed industry to treat animal feed rich in pectin and improve their digestibility for the animals.

For oil refinery, pectinases (and some other enzymes which degrade cellulose and hemicellulose) can be added to unprocessed olive oil, coconut oil, sunflower oil, and canola to improve the yield of the refined vegetable oil.

Pectinases are ubiquitous but the production of pectinases for industrial applications is mostly achieved through the use of microbes. Pectinases are mostly produced by fungi including *Aspergillus niger* MTCC [22], *Penicillium* spp., *Moniliella SB9*, *Streptomyces* spp. [19], *Aspergillus* spp. Gm, *Fusarium* spp. C, *Aspergillus* spp. T and *Penicillium* spp. Lco [20]. *Aspergillus kawachii* [25], *Aspergillus fumigatus* [26]. The *Bacillus subtilis* strain (*Bacillus subtilis* ABDR01) can also produce high yields of pectinase [17].

2.4. Xylanases (EC 3.2.1.8)

Xylanases (EC 3.2.1.8) are a group of enzymes that catalyse the hydrolysis of β-1,4 glycosidic bonds in xylan (a plant cell wall polysaccharide) into xylose (a five-carbon monosaccharide). A group of xylanolytic enzymes which depolymerize the heteropolysaccharide into monosaccharides for industrial applications include endo-(1,4)-β-d-xylanase, exoxylanase, xylan-1,4-β-xylosidase, α-l-arabinofuranosidase, α-glucuronidase, p-coumaric esterase, ferulic acid esterase and acetylxylan esterase [27] [28] [29]. Xylanases for industrial application are mostly derived from microbial sources.

During bread production, xylanase can be used to hydrolyse arabinoxylan present in the wheat, thereby solubilizing the arabinoxylan in water and making it extractable [30]. Subsequently, the gluten structure, dough, volume, viscosity, softness, and elasticity are improved. Cunha *et al.* (2018) produced extracellular

xylanase from genetically engineered *Pichia pastoris* carrying xynBS27 gene from *Streptomyces* sp. In their study, they showed that the xylanase was able to reduce the sugar content, stiffness and firmness of bread while also improving the bread volume [31].

Xylanases are used in fruit juice industry to hydrolyse hemicellulose in raw juice thereby enhancing juice extraction, stabilization, clarification, and overall yield of the fruit juice [29]. In their study, Adiguzel *et al.* (2019) showed that extracellular endo-(1,4)-*β*-d-xylanase can be produced by *Pediococcus acidilactici* GC25 and that the clarification of different fruit juice was significantly improved upon treatment with *Pediococcus acidilactici* GC25 xylanase [32].

Xylanases are used in processing ruminant animal feed as they can break down arabinoxylan in animal feed into a more digestible form. Similarly, exogenous xylanase is used in bird feed to improve the fibre content of the feed. Xylanase has also found application in the processing of animal feed for non-ruminant animals. As non-ruminant animals struggle to digest feed from plant sources because of the high content of cellulose and hemicellulose in the feed, xylanase can be used to hydrolyse the cellulolytic and hemicellulolytic contents of the feed thereby making them more digestible by the non-ruminant animals [16].

Xylanases are used in breweries to hydrolyse the complex cell wall of barley during beer production. The xylan in the cell wall of barley is hydrolysed by xylanase to shorter chain oligosaccharides and arabinoxylans. The hydrolysis of barley hemicellulose also improves the viscosity and clarity of the beer.

Xylanases are produced on an industrial scale by microbes such as bacteria. Some examples of xylanase-producing bacteria are *Clostridium acetobutylicum* [28], *Streptomyces* sp., *Pediococcus acidilactici* GC25 [32], *Bacillus subtilis* ABDR01 [17], *Bacillus licheniformis* DM5 [33], *and Bacillus pumilus* [34]. Xylanase-producing fungi include *Aspergillus japonicus*, *Penicillium occitanis* Pol6 [35], *Fusarium* sp., *Pichia pastoris* [31].

