International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(2): 1525-1529

Received: 18-01-2019
Accepted: 19-02-2019

Recent trends of enzymes in food industry: A review

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Abstract
Enzymes as industrial biocatalysts offer numerous advantages over traditional chemical processes with respect to sustainability and process efficiency. Enzymes offer potential for many exciting applications for the improvement of foods. There is still, however, a long way to go in realizing this potential. Economic factors such as achievement of optimum yields and efficient recovery of desired protein are the main deterrents in the use of enzymes. Changing values in society with respect to recombinant DNA and protein engineering technologies and the growing need to explore all alternative food sources may in time make enzyme applications more attractive to the food industry. Research is continuing on the commercially viable enzymes in use today to improve various properties such as thermo stabilities, specificities, and catalytic efficiencies. New and unique enzymes continue to be developed for use in enzymatic reactions to produce food ingredients by hydrolysis, synthesis, or biocatalysis. An aggressive approach is needed to open new opportunities for enzyme applications that can benefit the food industry.

Keywords: enzymes, food industry, biocatalysts, application

1. Introduction

Enzymes are proteins that act as catalysts in all living organisms - microorganisms, plants, animals, and humans. Catalysts are compounds that increase the rate of chemical reactions in biological systems. Very small quantities of enzymes can increase the rate of reactions up to ten million times. Enzymes operate within a narrow set of conditions, such as temperature and pH (acidity), and are subject to inhibition by various means. Enzymes are classified by the type of reaction they catalyse and the substance (called a substrate) they act upon. It is customary to attach the suffix "ase" to the name of the principle substrate upon which the enzyme acts. For example, lactose is acted upon by lactase, proteins by proteases, and lipids by lipases.

Food fermentation have been done since ancient times and fermentation processes are still applied in the preparation of many of the food items. These are important point about enzymes used food processing industry: [1] Microbial enzymes play a major role in food industries because they are more stable than plant and animal enzymes. They can be produced through fermentation techniques in a cost-effective manner with less time and space requirement, and because of their high consistency, process modification and optimization can be done very easily [2] Many of these enzymes find numerous applications in various industrial sectors, e.g. amylolytic enzymes find applications in food, detergent, paper and textile industries [3]. They are used for the production of glucose syrups, crystalline glucose, high fructose corn syrups, maltose syrups, etc. In detergent industry, they are used as additives to remove starch-based stains. In paper industry, they are used for the reduction of starch viscosity for appropriate coating of paper [4]. Similarly, enzymes like proteases, lipases or xylanases have wide applications in food sectors. The following sections give detailed and updated information about various enzymes in food industry (Figure 1).

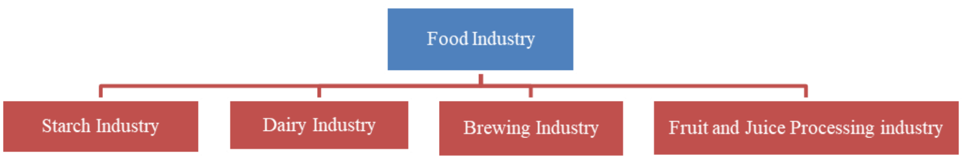

Fig 1: Different sectors of food industry

2. History of Enzyme Use in Food Production

Enzymes extracted from edible plants and the tissues of food animals, as well as those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in food manufacturing. Rennet is an example of a natural enzyme mixture from the stomach of calves or other domestic animals that has been used in cheese making for centuries. Rennet contains a protease enzyme that coagulates milk, causing it to separate into solids (curds) and liquids (whey). Alternatively, for centuries enzymes produced by yeast have been used to ferment grape juice in order to make wine (Table 1-2).

