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Microbial enzymes produced by fermentation and their applications in the food industry - A review

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***Abstract* – Microbial enzymes are widely used in different industries mainly because of vast availability of sources. Microbial enzymes could be genetically modified and are considered as economical in comparison to plant and animal enzymes. Production of microbial enzymes by application of fermentation procedures involves microbial propagation like bacteria, mold and yeast to get desired product. The process of fermentation is classified based on specific parameters. There are different techniques employed to produce microbial enzymes using downstream processing methods that are aimed at enzyme purification and recovery. The improvement in concentration, purity and percentage of recovery of enzymes can be achieved based on standard principles which are microbial sources, improvement of strain and application of membrane augmented downstream processing method to improve specific activity of enzyme. There are two methods of fermentation used to produce enzymes. These are submerged fermentation and solid-state fermentation. Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Solid-state fermentation is the cultivation of microorganisms, and hence enzymes on a solid substrate. Carbon containing compounds in or on the substrate are broken down by the microorganisms, which produce the enzymes either intracellular or extracellular. Microbial enzymes exhibit wide variety of applications in different industries. Industries that use enzymes generated by fermentation are the brewing, wine making, baking, cheese making, dairy, milling, beverages, and cereals.**

***Keywords* – Microbial Enzymes, Fermentation, Submerged Fermentation, Solid-State Fermentation.**

I. INTRODUCTION

Enzymes are “green” biological catalysts that have changed the way we prepare our food. Enzymes are worldwide used in various feed and food industries, widely covering dairy, brewing, meat, baking, juice and beverages, vegetable processing, dietary supplements, oils and fats [1, 2]. The application of enzymes and microorganisms to food processing is traditionally a known method. Enzymes and microorganism have been used in the brewing of bread baking, beer, wine and cheese making process for ages [3]. Biotechnology for food offers different ways to improve the processing of raw materials for conversion to food products of high nutritional value [4].

Fermentation is a microbial biotechnology whereby natural renewable substrates are converted to value-added products such as enzymes, organic acids, alcohols, polymers and more. Fermentation end-products such as ethanol and lactic acid are proton sinks, whereby NADH is recycled to NAD^+, which allows the cell to continue producing energy via glycolysis by substrate-level phosphorylation. Thus, microorganisms generate many end- or by-products to maintain energy balance. Today, enhanced production of economically important fermentation products has benefited from targeted genetic engineering techniques to established industrial microbial strains [5]. The formation of end or by-products is dependent on microbial strain and the environmental conditions employed. For an optimum fermentation process, a microbial strain should be selected and developed based on the desired product. Strain development technologies include mutation and recombinant DNA technology [6].

Growth of microorganisms and product formation are also affected by temperature, pH, dissolved oxygen, and fermentation medium composition. Therefore, the fermentation media and growth conditions need to be optimized. Moreover, the metabolic pathways should be determined. Knowledge of biochemical changes in fermented foods can help producers to manipulate the production by changing strains and/or conditions. In addition to growth conditions, types of strains, and media, fermentation modes affect the productivity. Batch, fed-batch, and continuous fermentation modes can be selected for high productivity. Fed-batch and continuous modes can overcome the substrate limitation during fermentation processes. Higher productivity can also be achieved by cell immobilization, which results in increases in the biomass concentration in the bioreactor, and thus an increased concentration of biocatalysts in the reactor. After the fermentation process, recovery methods of the product from the fermentation medium need to be evaluated and optimized, and are often an economic limitation factor. Indeed, purification of the end-product is often a high-cost step of the process. The microbial end-products can be biomass itself, extracellular products, or intracellular products. Filtration, homogenization, and extraction (liquid and solid) are examples of recovery methods [6].

Enzymes are products of living organisms and have been used in the industry for many years due to their catalytic activities. Enzyme activity depends on temperature, substrate, pH, inhibitors, etc. and should be optimized for each process. Since enzyme recovery is difficult, the application of enzyme immobilization may reduce process cost. Enzymes can be isolated from plants and mammalian tissues, or can be produced by microorganisms. However, microbial enzymes are preferred due to availability and specificity. Enzymes are used in the production of over 500 commercial products [7]. They have a broad range of applications from food to detergents. Most enzymes are commercially available and used to enhance the product quality in food, detergents, leather, paper, cosmetics, and pharmaceuticals. Commercial enzymes include lipase, amylases, proteases, pectic enzymes, and milk clotting enzymes (rennet) [6].

II. Microbial Sources

Microbial enzymes produced from industries are selected from different groups of microorganisms and they include bacteria, fungi and yeasts. Many enzymes are produced in industries but most predominant enzymes that are produced on large scale in industries include protease, α-amylase, glucose isomerase and glucamylase [8]. Enzymes produced in industries with the help of microorganisms were found to exhibit good biological activity. Microbial source is preferred over plants and animals for production of enzymes mainly because of the following reasons [9]. 1) Enzymes can be produced on large scale and are economical [10, 11]. 2) The process of extraction and purification of enzymes from microbial sources is easier in comparison with plant and animal sources [12]. 3) Microbial sources are capable of producing variety of enzymes in different environmental conditions in limited space and time period [13]. 4) Genetic manipulation is carried out to yield higher quantity of enzymes produced from microbial sources [14]. Some of the industrially produced enzymes produced in large scale using microorganisms as source are mentioned below in (Table 1) [9, 15, 16].

Table 1. Industrial enzymes and their source of microorganisms.

Source	Enzyme	Microorganism
Bacterial	Proteases	*Bacillus Subtilis*
	Amylases	*Bacillus subtilis*
	Pencillinase	*Bacillus subtilis*

Source	Enzyme	Microorganism
Yeast	Lactase	*Saccharomyces fragilis*
	Invertase	*Saccharomyces cerevisiae*
Fungal	Proteases	*Aspergillus niger*
	Amylases	*Aspergillus oryzae*
	Pectinases	*Aspergillus niger*
	Catalase	*Aspergillus niger*
	Glucose oxidase	*Penicillium notatum*
	Glucosidases	*Aspergillus flavus*

Source: [9]

III. Methods of Strain Improvement

Microorganisms are used as source for production of enzymes, biomolecules and proteins in industries. Few examples of source of microorganism include Saccharomyces cerevisiae and *Aspergillus niger* are widely used in industries for production of enzymes and alcohol [9]. A wild type strain is isolated for process of strain improvement and to increase productivity. To achieve growth rate faster, desirable downstream processing and behavior of fermentor is enhanced by altering cellular genetics and also it is important to understand the fundamentals of physiology and structure of organism. The strategies differ from each source of microorganism for example in case of fungal source the emphasis is more on porosity of cell wall, differentiation, secretion and branching. Whereas in case of yeast fermentation process involves gene regulation and ploidy through which carbon sources will play a predominant role in production of proteins associated with heterologous gene expression. Wild types of strains which are used for producing metabolic concentrations are not economical. Improvement of strains is considered as cost effective process and it is necessary to produce secondary metabolites [9, 12]. Desirable strain isolation depends on system and they exhibit following features like [17]. Rapid growth, Genetic stability, Nontoxic to humans, Large sized cells, Fermentation process time is less and Exhibit tolerance to carbon or nitrogen sources present in higher concentrations. Few methods that are associated with strain improvement process are Recombinant DNA technology [18], Recombination Protoplast fusion [19] and Mutations-Site-directed mutagenesis [20]. The successful application of these methods is enhanced by increasing a dose of gene concentration will increase the product activity which includes one or more number of genes, for example enzymes [9].

IV. Microbial Enzymes

Initially, enzymes were extracted from the stomach of calves, lambs, and baby goats, but now are produced by microorganisms like bacteria, fungi, yeast and actinomycetes. Enzymes obtained from microorganisms are better than those of animal and plant origin. Microorganisms can be genetically manipulated to improve the production of commercial scale [21-23]. Enzymes can hydrolyze complex molecules into simple monomer units, like carbohydrates into simple sugars, which are natural substances involved in all types biochemical processes. Every enzyme is substrate, pH, and temperature-specific for catalyzing the reaction to convert a reactant into a product [23, 24]. The food-processing industry uses more than 55 different microbial enzymes (Table 2).

Table 2. An overview of Enzymes Used in Food Processing Industry.

Class	Enzyme	Role
Hydrolases	Amylases	Starch liquefaction and sachcarification, increasing shelf life and improving quality by retaining moist, elastic and soft nature, Bread softness and volume, flour adjustment, ensuring uniform yeast fermentation, Juice treatment, low calorie beer.
	Galactosidase	Viscosity reduction in lupins and grain legumes used in animal feed, enhanced digestibility.
	Glucanase	Viscosity reduction in barley and oats used in animal feed, enhanced digestibility.
	Glucoamylase	Saccharification
	Invertase	Sucrose hydrolysis, production of invert sugar syrup.
	Lactase	Lactose hydrolysis, whey hydrolysis.
	Lipase	Cheese flavor, in-situ emulsification for dough conditioning, support for lipid digestion in young animals, synthesis of aromatic molecules.
	Proteases	Protein hydrolysis, milk clotting, low-allergenic infantfood formulation, enhanced digestibility and utilization, flavor improvement in milk and cheese, meat tenderizer, prevention of chill haze formation in brewing.
	Pectinase	Mash treatment, juice clarification.
	Peptidase	Hydrolysis of proteins (namely, soy, gluten) for savoury flavors, cheese ripening.
	Phospholipase	In situ emulsification for dough conditioning.
	Phytases	Release of phosphate from phytate, enhanced digestibility.
	Pullulanase	Saccharification
	Xylanases	Viscosity reduction, enhanced digestibility, dough conditioning.
Isomerases	Xylose (Glucose) Isomerase	Glucose isomerization to fructose.
Lyases	Acetolactate decarboxylase	Beer maturation.
Oxidoreductases	*Glucose oxidase*	*Dough strengthening.*
	Laccases	*Clarification of juices, flavor enhancer (beer).*
	Lipoxygenase	*Dough strengthening, bread whitening.*
Transferases	Cyclodextrin	Cyclodextrin production.
	Glycosyltransferase	
	Fructosyltransferase	Synthesis of fructose oligomers.
	Transglutaminase	Modification of viscoelastic properties, dough processing, meat processing.