2.5. A-Amylase (EC 3.2.1.1)

A-Amylase (EC 3.2.1.1) is a member of the endoamylase group of starch-converting enzymes that hydrolyses *α*-1,4 glycosidic bonds in polysaccharides to yield short-chain dextrins [36]. This enzyme is ubiquitously produced by a wide variety of living organisms including Archaea, fungi, bacteria, and animals. Amylase has found a wide range of applications in processed-food industry (brewing, livestock feed, baking, fruit juice, starch syrups, starch liquefaction, and so on).

A-amylase hydrolyses the starch in flour to fermentable sugars that will be fermented by yeast during bread production to improve the taste and quality of bread. In addition, it performs some anti-staling functions when it is used during bread-making.

Confectioners use *α*-amylase for starch liquefaction to make glucose and/or fructose syrup during the production of candy.

A-amylase catalyses the hydrolysis of starch into fermentable sugars which are acted upon by *Saccharomyces cerevisiae* to produce alcohol. *A*-amylase can also be used as a clarifying agent to improve clarification during beer production.

In animal feed industry, *α*-amylase is applied during the pre-treatment stage of animal feed processing to enhance the digestibility of the animal feed.

A-amylase is used in fruit juice industry to hydrolyse the polysaccharides in raw juice thereby enhancing juice extraction, clarification, and overall yield [37] [38].

The best sources for the production of *α*-amylase for industrial application are from *Bacillus* spp. such as *Bacillus stearothermophilus* [39], *Bacillus amyloliquefaciens* [40], *Geobacillus thermoleovorans* [41], *Anoxybacillus* sp. AH1 [42], *Bacillus licheniformis* [43], *Bacillus subtilis* JS-2004 [36] [44] and *Bacillus* sp. BCC 01-50 [45]. The *α*-amylase from *Bacillus* spp. is highly thermostable and their relatively lower doubling time implies a much higher production of *α*-amylase. Fungal sources for *α*-amylase production for industrial application is mostly from *Aspergillus* spp. However, *α*-amylase from *Aspergillus* spp. are not as thermostable as *α*-amylase from *Bacillus* spp. Some examples of *α*-amylase-producing *Aspergillus* spp. are *Aspergillus oryzae* [46], *Aspergillus terreus* NCFT4249.10 [47], *Aspergillus awamori* [48], *Aspergillus fumigatus* KIBGE-IB_{33} [49] and *Aspergillus niger* WLB42 [50].

2.6. Glucoamylase (EC 3.2.1.3)

Glucoamylase (EC 3.2.1.3) is an exoamylase that cleaves the glycosidic bonds in starch from the non-reducing terminus of polysaccharides thus yielding D-glucose. Glucoamylase is widely distributed in all living organisms especially in fungi and it has found huge application in food industry.

Glucoamylase is an important enzyme in the baking industry. Glucoamylase is usually added to the flour to improve its quality and to slow down the staling of dough for improved quality of baked products.

Glucoamylase can be used to make high glucose and/or fructose syrups during the production of candy in confectionery industry.

Just like *α*-amylase, glucoamylase can catalyse the hydrolysis of starch into fermentable sugars which are acted upon by *Saccharomyces cerevisiae* for the production of alcohol. Brewers now apply the starch-hydrolysing property of glucoamylase during the production of light beer by adding the enzyme to wort before or during fermentation [51].

Some microbial sources for the production of glucoamylase for application in food industry include *Rhizopus oryzae* F-923 [52], *Aspergillus niger* [53] and *Aspergillus awamori* [51] [54].

2.7. Lactase (EC 3.2.1.108)

Lactase (EC 3.2.1.108) is an enzyme that catalyses the hydrolytic cleavage of milk disaccharide lactose to simpler monomeric sugars like galactose and glucose.

Lactase, also known as β-galactosidase, can be obtained from a wide range of biosystems including plants, Archaea, fungi, bacteria, and animals. The importance of lactase in the dairy/food and pharmaceutical industries cannot be overemphasized.

In dairy industry, lactase is usually added to milk and/or milk-based products (such as whey) to improve their digestibility, especially for lactose-intolerant individuals thereby preventing diarrhoea and tissue dehydration [55]. Also, lactase is used to hydrolyse lactose in ice cream to improve the creamy nature of ice creams and their digestibility. Lactase is used during the production of some milk products to prevent crystallization of lactose thereby making the milk product more soluble.

