

5. Enzymes in Food Industry- Significance and Applications

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Abstract:

Enzymes have wide spread applications in food processing, because it can modify and improve the functional, nutritional and sensory properties of ingredients and products. It involves conversion of raw and perishable food materials into shelf stable and palatable food products. Enzymes are biocatalysts that speed up biochemical reactions in living organisms. They are unique processing aids in the food and beverage industry.

In food processing, enzymes are used for efficient utilization of raw materials, simplifying processing and for bringing about desirable quality attributes. Enzymes used in food processing are obtained from three primary sources, that are plants, animal tissues and microbes. Enzymes are extensively used for various food applications like brewing, baking, meat tenderization, milk coagulation, starch modification and also in coffee, tea and chocolate industries. Enzyme applications are environment friendly with lower energy consumption levels and yields biodegradable products. The potential of enzymes in efficient use of raw materials, improved product recovery and quality may lead to better applications of enzymes in the coming decades. Many of the enzymes are still unexplored and there are wider industrial applications for enzymes in food sector.

Keywords:

Enzyme, food processing, food applications

5.1 Introduction:

Enzymes are proteins that are produced by all living organisms. They speed up chemical reactions selectively as part of essential life processes such as digestion, respiration, metabolism and tissue maintenance. Enzymes find application in food, detergent, pharmaceutical and paper industries. 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. Food processing using biological agents is historically a well-established approach. Enzymes have wide spread applications in food processing, because it can modify and improve the functional, nutritional and sensory properties of ingredients and products.

It involves conversion of raw, perishable, edible food materials into shelf stable, palatable food products and beverages. Enzymes are biocatalysts that speed up biochemical reactions in living organisms. They are unique natural processing aids in the food and beverage industry.

5.2 Enzymes:

Enzymes are biological catalysts (also known as biocatalysts) that speed up biochemical reactions in living organisms (Robinson, 2015). The term enzyme is derived from the latin word meaning “in yeast”. Enzymes are proteins produced by living organisms to increase the rate of an immense and diverse set of chemical reactions required for life. In other words, they are highly specific biological catalysts. Enzymes are usually named according to the reaction they carry out. Typically, the suffix ‘ase’ is added to the name of the substrate (E.g. glucose-oxidase, an enzyme which oxidizes glucose) or the type of reaction (E.g. a polymerase or isomerase for a polymerization or isomerization reaction). The exceptions to this rule are some of the enzymes studies originally, such as pepsin, rennin and trypsin (Berg *et al.*, 2001).

5.3 Mechanism of Enzyme Activity:

Enzymes are protein molecules they speed up chemical reactions. An enzyme (E) molecule has a highly specific binding site or active site to which its substrate (S) bind to produce enzyme-substrate complex (ES). The reaction proceeds at the binding site to produce the products (P), which remain associated briefly with enzyme (enzyme-product complex). The product is then liberated and the molecule is then released in an active state to initiate another round of catalysis (Rabin, 1970). The affinity of binding site for the product is much lower than that for the substrate (Figure 5.1).