Source: *[23]*

V. INDUSTRIAL ENZYME PRODUCTION

Markets for traditional industrial enzymes remain to grow while the continued prominence on biotechnological accomplishments has generated demand for an ever increasing number of additional biocatalysts [25]. The emergences of genetic manipulations have now enabled the large-scale production of enzymes and other proteins which are produced naturally only in minute quantities. The level of downstream

processing to which any enzyme is subjected is depending on its proposed application. Industrial enzymes produced in bulk usually need little downstream processing, and hence are relatively crude preparations [25, 26]. The production of commercial enzymes from fungi are 60% followed by 24% bacteria, 4% yeast, 2% Streptomyces, 6% higher animals, and 4% plants. In earlier technologies animal and plant origin enzymes were largely used and till now for specific enzymes they are the main sources. The animal tissues and organs are very fine sources for enzymes such as proteases, lipases, and esterases for example, lysozyme is mostly get from hen eggs. Similarly, some enzymes are originated only from plants as brilliant sources such as papain (papaya) and bromelain (pineapple). There are several down sides related with the enzymes making from plant and animal sources. Due to limited quantities there is a wide disparity in distribution. Apart from all the difficulties, the most important is the isolation, purification of the enzymes, and the cost factor as regard to industrial enzymes extracting from bovine source which contains heavy risk of contamination due to bovine spongiform encephalopathy (BSE is a prion disease occurred due to the ingestion of abnormal protein) therefore, microbial production of enzymes is used. There exists a likelihood of producing commercial enzymes straight by mammalian cell cultures. Other than the most important limit is the cost factor which is extremely high. Although some therapeutic enzymes are prepared through cell culture technique like tissue plasminogen, microorganisms are the most noteworthy and suitable source of commercial enzymes. They can be made to prepare large amounts of enzymes under optimal growth conditions. Cultivation of microorganism by using low cost media as well as the growth of microorganism takes in short span of time. In addition, by using genetic engineering techniques on microorganism, desired product is produced. Isolation, purification, and recovery processes are easy with microbial enzymes as compared to plant and animal sources. In fact, most of the enzymes used in industrial processes are produced from microorganisms. Variety of fungi, bacteria, and yeast are produced for this purpose [25]. The microbial origin and the enzymes application in the food and beverages operations are listed in (Table 3) and (Fig. 1) [25].

Table 3. Industrial Applications of Enzymes Produced by Solid-State Fermentation Processes.

Process	**Enzyme**
Enzyme-assisted ensiling	Fungal cellulases and hemicelllulases
Bioprocessing of crops and crop residues	Fungal cellulases and hemicelllulases
Fiber processing (retting)	Fungal pectinases, cellulases, and hemicellulases
Feed supplement	Amylases, proteases, lipases, cellulases, and hemicellulases
Biopulping	Xylanases
Directed composting	Hydrolytic enzymes
Soil bioremediation	Laccases, ligninases
Postharvest residue decomposition	*Trichoderma harizanum* cellulases
Biopesticide	*T. harzianum* cellulase for helper function

Source: [25].

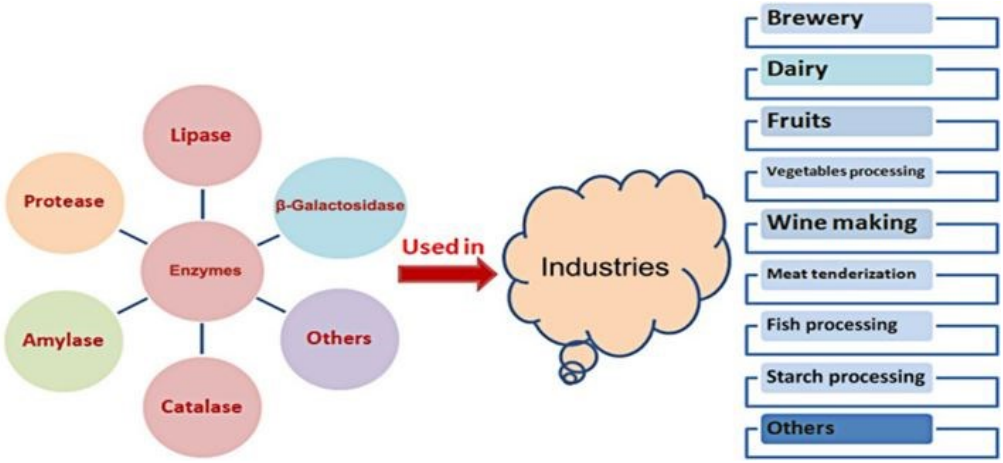

Fig. 1. Different microbial enzymes used in food and beverages processing. [25]

Recent developments in biotechnology are yielding efficient development of new enzymes particularly in the areas of protein engineering and directed evolution (Fig. 2). Solid-state fermentation carries enormous potential for the production of enzymes. It can be a particular attention in those processes where the basic fermented products may be used instantly at the enzymes sources [21, 25].

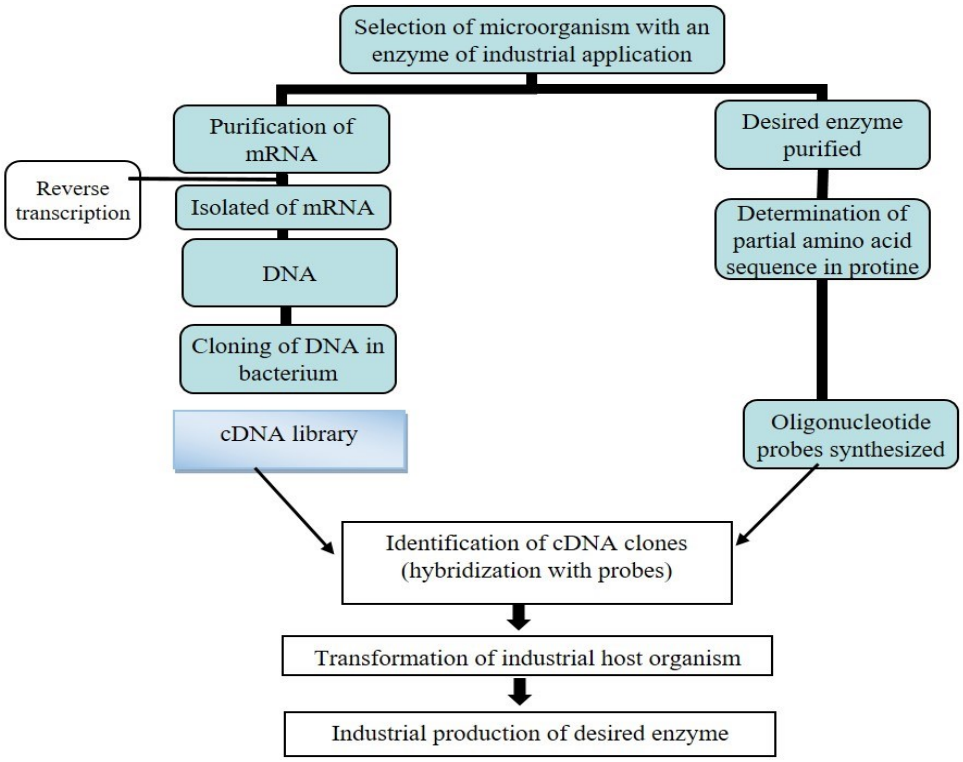

Fig. 2. Schematic representation of a cloning strategy for industrial production of enzymes. [25]

In industrial production of enzymes, the submerged liquid conditions are mostly preferred as compared to the solid-substrate fermentation because in the submerged culture methods the yields are more and contamination chances are less. However, historically solid-substrate fermentation is vital and immobile utilize for the fungal enzymes production for example, amylases, cellulases, proteases, and pectinases. The batch or continuous sterilization techniques are employed for medium sterilization. The growth conditions for fermentation viz., substrate, O_2 supply, pH, and temperature are maintained at optimal levels after inoculating the medium with desire culture [25].