Fungal sources for industrial lactase production include *Aspergillus niger* [56], *Aspergillus oryzae*, *Kluyveromyces lactis* and *Kluyveromyces fragilis* [57]. Bacterial sources for the production of lactase for industrial application include *Escherichia coli* [58], *Klebsiella oxytoca* ZJUH1705 [59], *Bifidobacterium infantis* CCRC 14633 and *Bifidobacterium longum* BCRC 15708 [60].

2.8. Phospholipase (EC 3.1.1.4)

Phospholipase (EC 3.1.1.4) is an enzyme that hydrolyses the cleavage of phospholipids into fatty acids and other lipophilic substances. Phospholipases are broadly classified into two. The acyl hydrolases (which consist of phospholipase A1, phospholipase A2, and phospholipase B) and the phosphodiesterases (which consist of phospholipase C and phospholipase D). Phospholipase can be produced at an industrial scale by microbes especially fungi. Its major industrial application is seen in the food industry where it is used for a wide variety of purposes.

Phospholipases are used for ensuring the stability of fat during the processing of some dairy products. During cheese production, phospholipases can be used to improve the flavour of the cheese. Also, phospholipase is added to milk during cheese production to reduce the huge loss of milk fat in whey thereby increasing cheese texture and yield [61].

Phospholipases can be used as degumming agents in vegetable oil producing companies to increase the yield and quality of refined vegetable oil. The application of phospholipases as degumming agents for refining vegetable oil have been reported in many studies. A chimeric enzyme called Lecitase Ultra produced through the genetic fusion of Phospholipase A1 from *Fusarium oxysporum* and lipase gene from *Thermomyces lanuginosus* was used to perform degumming of vegetable oils [62]. In a different study, Wang *et al.*, (2021) produced the first fungal alkaline cold-active phospholipase C from *Aspergillus oryzae* which they used to perform degumming of crude soybean oil [63]. In their study, Elena *et al.*, (2017) reported that the *Bacillus cereus* phospholipase C F66Y mutant efficiently removed about 90% of phosphatidylethanolamine from soybean oil during its degumming process [64].

Phospholipases can be used in food processing industry to improve the emulsifying characteristics of egg yolk used in making sauces, mayonnaise thereby

elongating the shelf life of the food products.

Phospholipases are used in baking industry to emulsify the dough and improve its flavour [65].

Phospholipases can be used to improve the nutritive value of fat-containing animal feed like soya bean lecithin.

Fusarium oxysporum [66], *Bacillus cereus* [64], *Streptomyces chromofuscus* [67], *Aspergillus oryzae* [63] are some of the microbial sources of phospholipase production.

2.9. Lipase (EC 3.1.1.3)

Lipase (EC 3.1.1.3) is the enzyme that catalyses the hydrolytic cleavage of fats/triglycerides into their component fatty acid and glycerol. Lipases are also involved in the biosynthesis of long-chain acylglycerides. Ideally, all animals including humans can secrete lipase in their stomach and pancreas to aid the digestion of fats [68]. Lipases are also produced in leaves, stems, latex, and seeds of many plants [69]. However, the production of lipase on an industrial scale is achieved through bacterial and fungal sources. The applications of lipase cut across a wide variety of industry including food, detergent/laundry, leather, textile, paper, and pulp processing industries.

During cheese production, lipase is used to hydrolyse milk fat into free fatty acids which gives a unique flavour to the cheese, improves its taste, promotes the ripening cheese, and enhances its texture [70].

Lipase is used in wine production and beverage-making companies to improve the aroma of wine and beverage, respectively.

Many products made in baking industry require the use of egg white as an ingredient. Egg white contains about 0.02% of lipid which is sufficient to decrease the quality of the dough [71]. Lipase can be used to catalyse the hydrolysis of the lipid content of egg white thereby improving the quality of the baked product. Lipase can also serve as a preservative in some baking products [70].