Table 1: Early Discovery of Enzymes

Year	Enzyme	Scientist
1831	Ptyalin (amylase)	Leuchs
1833	Diastase (amylase)	Payen and Persoz
1836	Pepsin activity	Schwann
1837	Emulsin activity	Leibig and Wholer
1846	Invertase activity	Dubonfant
1856	Trypsin activity	Corvisart
1897	Enzymes converting glucose to ethanol	Buchner

Neidleman, (1991)

Table 2: Early Discovery of Enzymes

Year	Early Enzyme	Industry Inventor	Title
1894	Amylases	J. Takamine	Process of Making Diastatic
1906	Amylases	J. Takamine	Enzyme
1911	Malt protease Proteases Pepsin Papain Bromelin	J. Takamine L. Wallerstein S. Franknel I. Pollak A. Boidin	Diastatic Substance and Method of Making Same Enzyme Beer and Method of Preparing Beer
1915	Yeast protease	H. S. Paine and	Preparation of Use in Brewing
1917	Amylases	J. Hamilton	Method of Treating Beer or Ale
1922	Amylases	J. Takamine	Method of Treating Beer or Ale
1923	Amylases Invertase Amylases, protease, lipase	M. Wailerstein	Method of Treating Beer or Ale Method of Treating Beer or Ale Manufacture of Diastase Diastase Preparation and Method of Making Same
1932	Amylases, papain	R.Douglas	Malt Extract and Method of Making Same
1933	Amylases Invertase	L. Wallerstein	Process for Preparing Fondant or Chocolate Soft Cream Center
1937	Proteases	L. Wallerstein	Enzymatic Substance and Process of Making Same Method of Making Chocolate Syrups Process of Preparing Pectin Invertase Preparation and Method of Making Same Process of Chillproofing and Stabilizing Beers and Ales

Adapted from Neidleman, (1991)

3. Modern Production of Food Enzymes

In the twentieth century, enzymes began to be isolated from living cells, which led to their large-scale commercial production and wider application in the food industry. Today, microorganisms are the most important source of commercial enzymes. Although microorganisms do not contain the same enzymes as plants or animals, a microorganism can usually be found that produces a related enzyme that will catalyse the desired reaction (Table 3-4). Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques. Some enzymes are:

4. Amylase

Alpha Amylases are extracellular enzymes that catalyze the hydrolysis of alpha 1, 4-glycosidic linkages in starch to release glucose and are important as industrial starch conversion eznymes. The optimal pH of alpha amylases ranges from 2 to 12 and they are thermostable. The application of these enzymes has been established in starch liquefaction, paper, food, sugar and pharmaceutical industries. In the food industry amylolytic enzymes have a large scale of applications, such as the production of glucose syrups, high fructose corn syrups, maltose syrup, reduction of viscosity of sugar syrups, reduction of turbidity to produce clarified fruit juice for longer shelf-life, solubilisation and saccharification of starch and delay the staling of baked products.

5. Lipase

Lipases are ubiquitous enzymes also called triacylglycerol acylhydrolases. Lipases catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids and has numerous activities. They are important flavouring agents and prolongs shelf life. A lipase to water –soluble enzyme that catalyzes the hydrolysis of ester bonds in water-insoluble, lipid substrates. Lipases (triacylglycerol acylhydrolases) are produced by microorganism in Individual or together with esterase. Micro-organisms that produce lipases are *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Bacillus subtilis*. Lipase is used as bio-catalyst to produce free fatty acid, glycerol and various esters, part of glycerides and fat that is modified or esterified from cheap substrate i.e. palm oil. Those products are extensively used in pharmacy, chemical and food industry. Various animal or microbial lipases gave pronounced cheese flavour, low bitterness and strong rancidity, while lipases in combination with proteinases and/or peptidases give good cheese flavour with low levels of bitterness.

6. Glucose Oxidase

The enzyme glucose oxidase was discovered in *Aspergillus niger* and *Penicillium glaucum* by Muller who proclaimed that the enzyme catalyzes the breakdown of glucose to gluconic acid in the presence of dissolved oxygen[1] and the most potent source of glucose oxidase enzyme are fungi. The largely used species for this enzyme is *Aspergillus niger* and their strains are capable producing very remarkable amount of glucose oxidase[2] It is used in the food industry to remove small amounts of oxygen from food products or glucose from diabetic drinks. It also imparts color flavour and texture also shelf life to a number of food products [2].