The froth formation can be controlled by adding antifoam agents and mostly the batch fermentation is used for production of enzymes, whereas continuous process used in lesser extent. Throughout the fermentation process the bioreactor system must be operated under sterile conditions. In most production processes, the fermentation duration is variable approximately 2–7 days. Several other metabolites are also produced besides the desired enzyme (s) and afterwards enzyme (s) contain to be healthier and purified. The preferred enzyme produced may be extracellular enzymes that are secreted into the culture medium or might be intracellular enzymes that bound within the cells. The commercial enzymes may be crude or highly purified depending on the requirements and also it might be in the liquid or solid form. During different steps involved in downstream processing, viz., improvement and decontamination steps engaged will depend on the degree of purity and desired nature of the enzyme. The recoveries of extracellular enzymes are very easy because it is present in the broth as compared to an intracellular enzyme. The different mechanisms are required for the cell disruption of microorganisms for the release of intracellular enzymes. Microbial cells membrane can be lysed by physical methods which included sonication, high pressure, glass beads, etc. Similarly, bacterial cell wall can also be lysed with the help of enzymes like lysozyme and for yeast, β-glucanase is used [25].

VI. Fermentation

Fermentation of foods dates back many thousands of years, with grape and barley fermentation for alcoholic beverage production [5, 6]. Fermentation can improve the sensory characteristics, increase the shelf life, and enhance the nutritional value of food. This section focuses on some of the results of actions of microorganisms, which cause biochemical and physical changes during the production of fermented foods [6].

Enzymes have been used for thousands of years to produce food and beverages, such as cheese, yoghurt, beer and wine. Yeast is a fungus whose enzymes aid the breakdown of glucose into ethanol and carbon dioxide anaerobically. This reaction, which takes place in the absence of oxygen, is called fermentation. The enzymes in yeast break down sugar (glucose) into alcohol (ethanol) and carbon dioxide gas [27]:

$$\textit{Glucose} \xrightarrow{\textit{yeast}} \textit{Ethanol + carbon dioxide}$$

$$C_6H_{12}O_6 \xrightarrow{yeast} 2\ C_2H_5OH\ (aq) + 2\ CO_2\ (g)$$

Fermentation works best when the yeast and glucose solution is kept warm. Enzymes will also become ineffective if the temperature becomes too high. Fermentation is used in all production of alcoholic drinks. For stronger alcohol, such as whiskey and vodka, these need to be distilled after fermentation to increase the concentration of ethanol in the fermented mixture. This is due to the fact that ethanol poisons the yeast and stops it working when the concentration builds up about 18% by volume. Fermentation is also used in the baking industry to make bread rise. After the dough has been prepared, it is left to rest in a warm place before going into the oven. This gives the enzymes in the yeast a chance to break down the sugar and make carbon dioxide [27].

6.1. *Methods of Fermentation*

6.1.1. *Submerged Fermentation*

Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. Industrial enzymes can be produced using this process. This involves growing carefully selected microorganisms (bacteria and fungi) in

closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the microorganisms break down the nutrients, they release the desired enzymes into solution (Fig. 3) [27].

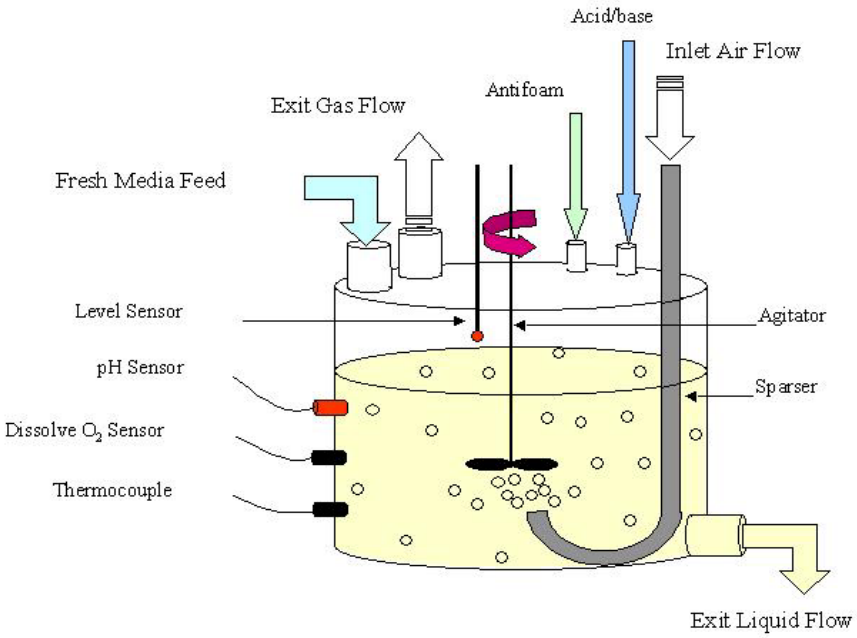

Fig. 3. Typical fermenter [27].

Due to the development of large-scale fermentation technologies, the production of microbial enzymes accounts for a significant proportion of the biotechnology industries total output. Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic metres. The fermentation media sterilizes nutrients based on renewable raw materials like maize, sugars and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources. Batch-fed and continuous fermentation processes are common. In the batch-fed process, sterilized nutrients are added to the fermenter during the growth of the biomass. In the continuous process, sterilized liquid nutrients are fed into the fermenter at the same flow rate as the fermentation broth leaving the system. This will achieve a steady-state production. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimize the fermentation process. Firstly, in harvesting enzymes from the fermentation medium one must remove insoluble products, e.g. microbial cells. This is normally done by centrifugation. As most industrial enzymes are extracellular (secreted by cells into the external environment), they remain in the fermented broth after the biomass has been removed. The biomass can be recycled as a fertilizer, but first it must be treated with lime to inactivate the microorganisms and stabilize it during storage. The enzymes in the remaining broth are then concentrated by evaporation, membrane filtration or crystallization depending on their intended application. If pure enzyme preparations are required, they are usually isolated by gel or ion exchange chromatography. Certain applications require solid enzyme products, so the crude powder enzymes are made into granules to make them more convenient to use. Sometimes liquid formulations are preferred because they are easier to handle and dose along with other liquid ingredients. Enzymes used in starch conversion to convert glucose into fructose are immobilized, typically on the surfaces of inert granules held in reaction columns or towers. This is carried out to prolong their working life as these enzymes normally go on working for over a year [27].

6.1.2. *Solid State Fermentation*

Solid-state fermentation (SSF) is another method used for the production of enzymes. Solid-state fermentation involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran, bagasse, and paper pulp [27, 28]. This method is an alternative to the production of enzymes in liquid by submerged fermentation. SSF has many advantages over submerged fermentation. These include, high volumetric productivity, relatively high concentration of product, less effluent generated and simple fermentation equipment. There are many substrates that can be utilized for the production of enzymes by SSF. These include wheat bran, rice bran, sugar beet pulp and wheat and corn flour. The selection of substrate depends on many factors, which is mainly related to the cost and the availability of the substrate. Other factors include particle size and the level of moisture. Smaller substrate particles have a larger surface area for the proliferation of the microorganisms, but if too small the efficiency of respiration will be impeded and poor growth and hence poor production of enzymes will result. Larger particles provide more efficient aeration and respiration, but there is a reduction in the surface area [27]. A compromise must be reached, regarding the particle size of the substrate for a particular process. SSF requires moisture to be present on the substrate, for the microorganisms to produce enzymes. As a consequence, the water content of the substrate must also be optimized, as a higher or lower presence of water may adversely affect the microbial activity. Water also has implications for the physicochemical properties of the solid substrate. Enzymes of industrial importance have been produced by SSF. Some examples are proteases, pectinases, glucoamylases and cellulases [27, 29].

6.2. *Types of Fermentation Process*

Fermentation in liquid media is of two types depending upon the mode of operation:

6.2.1. *Batch Fermentation*

Batch reactors are simplest type of mode of reactor operation. In this mode, the reactor is filled with medium and the fermentation is allowed to proceed. When the fermentation has finished the contents are emptied for downstream processing. The reactor is then cleaned, re-filled, re-inoculated and the fermentation process starts again [27].

6.2.2. *Continuous Fermentation*

Continuous reactors: Fresh media is continuously added and bioreactor fluid is continuously removed. As a result, cells continuously receive fresh medium and products and waste products and cells are continuously removed for processing. The reactor can thus be operated for long periods of time without having to be shut down. Continuous reactors can be many times more productive than batch reactors. This is partly due to the fact that the reactor does not have to be shut down as regularly and also due to the fact that the growth rate of the bacteria in the reactor can be more easily controlled and optimized. In addition, cells can also be immobilized in continuous reactors, to prevent their removal and thus further increase the productivity of these reactors. Continuous reactors are as yet not widely used in industry but do find major application in wastewater treatment. Fed batch reactor is the most common type of reactor used in industry. In this reactor, fresh media is continuous or sometimes periodically added to the bioreactor but unlike a continuous reactor, there is no continuous removal. The fermenter is emptied or partially emptied when reactor is full or fermentation is finished. As with the continuous reactor, it is possible to achieve high productivities due to the fact that the grow

-th rate of the cells can be optimized by controlling the flow rate of the feed entering the reactor [27].

VII. THE ENZYMES PRODUCED BY FERMENTATION

Enzymes are secreted by nearly all living cells for catalysis of their own specific biochemical reactions in the metabolic process. Enzymes are playing an important role in food processing techniques for improving nutritive value and flavor of processed food. The food processing industry the making of cheese, leavened bread, wine and beer, yogurt, and syrup is successfully using enzymes at the commercial level [23, 30].