Lipases can be used as degumming agents to remove phosphatides from crude soybean, rapeseed, and sunflower oil during the process of refining crude vegetable oil. The degumming of crude vegetable oil is a process that increases the yield and quality of refined vegetable oil. Lipase can also be used to treat crude vegetable oil to produce flavour esters which improve the organoleptic properties of the refined oil. Cong *et al.*, (2019) produced a novel lipase called An-lipase from *Aspergillus niger* strain F0125 which they used to esterify crude soybean oil to produce ethyl lactate, butyl butyrate and ethyl caprylate flavour esters [72]. The ω-3 polyunsaturated fatty acids content of salmon oil can be increased upon treatment of some oil like sardine oil and salmon oil with lipase [73] [74] [75].

Rhizomucor miehei [76] [77], *Aspergillus niger* F0215 [72], *Aspergillus repens*, *Aspergillus oryzae* [78], *Penicillium camemberti* [79], *Candida rugosa* (formerly called *Candida cylindracea*) [74] [80] [81], *Mucor javanicus* [81], *Thermomyces lanuginosus* (formerly known as *Humicola lanuginosus*) [82] are

known fungal sources for the industrial production of lipases. Bacteria sources for lipase production include *Bacillus subtilis* LP2 [83], *Pseudomonas aeruginosa* JCM5962(T) [84], *Staphylococcus aureus* [85], *Staphylococcus caprae* NCU S6 [86], *Serratia marcescens* [87] [88], *Burkholderia glumae*, *Burkholderia cepacia*, *Bacillus megaterium* reviewed in [89], *Pseudomonas alcaligenes*, *pseudomonas mendocina* reviewed in [90], *Staphylococcus hyicus*, *Staphylococcus simulans* PMRS35 [91], *Rhodothermus marinus* [92].

2.10. Glucose Oxidase (EC 1.1.3.4)

Glucose Oxidase (EC 1.1.3.4) is an oxidoreductase that catalyses the oxidation of glucose to D-glucono-δ-lactone and hydrogen peroxide [93] [94].

In food processing industry, glucose oxidase is added to glucose-containing food substances to produce the food additive, D-glucono-δ-lactone. Once produced, D-glucono-δ-lactone acts as a preservative to enhance the quality, flavour, and stability of the food.

The oxidizing property of glucose oxidase is employed in baking industry to produce stronger dough. Glucose oxidase can also be used to increase the volume of bread during baking. In a recent investigation by Ge *et al.*, (2020), they produced a novel glucose oxidase and showed that the enzyme significantly increased the volume of bread during baking [95].

Glucose oxidase is added to diabetic drinks to reduce the glucose content of the drinks thus making them favourable for consumption by diabetics.

In wine-making companies, glucose oxidase and catalase can be added during wine production to reduce the alcohol content of the wine [96].

Fungi are the most common microbial source for the production of glucose oxidase on an industrial scale. Examples of glucose oxidase-producing fungi include *Aspergillus niger* [93], *Mucor circinelloides*, *Aspergillus tubingensis*, *Aspergillus terreus*, *Aspergillus oryzae*, *Aspergillus carbonarius* and *Aspergillus nidulans* reviewed in [94], *Penicillium glaucum*, *Penicillium amagasakiense*, *Penicillium notatum*, *Penicillium purpurogenum* and *Penicillium adametzil* reviewed in [97], and *Cladosporium neopsychrotolerans* [95].

2.11. Transglutaminase (EC 2.3.2.13)

Transglutaminase (EC 2.3.2.13) is a naturally occurring transferase that catalyses the intramolecular and/or intermolecular crosslinking between the gamma-carboxamide group(s) of glutamine and the amino group of lysine. The isopeptide (ε-(γ-Glu)-Lys) bonds formed through the crosslinking make the newly formed molecule resistant to proteolysis. Transglutaminase is produced by plants, animals, and bacteria. The enzyme has found applications in baking, food processing and dairy industries.

Transglutaminase is used in baking industry to catalyse the crosslinking of glutens in wheat thereby improving the quality, structure, volume, stability, texture, and shelf life of the dough.

Transglutaminase can be used in the production of processed meat and fish as a protein-bonding agent. For instance, transglutaminase is applied in the processing of ham to improve its texture.

Polymerization of milk proteins is an essential process during the production of some dairy products. Transglutaminase is added to milk proteins to catalyse the polymerization of milk proteins to enhance the nutritional and functional properties of dairy products [98] [99].