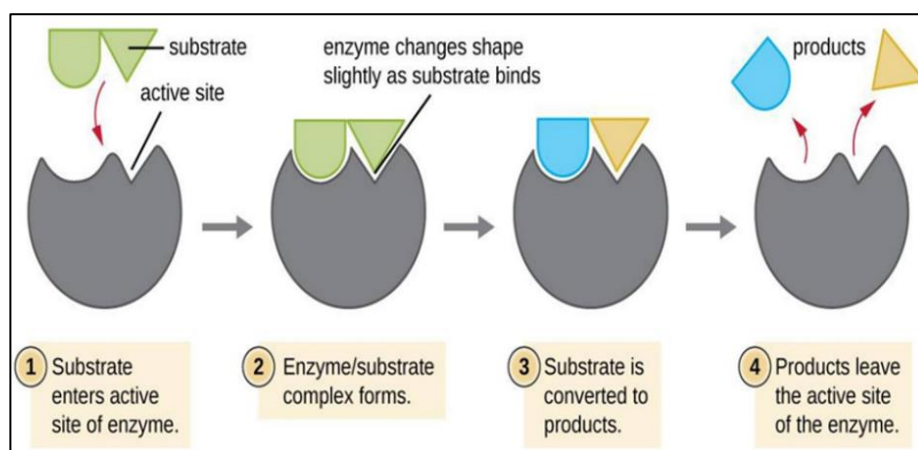


Figure 5.1: Mechanism of enzyme activity

5.4 Sources of Enzymes:

Enzymes have been used deliberately in food processing since ancient times to make a variety of food products, such as breads, fermented alcoholic beverages, fish sauces, cheeses and to produce several food ingredients. Enzymes have been traditionally produced by extraction and fermentation processes from plant and animal sources, as well as from a few cultivatable microorganisms. Industrial enzymes have traditionally been derived from plant, animal and microbial sources (Benjamin *et al.*, 2012) and described in Table 5.1.

Table 5.1: Sources of Industrial Food Enzymes

Sources	Enzymes
Plants	α -amylase, β -amylase, bromelain, β -glucanase, ficin, papain, chymopapain and lipoxxygenase
Animals	Trypsin, pepsin, chymotrypsin, catalase, pancreatic amylase and rennin (chymosin)
Microorganisms	α -amylase, β -amylase, glucose isomerase, pullulanase, cellulase, catalase, lactase, pectinases, pectolyase, invertase, raffinose, microbial lipases and proteases

5.5 Enzymes Used in Food Industries:

In food processing, enzymes are used for efficient utilization of raw materials, simplifying processing and also for bringing about desirable quality attributes. Enzymes are extensively used for various food applications like brewing, baking, meat tenderization, milk coagulation, starch modification and also in coffee, tea and chocolate industries. Enzymes have always been important to food technology because of their ability to act as catalysts, transforming raw materials into improved food products. The main values of enzymes are their substrate specificity (Salim *et al.*, 2017).

5.6 Dairy Industry:

Dairy enzymes are utilized for processing cheddar, yogurt, milk and milk products. The properties of these enzymes change broadly from coagulant, utilization in the making of cheese, bioprotective enzymes to improve shelf life aspects of dairy products processing. The utilization of enzymes in dairy technology and food technology is well known. Rennet (also known as rennin, which is a blend of pepsin and chymosin extracted from animals and microbiological sources) is utilized for milk curdling as the primary phase of cheese processing technology (Merheb-Dini *et al.*, 2010).

Proteases of different types are utilized for speeding up cheese aging, as a functional property and changing milk protein to decrease the allergic effects of cow milk products in infant foods (Fox, 2002). Lipase is mostly used in cheese maturing for the improvement of flavours. Lactase is usually applied to hydrolyse lactose to glucose and galactose sugars and increase the solubility and sweet flavour in different dairy items.

Milk contains protein, particularly casein that preserves the liquid structure. Rennet and rennin termed as enzymes are used to coagulate the milk. Rennet is isolated from the lining of a fourth part of the calf stomach. Animal rennet (bovine chymosin) is conventionally used as a milk-clotting agent in dairy industry for the manufacture of quality cheeses with good flavour and texture (Bhoopathy, 1994). Chymosin is the widely recognized enzyme separated from rennet. Chymosin is easy to acquire from animals, microbial and vegetable sources. microbial rennet is produced from the mold - *Mucor miehei*. Most common vegetable rennet is thistle. It is extracted from *Cirsium* plant, this thistle can be used for coagulation of milk (Eva-Maria *et al.*, 2017).

Lactase converts lactose into galactose and glucose sugars. Lactase can be obtained from various sources like plants, animal organs, bacteria, yeasts (intracellular enzyme) and molds. Lactase is typically obtained from *Aspergillus* species of fungi and *Kluyveromyces* species of yeasts. Lactase is mainly for people suffer from lactose intolerant who have less creation of lactase in small intestine. Lactase is utilized at a commercial level to develop products free from lactose for lactose intolerant people (Wilkinson *et al.*, 2003).