7.1. *α-Amylase*

Amylase enzymes hydrolyze complex starch molecules into simple monomer units of glucose. Sources of α-amylase are plants, animals and microorganisms, but commercially viable amylases are produced from microorganisms, especially bacterial and fungal species [23]. Thermostable α amylase is produced by some potential bacterial species like *Bacillus licheniformis and Bacillus stearothermophillus, Pseudomonas, and the Clostridium* family. Starch-converting properties of α-amylases are playing an important role in the food, beverage, and sugar industries. α-Amylase is improving the quality of breads that have reduced size and poor crust color, and compensates for the nutritional deficiencies of the grain. α-Amylase also degrades the starch in wheat flour into small dextrins, thus allowing yeast to work continuously during dough fermentation, proofing, and the early stages of the baking process. α-Amylases are also employed in many other aspects of the food industry like clarification of beer, fruit juices, and pretreatment of animal feed to improve the digestibility of fiber [23, 31].

7.2. *Lactase*

Lactase enzymes catalyze the breakdown of the milk sugar lactose into simple sugar monomer units like glucose and galactose. Lactases are obtained from plants, animal, bacteria, fungus, yeasts and molds. Commercial production of lactase enzymes is developed from *Aspergillus niger, A. oryzae, and Kluyveromyces lactis* [23, 32]. Fungal origin lactases have optimum activity at acidic pH ranges, and yeast and bacterial-originated lactases have optimum pH ranges near to neutral [33]. The lactase enzyme is predominantly rich in infancy and is called a brush border enzyme. Some people do not produce enough of the lactase enzyme so they do not properly digest milk. This is called lactose intolerant, and people who are lactose intolerant need to supplement the lactase enzyme to aid in the digestion of milk sugar. Another useful application of the lactase enzyme is it increases the sweetness of lactase-treated milk, and assists in the manufacturing of ice cream and yogurt preparation [23].

7.3. *Protease*

Proteolytic enzymes are also termed as peptidases, proteases, and proteinases, which are able to hydrolyze peptide bonds in protein molecules. Proteases are generally classified as endopeptidases and exopeptidases. Exopeptidases cut the peptide bond proximal to the amino or carboxy termini of the protein substrate, and endopeptidases cut peptide bonds distant from the termini of the protein substrate [23]. Proteases are obtained from diverse groups of organisms such as plants, animals, and microorganisms, but commercially viable proteases are obtained from microorganisms, especially bacterial and fungal species. Microorganisms secrete the extracellular and intracellular proteases in both the submerged and solid-state fermentation process. Bacillus

species of bacteria, like *Bacillus licheniformis*, Bacillus subtilis, and Aspergillus species of fungus like *Aspergillus niger, A. flavus, A. fumigatus, A. oryzae,* are the best sources of protease enzyme. Broad working range of temperature (10–80°C) and pH (4–12) of protease enzymes increases their application in the food-processing industry, the major role in cheese and dairy product manufacturing. Aminopeptidases are significantly improving the flavor in fermented milk products. Other basic applications of proteases in the food-processing industry are to increase the nutritive value of bread, baked goods, and crackers [23, 34].

7.4. *Pectinase*

Pectinase breaks down pectin components, which are found in the middle lamella of plant cell walls. Pectin is made up of complex colloidal acid polysaccharides with a back bone of galacturonic acid residue with a α-1-4 linkage. Pectinase therefore helps to break down plant cell walls to extract cell sap. Potential microbial strains like *Moniliella SB9, Penicillium* spp. *and Aspergillus* spp. are good sources of commercial pectinase [23, 35]. Where, Dupaign, (1974) mention that the Pectinases are now an essential part of the fruit juice industry, as well as having various biotechnological applications in the fermentation of coffee and tea, the oil extraction processes, and the treatment of pectic waste water from the fruit juice industry. Pectinase is lowering down the viscosity of fruit juice during the clarification process through the degradation of pectin substance in fruit juice and getting better pressing ability of pulp, simultaneously jelly structure are breaking down and increases the yields of fruit juice. Another significant application of pectinase enzymes in industrial processes is the refinement of vegetable fibers during the starch manufacturing process, such as the curing of coffee, cocoa and tobacco, canning of orange segments, and extracting sugar from date fruits [23].

7.5. *Lipase*

Lipases catalyze the hydrolysis of ester bonds in lipid substrates and play a vital role in digestion and the transport and processing of dietary lipids substrate [23, 36]. Lipases catalyze the biochemical reaction like esterification, interesterification, and transesterification in nonaqueous media which frequently hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. Microorganism like *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcoccus aureus, and Bacillus subtilis* are the best sources of lipase enzymes. Lipases are widely used in pharmacological, chemical and food industries. The commercial applications of lipases in the food industry are the hydrolysis of milk fats, pronounced cheese flavor, low bitterness and prevention of rancidity. Lipases may combine with many other enzymes like protease or peptidases to create good cheese flavor with low levels of bitterness [23, 37].

7.6. *Laccase*

Laccase enzymes were first obtained from the cell sap of the Japanese lacquer tree. Laccase enzymes are isolated from plants, bacteria, fungi, and insects [38]. Laccase is responsible for discoloration, haze, wine stabilization, baking, and flavoring in food processing [23, 39]. Laccase improves the baking process through an oxidizing effect, and provides an additional development in the strength of dough and baked products, including enhancing crumb structure and increasing softness and volume. Another diverse application of laccase is in environmental sectors, which degrade various ranges of xenobiotic compounds [23].

7.7. *Xylose (Glucose) Isomerase*

Xylose isomerase (d-xylose ketol-isomerase) catalyzes the isomerisation reaction of D-xylose into xylulose. This is initial step of xylose metabolism in microbial cell physiologies [23, 40]. Xylose isomerases are also referred to as glucose isomerases because of their capability to exchange d-glucose into d-fructose. Microorganisms are most suitable sources of xylose isomerase; some potential microbial species are *Streptomyces olivochromogenes, Bacillus stearothermophilus, Actinoplanes missouriensis, Thermotoga maritime and Thermotoga neapolitana,* known xylose isomerase procurers. Xylose isomerase loses its catalytic activities up to 50% under acidic conditions [41]. The greatest application for glucose isomerase is in the food-processing industry; it mainly catalyzes two significant reactions such as reversible isomerization of d-glucose to d-fructose, and d-xylose to d-xylulose [23].

7.8. *Cyclodextrin Glycosyl Transferase*

Cyclodextrin glycosyl transferase (CGTase) enzymes catalyze the change of starch into nonreducing cyclic sugars (cyclodextrin) [42]. Cyclodextrins (CD) are cyclic homogeneous oligosaccharides of glucose residues, which are composed of 6–8 d-glucose units linked by a -1,4 glycosidic bond. Cyclodextrins are being used in the food-processing industry for preparation of reduced-cholesterol products and rising bioavailability of desired molecules, because cyclodextrins facilitate hydrophobic-hydrophilic interactions within protein-protein and other molecules [23]. Production of Cyclodextrin glycosyl transferase (CGTase) is reported in different bacterial groups; major CGTase producers belong to the genus *Bacillus* spp. However, *Klebsiella pneumonia, Micrococcus luteus, Thermococcus, Brevibacterium sp., and hyperthermophilic archaea* are reported as major CGTase-producing strains [43-45].

7.9. *Catalase*

Catalase enzymes break down hydrogen peroxide (H_2O_2) to water and oxygen molecules, which protects cells from oxidative damage by reactive oxygen species. Commercial catalases are produced from *Aspergillus niger* through a solid-state fermentation process [23, 46]. The major applications of catalase in the food-processing industry include working with other enzymes like glucose oxidase, which is useful in food preservation and egg processing, and sulphydryl oxidase, which under aseptic conditions, can eliminate the effect of volatile sulphydryl groups, that is, they generate from thermal induction and are responsible for the cooked/off-flavor in ultra-pasteurized milk [23, 47].

7.10. *Glucose Oxidase*

The glucose oxidase enzyme is commercially produced from *Aspergillus niger* and *Penicillium glaucum* through a solid-state fermentation method. Muller [48] was the first one who reported that the catalyzation of glucose oxidase and the breakdown of glucose into gluconic acid in the presence of dissolved oxygen. Fungal strains *Aspergillus niger* are able to produce notable amounts of glucose oxidase. Glucose oxidase enzymes are used to remove small amounts of oxygen from food products or glucose from diabetic drinks [23]. Glucose oxidase is playing an important role in color development, flavor, texture and increasing the shelf life of food products [23, 49].

7.11. *Acetolactate Decarboxylase*

Acetolactate decarboxylase catalyzes the conversion of acetolactate into acitoine and release carbon dioxide,

a type of decarboxylation reaction. α-Acetolactate decarboxylase is commercially produced by the submerged fermentation of *Bacillus subtilis,* genetically improved *Bacillus brevis* and *Enterobacter aerogenes* strain 1033. In conventional brewing procedures, α-diacetyl is produced from α-acetolactate and this further reduces to acetoin over a 2–4 - week maturation period, but α-acetolactate decarboxylase causes direct decarboxylation of α-acetolactate to acetoin and avoiding maturation period [23].