Large scale production of transglutaminase for industrial application can be quite challenging and expensive especially transglutaminase from an animal source. However, bacteria sources are mostly relied upon as they are easier and faster to culture and their transglutaminase yield is much higher. Transglutaminase can be secreted by *Streptoverticillium mobaraense*, *Streptoverticillium lydicus*, *Streptoverticillium ladakanum*, *Streptomyces lydicus*, *Bacillus subtilis*, and *Bacillus spherules* reviewed in [100], *Streptomyces* sp. CBMAI 1617 [101].

2.12. Esterases (EC 3.1)

Esterases (EC 3.1) are a group of hydrolase enzymes that catalyse the hydrolysis of esters into alcohol and acid. Like lipases, esterases can hydrolyse glycerides into their component fatty acids and glycerol; except that esterases hydrolyse short-chain glycerides.

Feruloyl esterase is used in beverage industry to synthesize ferulic acid. Ferulic acid acts as a precursor for the phenolic aldehyde aroma compound, vanillin. Vanillin is a major constituent of vanilla which improves the taste of beverages [102]. Esterases are also used to modify and improve the flavours of fat in fruit juices [103].

Industrial-scale production of esterases is mostly achieved by microbes especially bacteria. Feruloyl esterase is produced by *Lactobacillus amylovorus*, *Lactobacillus acidophilus*, *Lactobacillus farciminis*, *Lactobacillus fermentum* [104], *Bacillus licheniformis* [105].

2.13. Laccase (EC 1.10.3.2)

Laccase (EC 1.10.3.2) is a multi-copper oxidase that catalyses the oxidation of phenolic compounds, aromatic amines, and ascorbic acid. Laccase is produced by fungi, bacteria, soil algae and some insects. However, the laccase produced by these organisms tends to perform different functions. For example, laccase from microbial sources (bacteria and fungi) catalyses the degradation of lignin in wood to release hemicellulose and cellulose components of the wood while laccase from plant sources tend to catalyse the biosynthesis of lignin in plants.

Laccase is used in wine industry for wine stabilization. Laccase has also been used to remove oxygen from wine to increase its shelf life. Laccase is also used in beverage-producing companies to modify the colour of beverages.

In brewing industry, laccase is used to minimize the formation of haze through the oxidation of polyphenols during beer production [106]. Addition-

ally, laccase is used to remove oxygen during beer production, thus acting as a preservative.

The oxidizing property of laccase is applied in baking industry as it makes the baking process much better. Laccase could serve as a crosslinker of biological polymers in baking industry, thus contributing to the strength and stability of dough. Manhivi *et al.*, (2018) have demonstrated that laccase can improve the overall rheological properties of bread [107].

In fruit juice industry, laccase can be used alone or with cellulase and pectinase for improved clarification and overall yield of fruit juice. In their study, Lettera *et al.*, (2016) used an immobilized fungal laccase to reduce the phenol content of fruit juice by 45%. In another study, Yin *et al.*, (2017) produced thermostable laccase from *Abortiporus biennis* strain J2 and further demonstrated that the enzyme was efficient for clarification of litchi juice [108].

Laccase can be used in dairy industry to treat skim milk yoghurt to improve the quality of the yoghurt by crosslinking milk proteins with laccase [109] [110].

Fungi are one of the best sources for the production of laccase for industrial applications. Some laccase producing fungi include *Trametes versicolor*, *Pleurotus flabellatus*, *Pleurotus eryngii*, *Funalia trogii*, *Pleurotus lampas* [110], *Abortiporus biennis* [108], *Pleurotus ostreatus* [111].

2.14. Naringinase (EC 3.2.1.40)

Naringinase (EC 3.2.1.40) is an example of a group of enzymes known as debittering enzymes. Naringinase is an enzyme complex that catalyses the degradation of naringin to prunin, rhamnose, naringenin, and glucose through the actions of α-rhamnosidase and β-glucosidase. Naringin is the compound present in most citrus fruits which gives the citrus fruits their bitter taste. α-rhamnosidase acts on naringin first and breaks it down to prunin and rhamnose. Then prunin is broken down to naringenin and glucose by β-glucosidase.