7. Proteases

Proteases are the most important type of enzymes in food processing and they hydrolyze the peptidic linkages in proteins. The application proteases are very diverse, including food science and technology, pharmaceutical industries, and detergent manufacturing. They are widely distributed in nature and plays important roles in biological processes [3]. The number of industrially used proteases of plant origin is small (Aehle., 2004) [1] and some cysteine proteases (CPs) such as papain, bromelain, and ficin are still being used in a variety processes. The optimum temperature and pH for enzyme activity were 70°C and 4, respectively. The protease also showed an excellent thermal stability at 60°C for 30 min [3]. Bromelain increases the tenderness and degraded collagen more than the contractile proteins, while ficin gives the most balanced degradation of both myofibrillar and collagen proteins [4]. Papain and bromelain are also used to manufacture different sauces (Díaz *et al*. 1996) [11] and dry cured ham [5]. One of the main applications of proteases is for the production of cheese. Due to the shortage of traditional rennet (enzymes derived from the stomachs of calves, lambs, or goats), other coagulant proteases have been investigated as substitutes for animal rennet. Microbial rennet has two hydrolytic action on casein: the first coagulant activity is represented by specific proteolysis, or the ability to recognize specific amino acid in the chain, breaking the κ-casein specifically between the units Phe (105) and Met (106); the second refers to nonspecific proteolytic activity, which hydrolyzes the κ-casein between other units of amino acids, leading to a reduction in yield and poor flavor development in some types of cheese.

8. Invertase

Invertases are seen intracellularly as well as extracellularly. This enzyme is produced by the controlled submerged aerobic fermentation of a non-pathogenic, non-toxigenic strain of *Saccharomyces cerevisiae* and extracted after washing and autolysis [6]. The enzyme has wide range of commercial applications like, the production of confectionery with liquid contents inside, like in the case of some chewing gums. It also helps in formation of ethanol from cane molasses. However, invertase is used very limitedly because another enzyme, glucose isomerase, can be used to convert glucose to fructose less expense [6]. For health and taste reasons; its use in food industry requires highly purified invertase. It is also used in the preparation of chocolates, digestive aid tablets, infant food formulas etc. It is also used in the assimilation of fortified wines.

9. Xylanase

Xylanases catalyze the hydrolysis of xylans and are industrially produced by submerged liquid culture or on a solid substrate. Xylanases is used in bread-making, together with α-amylase, malting amylase, glucose oxidase, protease etc. The xylanases, like the other hemicellulases, break down the hemicellulose in wheat-flour, helping in the redistribution of water and leaving the dough softer and easier to knead also they delay crumb formation, allowing the dough to grow. In biscuit-making, xylanase is recommended for making cream crackers lighter, improving its texture, providing palatability and uniformity of the wafers etc [7]. Xylanases are used to hydrolyze arabinoxylans to lower oligosaccharides reducing the beer‘s viscosity and muddy appearance. Xylanases, in conjunction with cellulases, amylases and pectinases, lead to an improved yield of juice by means of liquefaction of fruit and vegetables; stabilization of the fruit pulp; increased recovery of essential oils, vitamins, mineral salts, edible dyes, pigments etc., reduction of viscosity, hydrolysis of substances that hinder the physical or chemical clearing of the juice. Xylanase, in combination with endoglucanase isolates gluten from the starch in the wheat flour. This enzyme is also used in coffee bean mucilage. The main desirable property for xylanases is its high stability and optimum activity at an acid pH.