Milk contains protein, particularly casein that preserves the liquid structure. When protease enzyme is added to cheese processing, it hydrolyses casein into kappa casein, which stabilizes the micelle function preventing from coagulation in milk. Protease hydrolyses the peptide linkages of a protein. Bovine and human milk contain protease as a native ingredient. It hydrolyses the macro peptides in production of cheese. It provides flavour and desired texture to cheese (Razzaq *et al.*, 2019) (Fig. 2). Catalase has limited and specific usage in cheese processing. Catalase is obtained from bovine liver or microbial sources. It is usually added to dissolve hydrogen peroxide to water and oxygen. Therefore, it is utilized in making certain cheeses instead of pasteurization (E.g., swiss cheese) in order to save regular milk proteins that provide benefits to finished product and flavour enhancement of the cheese (Silva *et al.*, 2007).

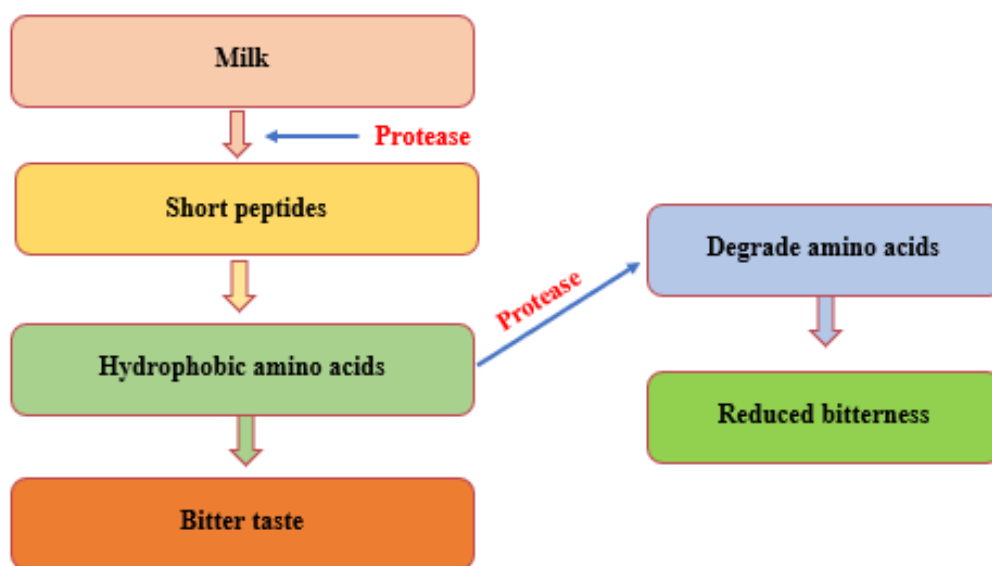


Figure 5.2: Role of Protease Enzyme in Cheese Preparation

5.7 Brewing Industry:

Beer and wine are both alcoholic beverages, produced by yeast fermentation of sugars. Beer is the world's most widely consumed alcoholic beverage (Nelson, 2005). Wine is based on grapes and beer is traditionally based on barley.

In the brewing process enzymes have an important role especially starch undergoes some transformations during the scarification process. The main enzymes used in beer brewing industry can be divided into four main processes which are germination, mashing, fermentation and clarification Some enzymes are already present in the barley (Figure 5.3), but the majority of enzymes are produced during germination. Different enzymes are used in brewing industry and described in Table 5.2.

In the final malt all the enzymes needed for the conversion of "grains" into a fermentable liquid (wort) is present.

Table 5.2: Brewing Enzymes and Their Effects

Enzyme	Sources	Function	Reference
α -amylase	Barley kernel <i>Bacillus licheniformis</i> <i>Bacillus subtilis</i>	Starch hydrolysis Improve clarification	Sammaritino (2015)
β -amylase	Barley kernel Wheat kernel <i>Bacillus licheniformis</i>	Starch hydrolysis Improve malting Improve saccharification Increase fermentation yield	Guerra <i>et al.</i> (2009)
β -glucanase	Barley kernel <i>Trichoderma</i> sp. <i>Orpinomyces</i> sp.	Improve malting Lower viscosity Aid in production of a clear wort	Tomasi <i>et al.</i> (2017)
Protease	<i>Aspergillus</i> sp. Pineapple latex	Improve fermentation Improve chilling and storage quality	Dulieu <i>et al.</i> (2000); Lei <i>et al.</i> (2013)
Amyloglucosidase	<i>Aspergillus niger</i>	Increase the amount of glucose in wort	Lei <i>et al.</i> (2013)