7.12. *Transglutaminase*

Transglutaminase enzymes catalyze reactions to alter proteins by merging amine, crosslinking, and deamination. Transglutaminase is responsible for acyl transfer, deamidation, and the inter- and intra-molecular crosslink between amino acid residues of glutamine and lysine [50-52]. The commercial application of transglutaminase enzymes in the food-processing industry is improving the protein-emulsifying capacity, gelatation, viscosity, and production of various types of protein ingredients to enhance the quality of food products [23]. Transglutaminase is enhancing the water-holding capacity, softness, foam formation, and stability of food products. Extracellular transglutaminase is isolated from cultural filtrate of *Strepto verticillium* spp., *Strepto verticillium mobarens, Strepto verticillium ladakanum, and Strepto verticillium lydicus* [53-55]. Intracellular transglutaminase is secreted by common microbial species Bacillus subtilis and spherules [23, 53].

VIII. Application of Enzymes in Food Processing

Enzymes are derived from natural sources and may be readily inactivated after a desired transformation has taken place [56, 57]. Unlike inorganic catalysts, enzymes are highly specific, catalyzing the transformation of only a single substrate or the splitting of a small group of closely related compounds or a specific bond. This minimizes byproduct formation in large-volume reactions. The capacity of enzymes to react under mild conditions of temperature and pH (up to 100°C and pH 3 to 10) achieves a reduction in energy costs [57]. Low usage levels make enzymes economical and practical for commercial application [56]. Because they are derived from plants, animals, or microbial sources, enzymes are perceived as natural, nontoxic food components and are preferred over chemical aids as food-processing aids by consumers [58]. Based on these properties, enzymes find numerous applications in industry [57].

8.1. *Dairy Industry*

Rennet is an exogenous enzyme extract that has a long history in the dairy industry. Originally sourced from calf stomachs, the proteolytic enzymes (chymosin and pepsin) quickly coagulate milk's casein micelles by stripping off kappa-casein molecules. The ensuing loss of steric stabilization causes micelles to aggregate into the particle gel that becomes cheese via a series of subsequent processing steps [59]. A typical addition of 1mL of a commercial liquid animal rennet will create cheese from 3L of milk. Very little rennet from calf stomachs is used in modern industrial cheese production, as fermentation-derived chymosin (EC 3.4.23.4) is predominantly employed for the commercial production of cheese in North America and Europe. A variety of cheese types are available, distinguished by flavor, aroma, appearance, and texture (Fig. 4). Enzymatic conversions are instrumental in generating this diversity from a single main ingredient. Traditionally, these enzymes have been obtained from bacterial and fungal sources, but now industrial exogenous enzymes are the means of attaining a distinctive character in a shorter time than through traditional aging processes. An example is the production of cheddar, where traditional aging times may be as long as 18 months [60]. Added proteases reduce the length of

time for cheese aging by accelerating flavor modifications as a result of enzymatic breakdown of protein structure. Of particular interest in flavor modifications is the liberation of volatiles by carbon–sulfur lyases (EC 4.4) [61]. Bioactive peptides are enzymatic products potentially derived from dairy proteins. The discovery that certain peptides generated from the peptidase scission of dairy proteins possess opioid and anti-hypertensive properties has been the impetus for growth of this sector [59, 62]. Of particular economic interest are bioactive peptides created from peptidase action on whey proteins. Whey was once a long-standing waste issue for the cheese industry [63], but now is rendered into a spectrum of value-added commodities with an estimated global value of $9 billion. A typical manufacturing route for bioactive peptides uses membrane filtration to produce a whey-enriched retentate stream that can be enzymatically valorized using alcalase (EC 3.4.21.62), chymotrypsin (EC 3.4.21.1), pepsin (EC 3.4.23.1), or thermolysin (EC 3.4.24.27) [64].

The number of people exhibiting symptoms of intolerance to lactose is growing. Yet, milk's roles as an important nutritional source in its own right, and as the base ingredient for a wide range of other foods, means that products that allow dairy nutrients to be accessible to lactose-intolerant consumers are sought-after for product development strategies [59].

An enzymatically derived answer arises when β-galactosidase (EC 3.2.1.23) is used to hydrolyze the lactose in milk; operation in a continuous mode facilitates consistent reduction of lactose levels in the milk. Using a continuous immobilized β-galactosidase reactor, a 92% efficiency (after 1 month of operation) was reported in converting lactose into galactose and glucose [65].

Fig. 4. Enzymatic conversions creating cheeses of different appearances and textures; from 9 o'clock progressing clockwise: Gouda, Parmesan (both created from enzymes from lactic acid bacteria), Jarlsberg (lactic acid bacteria and propionibacteria), and Danablu (lactic acid bacteria and Penicillium) [59].

8.2. *Fruit, Vegetable and Oilseed Industries*

A portion of a review cannot do justice to the benefits of enzymes in industries employing a diverse range of processes to produce a variety of products [59]. Nevertheless, the breadth of exogenous enzyme uses in the fruit and vegetable industries for enhancing process efficiency, creating new products, and improving the yield and/or quality of food products can be gleaned from some of the enzymes reported later. Readers can consult

reviews of enzymatic applications in processing of fruits [66-68], vegetables [68], and oilseeds [67, 69] for more details. In the oilseeds industry, exogenous enzymes are used as process aids for better processing options, to optimize product yield and quality, and to develop new products where inorganic catalysts have failed. In optimizing product yield and quality, degumming of crude vegetable oils is facilitated by dispersing phospholipases (EC 3.1.1.4) into water and using high shear forces to create a water-in-oil emulsion [59]. The surface-active phospholipids diffuse to the interfaces of the water droplets in this emulsion because it is an energetically favorable location for their repositioning. Sufficient reaction time is allowed for the enzyme to act on the phospholipids to generate reaction products (gums) in the water phase which are then removed through centrifugal separation of the oil and aqueous phases [70]. In optimizing product yields, glycerolysis conversions can be carried out with fewer steps and without the need for expensive higher process temperatures if lipases (EC 3.1.1.3) are employed in place of inorganic catalysts [71]. One desired product from glycerolytic conversions is a suite of diacylglycerol molecules. The yield of these body-fat suppressing fat substitutes by lower-temperature enzymatic catalysis can be further enhanced by conducting the reaction in supercritical carbon dioxide [59]. Up until recently, the production of solid fats from oils with desirable physical properties (so as to confer good functionality in products such as bakery shortenings) relied on chemical interesterification techniques with inorganic catalysts. However, the link between trans-fat production by this process and increased heart disease risks has now been firmly established. As a result, enzymatic interesterification methods have superseded chemical methods because the precise nature of lipase catalysis in a limited water environment significantly reduces trans-fat generation [67].

8.3. *Bakery Industry*

Enzymes play a major role in today's baking industry, with a growth rate estimated at 6%–8% annually [12]. The main products from the baking industry bread, pastries, cakes, and cookies all can have exogenous enzymes added to attain specific quality attributes in the baked product. For bread, three of the six enzyme types can be added as improvers to dough formulations [59, 72]. Amylases play a number of roles in optimizing the quality of a loaf of bread. Exogenous α-amylases (EC 3.2.1.1) that are still active at the beginning of baking improve the crumb structure and the volume of the loaf. In addition, the modification of amylose and amylopectin molecules by maltogenic α-amylase (EC 3.2.1.133) reduces the rate of staling of the breadcrumb as a result of how the enzymatically-derived molecules interact with water and gluten. A reduced rate of staling extends the shelf life of the baked product and can cut down on food waste. Lipases (EC 3.1.1.3) have an interesting functional role in baked goods such as bread, where the control of aeration in a product that can be as high as 85% air by volume [73, 74] directly governs product quality [73]. Phospholipases (e.g., EC 3.1.1.4) that target polar lipids in flour are being increasingly used for this purpose [70], due to lower propensity for generating off-flavors. The cleaving of acyl chains from any of the carbon positions of the triacylglycerol molecules of phospholipids and triglycerides creates highly surface-active monoacylglycerols, molecules that easily position themselves at the interface of a bubble and its surrounding dough liquor [75]. A reservoir of such surface-active molecules provides better stability for the bubbles as they expand slowly in the dough during fermentation, and rapidly during the oven spring phase of baking. A reduction in the extent of bubble coalescence leads to increased stability during processing as well as a finer crumb structure in the resulting loaf of bread. The in situ nature of enzymatic formation of the monoacylglycerol obviates declaration of added surface-active ingredients (e.g., emulsifiers) on the food product label. A greater degree of enhanced product quality can be attained with

bakery improvers that contain a selected combination of enzyme functionalities. This can be seen in (Fig. 5), where loaves of bread that contain increasing amounts of a bakery improver (comprised of amylase and xylanase) demonstrate the clear effect of increasing enzymatic dosage on loaf volume. Increased demand for freshness coupled with convenience (both at home and at retail outlets) has driven growth of the frozen or refrigerated bakery products sector [59]. The unique quality challenges associated with freezing and changes in ice crystal structure during frozen storage are amenable to mitigation, and thus improvement in the quality of the product, by formulations that incorporate exogenous enzymes [72].