Fruit juice producers add naringinase to citrus fruit juice to act as a debittering agent and to improve the flavour, aroma, and taste of fruit juice. Zhu *et al.*, (2017) produced intracellular naringinase from *Bacillus amyloliquefaciens* strain 11568 and showed that the enzyme reduced the bitterness in a citrus fruit juice by removing naringin from the citrus fruit [112].

In food processing industry, naringinase can be used for making food additives like sweeteners to improve the taste of food.

The production of naringinase for industrial application is usually obtained through the use of microbes especially bacteria and fungi. Some known bacterial sources of naringinase are *Burkholderia cenocepacia* [113], *Thermomicrobium roseum*, *Bacillus amyloliquefaciens*-D1 [114], *Bacillus amyloliquefaciens* strain 11568 [112], *Cryptococcus albidus* [115], *Thermoclostridium stercorarium* DSM 8532, *Caldicellulosiruptor bescii* DSM 6725, *Thermotoga neapolitana* Z2706-MC24, *Thermotoga maritima* MSB8 [116]. Fungi sources for naringinase production are *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus flavus*, *Aspergillus usamii*,

Cochliobolus miyabeanus, *Penicillium decumbens*, *Rhizopus nigricans*, *Rhizoctonia solani*, *Coniothyrium diplodiella*, *Lasiodiplodia theobromae* [113].

2.15. Catalase (EC 1.11.1.6)

Catalase (EC 1.11.1.6) is an enzyme that catalyses the reduction of hydrogen peroxide to water and oxygen. Catalase is found in every aerobic living organism that is exposed to oxygen. Consequently, it can be produced by plants, animals, and microbes.

Like laccase, catalase is used in wine-making companies to remove oxygen from wine to further elongate its shelf life and also for reducing alcohol in wines [96].

In dairy industry, catalase is used to remove peroxide from milk products to prevent milk rancidity [117].

Oxidation of food items inside food wrappers is one of the causes of food spoilage. Catalase is used during food packaging to prevent oxidation of food items thereby elongating their shelf life.

The production of catalase for industrial application are mostly from microbial sources. Some identified microbial sources of catalase for industrial application are *Enterococcus faecalis* [118], *Aspergillus niger*, *Micrococcus luteus*, *Bacillus maroccanus* [119], *Pyrobaculum calidifontis*, *Rhizobium radiobacter* 2-1 (previously called *Agrobacterium tumefaciens*) [120], *Ureibacillus thermosphaericus* FZSF03 [121], *Bacteroides fragilis* [122].

2.16. Phytase (EC 3.1.3.8)

Phytase (EC 3.1.3.8) is a kind of phosphatase that catalyses the hydrolytic release of inorganic phosphorus from phytic acid contained in grains and oilseeds. The enzymatic activity of phytase enhances the uptake of minerals like calcium, zinc, and iron which are sometimes bound to phytic acid in the grains and oilseeds. Thus, the hydrolytic activity of phytase on phytic acid increases the bioavailability of calcium, zinc, and iron to monogastric animals. Phytase is a natural enzyme that is found in fungi, bacteria, plants, and ruminant animals.

Phytase is added to the feed of monogastric animals to improve the nutritive value of animal feed by enhancing the uptake of important minerals bound to phytic acid in their feed [123] [124].

Fungi and bacteria are the predominant sources for the industrial production of phytase. Microbial sources of phytase include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus ficuum*, *Schizosaccharomyces pombe*, *Klebsiella terrigena*, *Klebsiella oxytoca*, *Escherichia coli*, *Bacillus amyloliquefaciens*, *Bacillus subtilis* [123] [124] [125].

3. Conclusion and Future Perspectives

With the continuous rise in the global population, the demand for products from food industry is expected to continue to increase. Consequently, the de-

mand for enzymes with applications in food industry and the value of the global food enzyme market is expected to maintain an upward trend as projected. Therefore, research geared towards the production of novel enzymes with applications in food industry has to be intensified to meet the demand for industrial enzymes. With the aid of biotechnological advancements, microbes can be genetically modified to overproduce enzymes with desired biochemical characteristics and higher enzymatic activity. Also, the production of chimeric enzymes through the genetic combination of genes for enzymes from different microbes can be promoted. With this, a fusion enzyme can be used in several industrial production processes thus saving cost, time, and consumables for producing several enzymes with different functions.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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