10. Laccase

Laccase was first discovered in the sap of the Japanese lacquer tree. The metal containing oxidase property of laccase was found out in 1985. Laccases are seen in various fungi thus forming the most important group of multicopper oxidases (MCOs) with respect to number and extent of characterization. Laccases display other functions that are the foundation of several industrial applications like degrading a range of xenobiotics (industrial colored waste waters for instance). They are almost ubiquitary enzymes as they have been isolated from plants, from some kinds of bacteria, and from insects too [9]. Laccase is used to improve the quality of drinks and for the stabilization of products containing plant oils. In food industry, laccase is involved in wine stabilization. This enzyme is also responsible for discoloration, haze, and flavor changes. In the bread-making process laccases affix bread and or dough-enhancement additives to the bread dough, these results in improved freshness of the bread texture, flavor etc. [10]. Laccase enzyme is added in the baking process which results in the oxidizing effect, and further improvement in the strength in dough and or baked products. Laccase imparts many characteristics to the baked products including an improved crumb structure, increased softness and volume. A flour of poor quality can be also used in this process using laccase enzyme.

11. Catalase

Catalase helps in the breakdown of hydrogen peroxide to water and oxygen along with the formation of bubbles the source for industrial purpose is mostly fruit or vegetable source. They have high content of this enzyme. Earlier, catalase was isolated from liver also. Since this method is no longer feasible. Industries isolate them from bacteria and immobilized cultures. The optimum pH and temperature also varies from species to species that are used as the source. This enzyme was first identified by Louis Jacques Thenard in the 1900s and later catalase it was crystallized by Sumner and Dounce in 1937. In 1938, Sumner worked out the weight of this enzyme as well. This enzyme is used to remove hydrogen peroxide from milk prior to cheese production [11]. Another use is in food wrappers to prevent food from oxidizing. Catalase is also used in the removal of glucose from egg white before drying for the use in baking industry. It controls the perishability of the food [12].

12. Lipoxygenase

Lipoxygenases (linoleate: oxygen, oxidoreductases, LOXs) are found in a number of plants animals and fungi. They are a large group of monomers with iron cofactor containing dioxygenases that catalyses the breakdown of PUFA (Gardner (1991) [14]. Theorell *et al*. in 1947[13] crystallized and characterized lipoxygenase. Plant lipoxygenases are of significant importance to the food industry, due to the generation of the 9832lavor and aroma in many plant products [15]. Lipoxygenases play a very huge role in baking industry by serving the function of bleaching agents. Besides this they increase the mixing tolerance and improve the texture of the dough [16].

13. Asparaginase

L- Asparaginase is a hydrolytic enzyme that breakdowns of L-asparagine to L-aspartic acid with the release of ammonia (Astrid. L, 1999) [3]. It is produced by various organisms like Serratia, Actinomycetes (Gulati *et al*, 1997) [15]. It is used to reduce the circulatory L-asparagine level, thereby stopping tumor cell growth in the body .Acrylamide is formed from L-asparagine and reducing sugars in carbohydrate-containing foods that are heated above 120 °C. Examples of such foods include bread and other baked goods, fried or baked potato products, and reaction flavors. Asparaginase will be added to food prior the heating step. When asparaginase is added before baking or frying the food, asparagine is converted into another common amino acid, aspartic acid and ammonia. As a result, asparagine cannot take part in the Maillard reaction (reaction that is seen as a part of browning of bread), and therefore the formation of acrylamide is significantly reduced (Kornbrust, *et al* B.A. 2010) [20].

14. Pectinase

Pectinases were the first domestically used enzymes and their commercial application was first studied in for the preparation of wines and fruit juices during 1930s [17]. They are one of the most important enzymes in the industry especially in fruit juice industry since they help in obtaining well clarified and stable juices with higher yields (Dupaigne, 1974) [12]. With the addition of pectinases the viscosity of the fruit juiceis lowered, the press ability of the pulp is improved, the jelly structure breaksdown and the fruit juice is easily obtained with higher yields. Pectinases are now an integral part of fruit juice industries as well as having various biotechnological applications. Other uses of pectinases in fruit processing are the manufacture of better quality purees from a number of fruits. The enzymatic process makes the use of enzymes to soften skins and tissues. The pectinolytic enzymes are also used in canning of orange segments. Pectinases are also used in sugar extraction process from date fruits. Other important processes where pectic enzymes are utilized are: in the preparation of hydrolysed products of pectin in the refinement of vegetable fibres during starch manufacture, in the curing of coffee, in cocoa and tobacco etc.