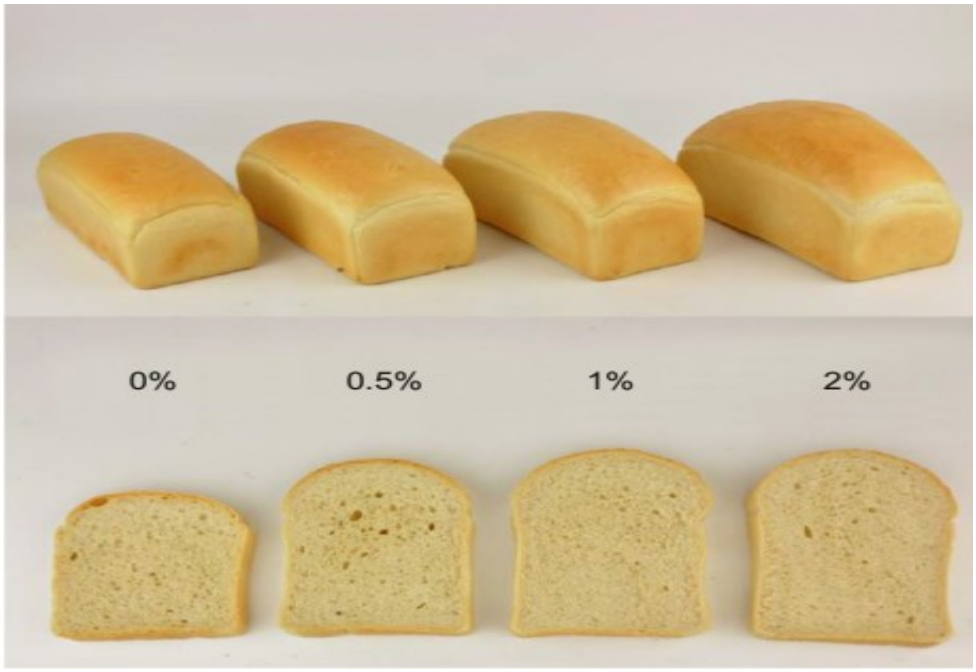

Fig. 5. Loaves of bread containing increasing dosages of a pure enzyme improver (amylase and xylanase), added as percentages of flour weight. [59]

For example, enzymes such as glucose oxidase (EC 1.1.3.4), that improve the gas retention capacity of the gluten network via protein cross-linking, have been shown to improve dough's resilience to prolonged frozen storage [76].

Cakes, like bread, should undergo minimal staling throughout their intended shelf life. But, enzymes proposed for shelf-life extension in cakes have to produce outcomes that last considerably longer. The enzymes must also thrive in the high sugar and/or lipid levels that are not prevalent in bread doughs. To address this demand, enzyme manufacturers have used modern biotechnology techniques to develop enzymes that are less inhibited by the rich environment that a cake batter provides [59]. One example is the development of sugar-tolerant maltogenic α-amylases (e.g., EC 3.2.1.133) that support softness and tenderness in long shelf-life cake applications. As a further example in cake production, the lipid-rich batter must incorporate the appropriate distribution of air bubbles and retain them during baking so that specific cake crumb structures are developed. The action of phospholipase (EC 3.1.1.4) on egg-yolk lipids stabilizes bubble sizes within the batter because surface-active monoacylglycerols are available for the bubble–batter interface [70, 77]. In contrast to what is required of gluten proteins for bread making, cookie manufacturing requires gluten that is rather weak so that the cookie dough spreads well during the initial stages of baking. In North America, gluten "weakness" is a primary consideration if new wheat varieties are to be recommended for registration for cookie production. In

the cookie spread test, a given volume of dough is transformed into a cookie by baking, and the cookie dimensions are measured. Because flour from a given wheat variety is variable in its properties (from year to year and depending on the location where the wheat is grown), the innate capacity for dough spreading also varies. Consequently, proteases (e.g., EC 3.4.22.2) can be added to control the "weakness" of the flour [72] and thus attain a desired degree of spread. Control of spread to attain consistent cookie height and width while meeting a legal weight target is essential in automated packaging operations where tolerances to cookie height variation are severely limited. Consumer concern about the danger of acrylamide [78] has caused the food industry to look for solutions to this food safety issue. Since the amino acid asparagine is required for acrylamide formation during baking and frying [79], asparaginase (EC 3.5.1.1) is a potential enzymatic solution. In the reaction, ammonia and asparate are generated from asparagine as a result of the activity of the enzyme, thus reducing precursor supply for acrylamide formation [59].

$$L\text{- asparagine} + H_2O \longrightarrow L\text{- asparate} + NH_3$$

Asparagine reduction is also relevant to the frozen French fry industry where high frying temperatures facilitate asparagine's reaction with reducing sugars and where levels of asparagine can range considerably between potato varieties (from 1 to 58 mmol kg^{-1} of fresh weight) [80]. Pretreatments with asparaginase have led to reductions in acrylamide exposure by as much as 92% in crispbread and up to 85% in French fries [81].

8.4. *Meat and Fish Industries*

Exogenous enzymes are utilized for a myriad of reasons in the meat and fish industries [82]. Even prior to slaughter, enzymes can influence outcomes in animal food systems as manipulation of feed properties affects how muscles are converted into meat. Two examples are xylanases and phytase. Xylanases (e.g., EC 3.2.1.8) break down arabinoxylans in cereals so that the reaction products can be digested by the animal, or the viscosity of the digesta originating from the animal's feed is sufficiently lowered by xylanase action that nutrients can be more readily absorbed [83]. In this way, the feed conversion efficiency (mass of animal created from a unit mass of feed) is increased. Phytase (EC 3.1.3.26) acts on phytic acid to liberate phosphorus, so that this vital mineral is available to build bone strength in the growing animal. By enzymatically liberating phosphorus in situ, there is not the need to add as much supplemental phosphorus to the animal's ration [59]. Since some phosphorus is always excreted, the phosphorus-liberating capacity afforded by phytase reduces the environmental load of the animal operation by limiting phosphorus inputs into the system [84]. One example from the meat industry proper is the use of proteases to upgrade the value of processed animal by-products. Tenderness is a primary textural quality attribute for meat products. The infusion or injection of proteases (e.g., EC 3.4.22.32) into cuts of meat that are naturally tougher allows the connective tissues in the meat to break down and so the meat products are tenderized [82]. Upgrading the value of by-products in the fish industry is a further example. In this case, catalase (EC 1.11.1.6) and glucose oxidase are added in addition to protease. The catalase and glucose oxidase eliminate oxidizing agents (including free oxygen) in the system, a critical outcome for good fish product quality because even 100 parts per billions of oxidized fatty acids give rise to off-flavors [85].

8.5. *Starch Industries*

Starch, after lignucellulosic polymers, is the planet's most widely utilized renewable biopolymer. In the food industry, starch is the base material for many food ingredients, and starch (or its derivatives) is also found in

multiple industrial and pharmaceutical products. Enzymes are broadly employed in starch conversion processes [86]. Their first use is to liberate starch granules from the proteinaceous matrix in grain endosperms in which they are embedded. In maize and wheat endosperm (in which there may be as much as 75% starch granules), proteases substantially reduce the time required to soften the matrix during steeping. As a result of enzymatic weakening, the denser starch granules can be more easily separated out from a lower viscosity matrix at very high purity (>99%) by centrifugal forces. Starch granules can then be sold after drying as corn (maize) or wheat starch, or they can be used in subsequent processing operations. Where protein yield is as important as starch granule yield, for example, in the production of vital gluten from wheat, lipases can be useful process aids [87]. The rendering of starch granules into a variety of products typically requires judicious enzyme use to attain a desired profile of carbohydrate molecules in the finished product [86]. These molecules range from monosaccharides (DP = 1) to oligosaccharides (DP = 2 to ~17) to dextrins (DP = <31), where the latter may be linear or branched glucose polymers. A variety of EC 3.2.1 enzymes (glycosylases) are used to produce glucose syrups. For high-fructose syrups, enzymatic conversion systems are immobilized [67, 86]. In fact, the largest commercial application of immobilized enzyme technology uses glucose isomerase (EC 5.3.1.5) for this purpose [67].

Control of process time, temperature, cation concentration and pH define the fructose content of the syrup (up to 90% with additional processing). A schematic of enzyme additions in the initial process steps in a maize (corn) wet-milling plant is shown in (Fig. 6). An alternative to production of sweetened carbohydrates is enzymatic conversion of starch hydrolyzates to create cyclodextrins and prebiotic molecules. In the former, cyclodextrin glycosyl transferase (EC 2.4.1.19) creates ring-structured oligomers comprised of six to eight α-d-glucopyranosyl units. The ring structure can accommodate hydrophobic molecules, with the hydrogen bonding capacity of the outer part of the ring conferring aqueous solubility [59, 88].

