

Enzymes used in Food Industry A Systematic Review

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ABSTRACT: This article overviews the enzymes used in food processing, which have been extensively studied worldwide for their isolation, purification and characterization of their specific properties to use in food processing. The special characteristics of enzymes are exploited for their commercial interest and industrial applications, which include: thermo-tolerance, thermophilic nature, tolerance to a varied range of pH, stability of enzyme activity over a range of temperature and PH. Such enzymes have proven their utility in food processing.

KEYWORDS: Proteases, Amylase, Cellulase, Lipase, Catalase, Glucose oxidase, Invertase, Laccase, Lipoxigenase, Pectinase, Xylanase, Asparaginase, Pullulanase

I. INTRODUCTION

Food processing can be defined as the practices and approaches followed in the food and beverage industry to transform raw food materials of plant and animal origin to make it fit for consumption.(Heldman&Hartel ,2007; Monteiro& Levy,2010). The methods practised to process one food material is different from that practised for another material. On the basis of degree of processing, the food materials we consume can be divided into minimally processed foods , processed food ingredients and highly processed foods (Ohlsson& Bengtsson,2002); Monteiro*et.al* ,2011). The food processing techniques are done with the support of a variety of chemical and biological agents. the so called biological agents are what we call enzymes today. The usage of biological agents in food processing dates back to 6000BC with the brewing of beer, bread making, cheese and wine making while the first known purposeful microbial oxidation dates from 2000 BC, with the production of vinegar (Vasic-Rackiet*et.al* , 2006; Schäfer *et.al* ,2002). Later in the 19th century, Christian Hansen reported the use of rennet (mixture of chymosin and pepsin) for cheese making moreover the production of bacterial amylases was also done. Even though enzyme usage was a part of the day to day life, no one was particularly sure about the chemistry behind all these reactions. The usage of the enzymes, invertase and pectinases were used to process foods from 1930s.the usage of invertase pioneered the immobilized enzymes in 1960s.Since then the large scale application of enzymes in food industry began also the trend for designing and implementing of processes and production of goods based on the enzyme usage has increased rapidly. They are currently the mostly established products in biotechnology industry. The profit in this particular industry rose from 1.3 billion dollars in 2002 was expected to become 7 billion dollars in the year 2013 (Leisola *et.al.*,2002); Bon and Ferrara,2007).This the particular scenario in the United States. Part of this market is dedicated to usage in food and feed industry and includes those used in dairy, beverages and brewing, dietary. supplements etc.(Berka& Cherry,2006);Ogawa & Shimizu,2002).The other usage of enzymes include those used in detergent, personal care, leather, textile ,pulp and paper industry etc.

II. COMMONLY USED ENZYMES IN INDUSTRIES

1.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 (Rao *et al.* 1998). The number of industrially used proteases of plant origin is small (Aehle2004) and some cysteine proteases (CPs) such as papain, bromelain, and ficin are still being used in a variety processes. The optimum

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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. (Maryam Beigomi et al. 2014). 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 (Sullivan and Calkins, 2010). Papain and bromelain are also used to manufacture different sauces (Díaz et al. 1996) and dry cured ham (Scannell et al. 2004). 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.

2. 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 enzymes. 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

3. 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.

4. 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 (Tamara, 2011). 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 (Henge, 1999).

5. 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 (Muller (1928) 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. (Shazia, 2008) 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 (Shazia, 2008)

6. INVERTASE

Invertases are seen intracellularly as well as extracellularly. This enzyme is produced by the controlled submerged aerobic fermentation of a non-pathogenic, non-toxic strain of *Saccharomyces cerevisiae* and extracted after washing and autolysis. (Uma et al., 2010).

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 at less expense (Uma, 2010). For health and taste reasons; its use in food industry requires highly purified invertase. It is

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also used in the preparation of chocolates, digestive aid tablets, infant food formulas etc. It is also used in the assimilation of fortified wines.

7.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 ubiquitous enzymes as they have been isolated from plants, from some kinds of bacteria, and from insects too (Saqib (2012)). 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 (Rosana et.al 2002). 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.

8.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)). Theorell et al. in 1947 crystallized and characterized lipoxygenase. Plant lipoxygenases are of significant importance to the food industry, due to the generation of the 9832 flavor and aroma in many plant products. (Theorell et.al (1947)). 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 (Nicolas & Potus, 1994)

9.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 (Oslen, 2000). 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). With the addition of pectinases the viscosity of the fruit juice is lowered, the press ability of the pulp is improved, the jelly structure breaks down 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.