15. Pullulanase

Pullulanase also known as α-dextrin 6-glucanohydrolase, pullulan 6-glucanohydrolase, limit dextrinase, and amylopectin 6-glucanohydrolase is derived from various microorganisms such as *Bacillus acidopullulyticus*, *Klebsiella planticola*, *Bacillus deramificans*, *Bacillus* sp. AN-7, *Bacillus cereus* FDTA-13, and *Geobacillus stearothermophilus* (Chaplin, 2002) [7, 17]. This enzyme is most active at pH ranging from pH 3 to pH 4 and has maximum temperature stability at 45 °C to 55 °C. The primary application of pullulanase is in starch saccharification and the most important industrial application of pullulanase is in the production of high-glucose (30% to 50% glucose; 30% to 40% maltose) or high-maltose (30% to 50% maltose; 6% to 10% glucose) syrups [18, 19]. In the saccharification process, pullulanase is normally used in combination with glucoamylase or β-amylase. It is well suited for numerous applications in food processing such as in the manufacturing of high-quality candy and ice cream [20]. Pullulanase has also been used to prepare high amylose starches as well as high fructose corn syrup.

16. Rennet

The use of rennet in cheese manufacture was among the earliest applications of exogenous enzymes in food processing, dating back to approximately 6000 BC. The use of rennet, as an exogenous enzyme, in cheese manufacture is perhaps the largest single application of enzymes in food processing. In recent years, proteinases have found additional applications in dairy technology, for example in accelerations of cheese ripening, modification of functional properties and preparation of dietic products. Animal rennet (bovine chymosin) is conventionally used as a milk-clotting agent in dairy industry for manufacture of quality cheeses with good flavour and texture Marty microorganisms are known to produce rennet like proteinases which can substitute the calf rennet. Microorganisms like *Rhizomucor pusillus, R. miehei*, *Endothia parasitica*, *Aspergillus oryzae* and *Irpex lactis* are used extensively for rennet production in cheese manufacturing [21].

Table 3: Marketed enzymes produced from gene technology used in different food industry

Principal enzyme activity	Application
Alpha-acetolactate decarboxylase	Brewing
Alpha-amylase	Baking, brewing, distilling, starch
Catalase	Mayonnaise
Chymosin	Cheese
Beta-glucanase	Brewing
Alpha glucanotransferase	Starch
Xylanase	Baking, starch
Pullulanase	Brewing, starch
Protease	Baking, brewing, diary, distilling, fish, meat, starch, vegetable
Phytase	Starch
Microbial rennet	Dairy
Maltogenic amylase	Baking, starch
Lipase	Fats, oils

Table 4: Some uses of enzymes in food production

Market	Enzyme	Purpose/function
Dairy	Rennet (protease)	Coagulant in cheese production
	Lactase	Hydrolysis of lactose to give lactose-free milk products
	Protease	Hydrolysis of whey proteins
	Catalases	Removal of Hydrogen peroxide
Brewing	Cellulases,	For liquefaction,
	betaglucanases,	clarification and
	alpha	to supplement
	amylases, proteases, maltogenic amylases	malt enzymes
Alcohol Production	Amyloglucosidase	Conversion of starch to sugar
Baking	Alphaamylases	Breakdown of starch, maltose production
	Amyloglycosi dases	Saccharification Maltogen
	amylase (Novamyl)	Delays process by which bread becomes stale
	Protease	Breakdown of proteins
	Pentosanase	Breakdown of pentosan, leading to reduced gluten production
	Glucose	Stability of
	oxidase	dough
Wine and fruit juice	Pectinase	Increase of yield and juice clarification
	Glucose	Oxygen removal
	oxidase	Betaglucanases
Meat Protein Starch	Protease	Meat tenderizing Papain Breakdown of various components

17. Conclusions

Nowadays, the enzymatic hydrolysis and enzyme-based processes are preferred to the chemical ones due to the environmentally friendly nature, efficient process control, high yield, low refining costs and process safety. In comparison with plant and animal enzymes, microbial enzymes can be produced very effectively by different fermentation techniques like solid-state and submerged fermentations. It is also easy to produce microbial enzymes on a large scale. The microbial enzymes can be easily modified through various molecular and biochemical approaches. Hyperproduction of microbial enzymes with high specific activity can be achieved by overexpression of their genes. Many of the enzymes of microbial origin are still unexplored and there are many opportunities for finding wider industrial application of microbial enzymes, especially in food sector.