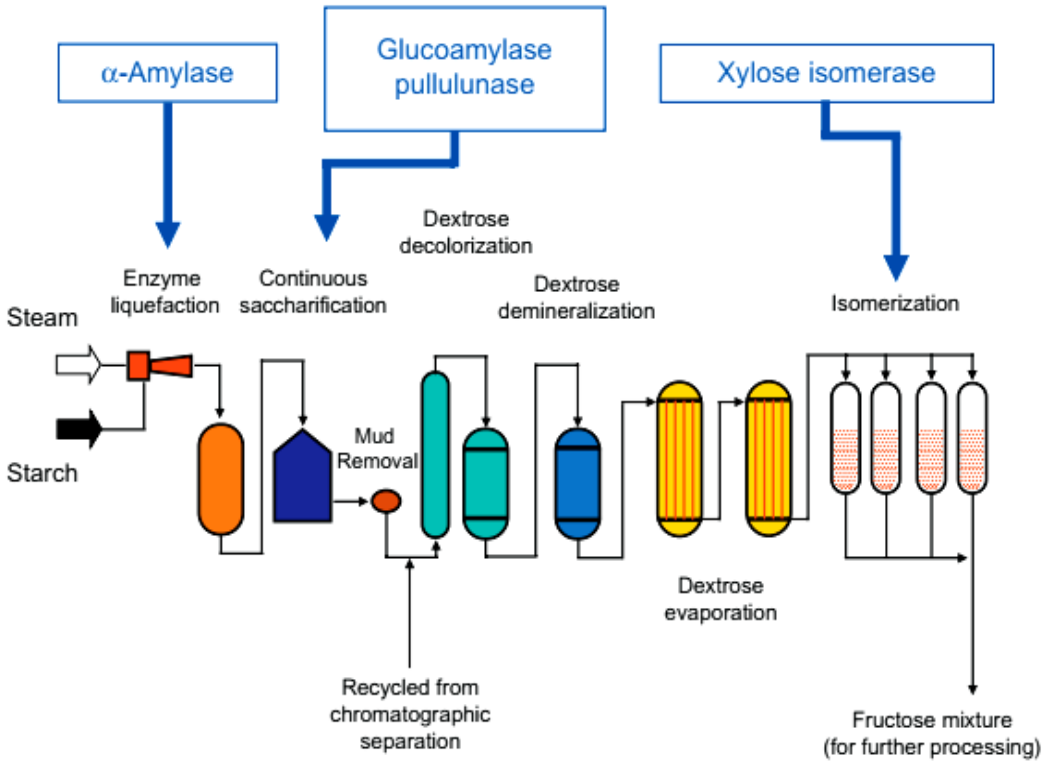

Fig. 6. Enzyme use in the initial operations of a maize wet milling plant targeting carbohydrate conversions [59].

Pharmaceutical as well as food applications are the target market. Prebiotic oligosaccharides created from starch using immobilized enzyme technologies with glycosidases (EC 3.2) or glycosyl transferases (EC 2.4), or fructooligosaccharides from sucrose using β-fructosyltransferases (e.g., EC 2.4.1.99) are indigestible in the small intestine, and so qualify as fiber. Commensal bacteria in the colon can ferment the oligosaccharides to generate short-chain fatty acids that maintain gut health [59].

IX. Conclusion

There are many products that are derived from the process of fermentation and the use of enzymes, alcohol is one product produced by enzymes and fermentation. The process of brewing and wine making produces alcohol. Other products include, cheese, yoghurt and bread. The microorganisms and enzymes cause the release of carbon dioxide and lactic acid. Fermentation changes the characteristics of the food by the action of the enzymes produced by bacteria, mould and yeasts, which can occur in aerobic or anaerobic conditions. Fermentation can yield acetic acid, lactate, ethanol and other simple products.

References

[1] Robinson, P.K., *Enzymes: principles and biotechnological applications.* Essays in biochemistry, 2015. **59**: p. 1-41.
[2] Schäfer, T., *Discovering new industrial enzymes for food applications*, in *Novel Enzyme Technology for Food Applications*. 2007, Elsevier. p. 3-15.
[3] Fernandes, P., *Enzymes in food processing: a condensed overview on strategies for better biocatalysts.* Enzyme research, 2010.
[4] Underkofler, L., R. Barton, and S. Rennert, *Production of microbial enzymes and their applications.* Applied microbiology, 1958. **6**(3): p. 212.
[5] Campbell-Platt, G., *Fermented foods-a world perspective.* Food Research International, 1994. **27**(3): p. 253-257.
[6] Demirci, A., G. Izmirlioglu, and D. Ercan, *Fermentation and enzyme technologies in food processing.* Food processing: principles and applications. 2nd ed. New York: Wiley, 2014: p. 107-36.
[7] Johannes, T.W. and H. Zhao, *Directed evolution of enzymes and biosynthetic pathways.* Current opinion in microbiology, 2006. **9**(3): p. 261-267.
[8] Nigam, P., *Microbial enzymes with special characteristics for biotechnological applications.* Biomolecules, 2013. **3**(3): p. 597-611.
[9] Vittaladevaram, V., *Fermentative Production of Microbial Enzymes and their Applications: Present status and future prospects.* Journal of Applied Biology & Biotechnology Vol, 2017. **5**(04): p. 090-094.
[10] Seo, Y.B., et al., *Agarose hydrolysis by two-stage enzymatic process and bioethanol production from the hydrolysate.* Process Biochemistry, 2016. **51**(6): p. 759-764.
[11] Rodríguez, V., J.A. Asenjo, and B.A. Andrews, *Design and implementation of a high yield production system for recombinant expression of peptides.* Microbial cell factories, 2014. **13**(1): p. 65.
[12] Singh, R., et al., *Microbial enzymes: industrial progress in 21st century.* 3 Biotech, 2016. **6**(2): p. 174.
[13] Adrio, J. and A. Demain, *Microbial enzymes: tools for biotechnological processes.* Biomolecules, 2014. **4**(1): p. 117-139.
[14] Demain, A.L. and P. Vaishnav, *Production of recombinant proteins by microbes and higher organisms.* Biotechnology advances, 2009. **27**(3): p. 297-306.
[15] Bueno, M.M., R.C.S. Thys, and R.C. Rodrigues, *Microbial enzymes as substitutes of chemical additives in baking wheat flour-Part II: combined effects of nine enzymes on dough rheology.* Food and bioprocess technology, 2016. **9**(9): p. 1598-1611.
[16] Liu, Y., et al., *A novel approach for improving the yield of Bacillus subtilis transglutaminase in heterologous strains.* Journal of industrial microbiology & biotechnology, 2014. **41**(8): p. 1227-1235.
[17] Garg, G., et al., *Microbial pectinases: an ecofriendly tool of nature for industries.* 3 Biotech, 2016. **6**(1): p. 47.
[18] Aguilar-Toalá, J., et al., *Assessment of multifunctional activity of bioactive peptides derived from fermented milk by specific Lactobacillus plantarum strains.* Journal of dairy science, 2017. **100**(1): p. 65-75.
[19] Agyei, D., et al., *Bioprocess challenges to the isolation and purification of bioactive peptides.* Food and Bioproducts Processing, 2016. **98**: p. 244-256.
[20] Zhang, Q., Y. Han, and H. Xiao, *Microbial α-amylase: A biomolecular overview.* Process Biochemistry, 2017. **53**: p. 88-101.
[21] Pandey, A., et al., *Solid state fermentation for the production of industrial enzymes.* Current science, 1999: p. 149-162.
[22] Sabu, A., *Sources, properties and applications of microbial therapeutic enzymes.* 2003.
[23] Singh, P. and S. Kumar, *Microbial Enzyme in Food Biotechnology*, in *Enzymes in Food Biotechnology*. 2019, Elsevier. p. 19-28.
[24] Qureshi, M., et al., *Enzymes used in dairy industries.* Int J Appl Res, 2015. **1**(10): p. 523-527.
[25] Kaur, H. and P.K. Gill, *Microbial Enzymes in Food and Beverages Processing*, in *Engineering Tools in the Beverage Industry*. 2019, Elsevier. p. 255-282.
[26] Headon, D. and G. Walsh, *The industrial production of enzymes.* Biotechnology advances, 1994. **12**(4): p. 635-646.
[27] Renge, V., S. Khedkar, and N.R. Nandurkar, *Enzyme synthesis by fermentation method: a review.* Sci Rev Chem Comm, 2012. **2**(4): p. 585e90.
[28] Subramaniyam, R. and R. Vimala, *Solid state and submerged fermentation for the production of bioactive substances: a comparative study.* Int J Sci Nat, 2012. **3**(3): p. 480-486.
[29] Suganthi, R., et al., *Amylase production by Aspergillus niger under solid state fermentation using agroindustrial wastes.* International Journal of Engineering Science and Technology, 2011. **3**(2): p. 1756-1763.
[30] Dewdney, P., *Enzymes in food processing.* Nutrition & Food Science, 1973. **73**(4): p. 20-22.
[31] Ziegler, P., *CerealBeta-Amylases.* Journal of Cereal Science, 1999. **29**(3): p. 195-204.
[32] Mehaia, M. and M. Cheryan, *Production of lactic acid from sweet whey permeate concentrates.* Process biochemistry, 1987.