10.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, malted 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 (Polizeli et.al, 2005). 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.

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11.ASPARAGINASE

L-asparaginase is a hydrolytic enzyme that breakdowns of L-asparagine to L-aspartic acid with the release of ammonia (Astrid. L, 1999).It is produced by various organisms like Serratia,Actinomycetes (Gulati et.al,1997). 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) .

12.PULLULANASE

Pullulanase also known as α -dextrin 6-gluconohydrolase, pullulan 6-gluconohydrolase, limit dextrinase, and amylopectin 6-gluconohydrolase is derived from various microorganisms such as *Bacillus acidopullulyticus*, *Klebsiellaplanticola*, *Bacillus deramificans*, thermophilic*Bacillus* sp. AN-7 , *Bacillus cereus* FDA-13, and *Geobacillusstearothermophilus* (Chaplin,2002 Teague & Brumm,2000). 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 (Van Der Maarel et.al ,2002;Gomes et.al (2003). 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 (Gomes et.al,2003). Pullulanase has also been used to prepare high-amylose starches as well as high fructose corn syrup.

Table3. Enzyme name their properties and source

ENZYME	PROPERTIES	SOURCE
PROTEASE	Acidic,Neutral,Alkaline, Thermophilic, Active in presence of inhibitory compounds	<i>Bacilli</i> ; <i>Pseudomonas</i> ; <i>Clostridium</i> ; <i>Rhizopus</i> ; <i>Penicillium</i> ; <i>Aspergillus</i>
AMYLASE	Thermotolerant, Thermostable, Alkali-resistant-Exo-, endo-, debranching,cyclodextrin- producing enzymes	<i>Bacillus sp.</i> ; <i>Geobacillus</i>
CATALASE	Acidic,neutral,alkaline Breaksdown hydrogen peroxide Optimum pH:4-11 Optimum temperature:Humans(37°C); Bacteria(upto 90°C)	All aerobic organisms
GLUCOSE OXIDASE	Catalyses the formation of gluconic acid	<i>Penicilliumnotatum</i> <i>Aspergillusniger</i>

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	Mol: weight :186000 Da Max catalytic activity pH:3-10	<i>Penicilliummagasakiense</i>
INVERTASE	Breaks down sucrose Optimum pH: 5-5.5 Highly stable groups	<i>Saccharomyces Cerevisiae</i> <i>Pseudozyma</i> sp.
LACCASE	Specific to a large number of substrates:mono and di phenols, Reduces the toxicity	Deuteromycetes, Ascomycetes Basidiomycetes,
LIPOXYGENEASE	Oxidizes PUFA Isoelectric point varies from species to species(5.7-6.4) Molecular weight 94-97 KDa Best activity at pH 7	Plants, fungi, animals
PECTINASE	Breaks down pectin of fruits Optimum pH between 3 and 6.5 Temperature for activation 55- 60°C after which degradation happens	<i>Acidic pectinase</i> <i>Aspergillusniger</i> CH4 <i>Penicilliumfrequentans</i> <i>Sclerotiumrolfsii</i> <i>Rhizoctoniasolani</i> <i>Alkaline pectinases</i> <i>Bacillus</i> s sp. RK9 <i>Bacillus</i> sp.NT-33.
XYLANASE	Extremophilic characteristics– Alkalophilic, Thermophilic&Thermostable	<i>Thermoactinomycesthalophilus</i> ; <i>Bacillus</i> sp.;; <i>Humicolainsolens</i> . <i>Bispora</i> (acidophilic fungus)
ASPARAGINASE	Breakdown acrylamide Prevents tumor in body Highly stable at high temperatures	<i>Serratiasps</i> <i>Actinomycetessps</i> <i>Aspergillusps</i>
PULLULANASE	Thermostable Production of high carbohydrate syrops Optimum pH 3-4 Optimum temperature 45-55°C	<i>Bacilli</i> <i>Geobacillus</i> <i>Klebsiella</i>

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LIPASE	Fat- splitting; Stereoselectivity; Racemic-Resolution activity; Solvents-resistant; Thermotolerant	Yeasts and Fungal strains- <i>Candida</i> sps., <i>Aspergillus</i> sps., <i>Penicillium</i> sps., <i>Rhizopus</i> , <i>Mucor</i>
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III. CONCLUSION

This review article is based on the enzymes that are commonly used in industries for a number of uses. They all thus play a crucial role in our lives directly or indirectly.

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