18. References

1. Aehle W. Industrial enzymes: Enzymes in food applications, Enzymes in industry: Production and applications. Chichester: Wiley, 2004.
2. Antranikian G. Microbial degradation of starch‖, Microbial degradation of natural products‖ Weinheim, Germany: VC, 1992, 27-56.
3. Astrid L. Preparation of Lacto phenol Cotton Blue Slide Mounts‖. Community Eye Health. 1999; 12(30):24.
4. Silva BL. The Production and Characterization of a Milk-clotting Protease Produced in Submerged Fermentation by the Thermophilic Fungus Thermomucorindicae-seudaticae N31‖, Appl Biochem Biotechnol, 2014, 172.
5. Berka RM, Cherry JR. Enzyme biotechnology‖ Basic Biotechnology. Cambridge University Press, UK, 3rd edition, 2006, 477-498.
6. Bon EPS, Ferrara MA. Bioethanol production via enzymatic hydrolysis‖. The Role of Agricultural Biotechnologies for Production of Bioenergy in Developing Countries, 2007.
7. Chaplin M. Production of syrups containing maltose, 2002.
8. Chowdary GV, Ramesh MN, Prapulla SG. Enzymic synthesis of isoamyl isovalerate using immobilized lipase from *Rhizomucor miehei:* multivariate analysis‖, Process Biochem, 2001.
9. De Clerck J. The use of proteolytic enzymes for the stabilization of beer. Technology Q Master Brew Association American, 1969, 136-140.
10. Díaz O, Fernandéz M, Gracia de Fernando CD, de la Hoz L, Ordóñez JA. Effect of the addition of papain on the dry fermented sausage proteolysis. Journal of the Science of Food and Agriculture. 1996; 71(1):13-21.
11. Dupaigne. The aroma of bananas‖. Fruits. 1974; 30(12):783-789.
12. Effects of lipoxygenase in bread making and of polyphenol oxidase in fruit technology‖. Sciences des Aliments. 14:627-642.
13. Gardner HW, Recent investigations into thelipoxygenase pathway in plants, Biochimica Biophysica Acta. 1991; 1084(3):221-239.
14. Gulati R, Saxena RK, Gupta R. A rapid plate assay for screening L-asparaginase producing micro-organisms., Letters in Applied Microbiology. 1997; 24:23-26.
15. Heldman DR, Hartel RW. Principles of Food Processing, 1997.
16. Jin F, Toda K. Preparation of immobilized papain covalently bound on natural cellulose for treatment of beer‖. Biotechnology Letters. 1988; 10(3):221-223.
17. Jin F, Toda K. Preparation of immobilized papain covalently bound on natural cellulose for treatment of beer.‖ Biotechnology Letters. 1988; 10(3):221-223.
18. Kennedy JF, Pike VW. Papain, chymotrypsin and related proteins—a comparative study of their beer chill-proofing abilities and characteristics. Enzyme and Microbial Technology. 1981; 3(1):59-63.
19. Kornbrust BA, Stringer MA, Lange NK, Hendriksen HV. Asparaginase – an enzyme for acrylamide reduction in food products. Enzymes in Food Technology, 2nd Edition, 2010, 59-87.
20. Malik Muhammad Hashim. Ginger protease used as coagulant enhances the proteolysis and sensory quality of Peshawari cheese compared to calf rennet, Dairy Sci. & Technol. 2011; 91:431-440.
21. Monteiro CA, Levy RB. A new classification of foods based on the extent and purpose of their processing, Public Health Nutrition. 2010; 26(11):2039-2049.