[33] Gekas, V. and M. Lopez-Leiva, *Hydrolysis of lactose: A literature review.* Process biochemistry, 1985. **20**(1): p. 2-12.
[34] Law, J. and A. Haandrikman, *Proteolytic enzymes of lactic acid bacteria.* International Dairy Journal, 1997. **7**(1): p. 1-11.
[35] Priya, V. and V. Sashi, *Pectinase enzyme producing Microorganisms 1*. 2014.
[36] Svendsen, A., *Lipase protein engineering.* Biochimica et Biophysica Acta (BBA)/Protein Structure and Molecular Enzymology, 2000. **1543**(2): p. 223-238.
[37] Fox, P.F., et al., *Cheese: Chemistry, Physics and Microbiology, Volume 1: General Aspects*. 2004: Elsevier.
[38] Imran, M., et al., *Production and industrial applications of laccase enzyme.* Journal of Cell & Molecular Biology, 2012. **10**(1).
[39] Minussi, R.C., G.M. Pastore, and N. Duran, *Potential applications of laccase in the food industry.* Trends in Food Science & Technology, 2002. **13**(6-7): p. 205-216.
[40] Wovcha, M.G., D.L. Steuerwald, and K.E. Brooks, *Amplification of D-xylose and D-glucose isomerase activities in Escherichia coli by gene cloning.* Appl. Environ. Microbiol., 1983. **45**(4): p. 1402-1404.
[41] Oshima, T., *Properties of heat stable enzymes of extreme thermophiles*, in *Enzyme engineering*. 1978, Springer. p. 41-46.
[42] Coelho, S.L.d.A., et al., *A new alkalophilic isolate of Bacillus as a producer of cyclodextrin glycosyltransferase using cassava flour.* brazilian journal of microbiology, 2016. **47**(1): p. 120-128.
[43] Mori, S., *Studies on cyclodextrin glucanotransferase from Brevibacterium sp. No. 9605.* Journal of Applied Glycoscience, 1999. **46**(1): p. 87-95.
[44] Szerman, N., et al., *Cyclodextrin production by cyclodextrin glycosyltransferase from Bacillus circulans DF 9R.* Bioresource technology, 2007. **98**(15): p. 2886-2891.
[45] Tachibana, Y., et al., *Purification and characterization of an extremely thermostable cyclomaltodextrin glucanotransferase from a newly isolated hyperthermophilic archaeon, a Thermococcus sp.* Appl. Environ. Microbiol., 1999. **65**(5): p. 1991-1997.
[46] Fiedurek, J. and A. Gromada, *Production of catalase and glucose oxidase by Aspergillus niger using unconventional oxygenation of culture.* Journal of applied microbiology, 2000. **89**(1): p. 85-89.
[47] MUIR, D.D., *The shelf- life of dairy products: 1. Factors influencing raw milk and fresh products.* International Journal of Dairy Technology, 1996. **49**(1): p. 24-32.
[48] Muller, D., *Detection of glucose oxidase from Aspergillus niger.* Biochem. Z, 1928. **199**: p. 136-170.
[49] Khurshid, S., et al., *Optimization of glucose oxidase production by Aspergillus niger.* African Journal of Biotechnology, 2011. **10**(9): p. 1674-1678.
[50] CHANYONGVORAKUL, Y., et al., *Physical properties of soy bean and broad bean 11S globulin gels formed by transglutaminase reaction.* Journal of food science, 1995. **60**(3): p. 483-488.
[51] Christensen, B.M., et al., *Localization of potential transglutaminase cross-linking sites in bovine caseins.* Journal of Agricultural and Food Chemistry, 1996. **44**(7): p. 1943-1947.
[52] Kuraishi, C., et al., *Production of restructured meat using microbial transglutaminase without salt or cooking.* Journal of Food Science, 1997. **62**(3): p. 488-490.
[53] TSAI, G.J., S.M. LIN, and S.T. JIANG, *Transglutaminase from Streptoverticillium ladakanum and application to minced fish product.* Journal of food science, 1996. **61**(6): p. 1234-1238.
[54] Jiang, S.T., et al., *Microbial transglutaminase affects gel properties of golden threadfin - bream and pollack surimi.* Journal of Food Science, 2000. **65**(4): p. 694-699.
[55] Dickinson, E., *Enzymic crosslinking as a tool for food colloid rheology control and interfacial stabilization.* Trends in Food Science & Technology, 1997. **8**(10): p. 334-339.
[56] Dziezak, J., *ENZYMES-CATALYSTS FOR FOOD PROCESSES.* Food technology, 1991. **45**(1): p. 78-&.
[57] James, J., B.K. Simpson, and M.R. Marshall, *Application of enzymes in food processing.* Critical Reviews in Food Science & Nutrition, 1996. **36**(5): p. 437-463.
[58] Simpson, B. and N. Haard, *Cold-adapted enzymes from fish.* 1987.
[59] Scanlon, M., A. Henrich, and J. Whitaker, *Factors affecting enzyme activity in food processing*, in *Proteins in Food Processing*. 2018, Elsevier. p. 337-365.
[60] Kilcawley, K., et al., *Evaluation of commercial enzyme systems to accelerate Cheddar cheese ripening.* International Dairy Journal, 2012. **26**(1): p. 50-57.
[61] Allegrini, A., et al., *Characterization of CS lyase from Lactobacillus delbrueckii subsp. bulgaricus ATCC BAA-365 and its potential role in food flavour applications.* The Journal of Biochemistry, 2017. **161**(4): p. 349-360.
[62] Korhonen, H. and A. Pihlanto, *Food-derived bioactive peptides-opportunities for designing future foods.* Current pharmaceutical design, 2003. **9**(16): p. 1297-1308.
[63] Smithers, G.W., *Whey and whey proteins-from 'gutter-to-gold'.* International Dairy Journal, 2008. **18**(7): p. 695-704.
[64] Mohanty, D., et al., *Milk derived bioactive peptides and their impact on human health-A review.* Saudi journal of biological sciences, 2016. **23**(5): p. 577-583.
[65] Ansari, S.A. and Q. Husain, *Lactose hydrolysis from milk/whey in batch and continuous processes by concanavalin A-Celite 545 immobilized Aspergillus oryzae β galactosidase.* Food and Bioproducts processing, 2012. **90**(2): p. 351-359.
[66] Aehle, W., *Enzymes in industry: production and applications*. 2007: John Wiley & Sons.
[67] DiCosimo, R., et al., *Industrial use of immobilized enzymes.* Chemical Society Reviews, 2013. **42**(15): p. 6437-6474.
[68] Bayindirli, A., *Enzymes in fruit and vegetable processing: chemistry and engineering applications*. 2010: CRC Press.
[69] Hayes, D.G., *Enzyme- catalyzed modification of oilseed materials to produce eco- friendly products.* Journal of the American Oil Chemists' Society, 2004. **81**(12): p. 1077-1103.
[70] Borrelli, G. and D. Trono, *Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications.* International journal of molecular sciences, 2015. **16**(9): p. 20774-20840.
[71] Phuah, E.-T., et al., *Review on the current state of diacylglycerol production using enzymatic approach.* Food and Bioprocess Technology, 2015. **8**(6): p. 1169-1186.
[72] Kornbrust, B., T. Forman, and I. Matveeva, *Applications of enzymes in breadmaking*, in *Breadmaking*. 2012, Elsevier. p. 470-498.
[73] Campbell, G.M. and E. Mougeot, *Creation and characterisation of aerated food products.* Trends in food science & technology, 1999. **10**(9): p. 283-296.
[74] Scanlon, M. and M. Zghal, *Bread properties and crumb structure.* Food Research International, 2001. **34**(10): p. 841-864.
[75] Sroan, B.S. and F. MacRitchie, *Mechanism of gas cell stability in breadmaking*, in *Bubbles in Food 2*. 2008, Elsevier. p. 299-306.
[76] Strubbe, L.G., et al., *Method for preparing a dough comprising addition of penicillium glucose oxidase*. 2017, Google Patents.
[77] Gerits, L.R., et al., *Lipases and their functionality in the production of wheat- based food systems.* Comprehensive Reviews in Food Science and Food Safety, 2014. **13**(5): p. 978-989.
[78] SANCO, D., *Summary Record of the Standing Committee on the Food Chain and Animal Health held in Brussels on 25 September 2008.* SANCO–D1 (2008) D/411968, 2008.

[79] Mottram, D.S., B.L. Wedzicha, and A.T. Dodson, *Food chemistry: acrylamide is formed in the Maillard reaction.* Nature, 2002. **419**(6906): p. 448.
[80] Vivanti, V., E. Finotti, and M. Friedman, *Level of acrylamide precursors asparagine, fructose, glucose, and sucrose in potatoes sold at retail in Italy and in the United States.* Journal of food science, 2006. **71**(2): p. C81-C85.
[81] Hendriksen, H.V., et al., *Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from Aspergillus oryzae.* Journal of agricultural and food chemistry, 2009. **57**(10): p. 4168-4176.
[82] Chandrasekaran, M., *Enzymes in food and beverage processing*. 2015: CRC Press.
[83] Bedford, M.a. and H. Schulze, *Exogenous enzymes for pigs and poultry.* Nutrition research reviews, 1998. **11**(1): p. 91-114.
[84] Jarvie, H.P., et al., *The pivotal role of phosphorus in a resilient water–energy–food security nexus.* Journal of environmental quality, 2015. **44**(4): p. 1049-1062.
[85] Børresen, T., *Improving seafood products for the consumer*. 2008: Elsevier.
[86] Hobbs, L., *Sweeteners from starch: production, properties and uses*, in *Starch*. 2009, Elsevier. p. 797-832.
[87] Melis, S., et al., *Lipases as processing aids in the separation of wheat flour into gluten and starch: Impact on the lipid population, gluten agglomeration, and yield.* Journal of agricultural and food chemistry, 2017. **65**(9): p. 1932-1940.
[88] Hedges, A., *Cyclodextrins: properties and applications*, in *Starch*. 2009, Elsevier. p. 833-851.

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