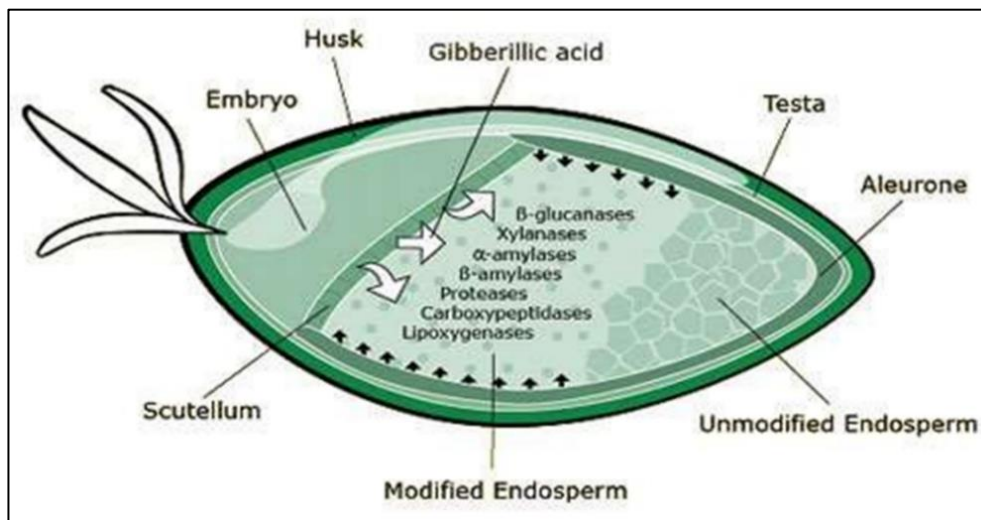


Figure 5.3: Structure of barley kernel

Wine is the product of the biochemical transformations of the compounds present in grape juice by means of a controlled alcoholic fermentation. Yeasts convert sugars into ethanol and other metabolites, as well as into a wide range of volatile and non-volatile compounds that significantly contribute to the sensory properties of wine such as colour, flavour, bitterness, sourness and aroma. Enzymes play a vital role in the wine making process. Many of these enzymes originate from the fruit itself. The indigenous microflora on the fruit and the microorganisms presents during wine making increase the fermentation. The most widely used enzymes for wine making are pectinase, glucanase, xylanase and protease are used to improve the clarification and processing of wine. Glycosidase is used to release of varietal aromas from precursor compounds. Urease is used to reduce the ethyl carbamate formation. Glucose oxidase play a vital role in the regulation of alcohol levels (Table 5.3).

Table 5.3: Enzymes used in wine industry

Application	Enzymes	References
Filtration and clarification	Pectinolytic enzymes	Mojsov (2013)
Mash fermentation	Pectinase	Ramirez <i>et al.</i> (2015)
Late fermentation (white wine)	Glycosidases	Merin <i>et al.</i> (2015)
Young wine	Glucanases	Ramirez <i>et al.</i> (2015)
Ageing	Ureases	Cerreti <i>et al.</i> (2016)

The use of commercial enzymes has proved to be quite advantageous in modern wine making. Wine production considers four main stages schematized in Figure 5.4. First, the grapes are crushed by pressing and kept in maceration with the purpose of extracting as much as juice as possible for must formation. Here, the use of enzymes is considered as a pre-treatment step, which precedes wine making. Second, the alcoholic fermentation takes

place, where the main specific features of the wine are obtained such as aroma release is an important feature that can be enhanced by the use of some specific enzymes, as explained below. Third, clarification step has the purpose of reducing the turbidity and viscosity of wine, as well as avoiding operational issues such as filter stoppages due to the high concentration of polysaccharides. The addition of pectic enzymes facilitates the clarification and filtration process. Lastly, aging and stabilization operations aim at obtaining the physicochemical properties of the final product, which can be improved by the use of suitable enzymes (Ottone *et al.*, 2020).

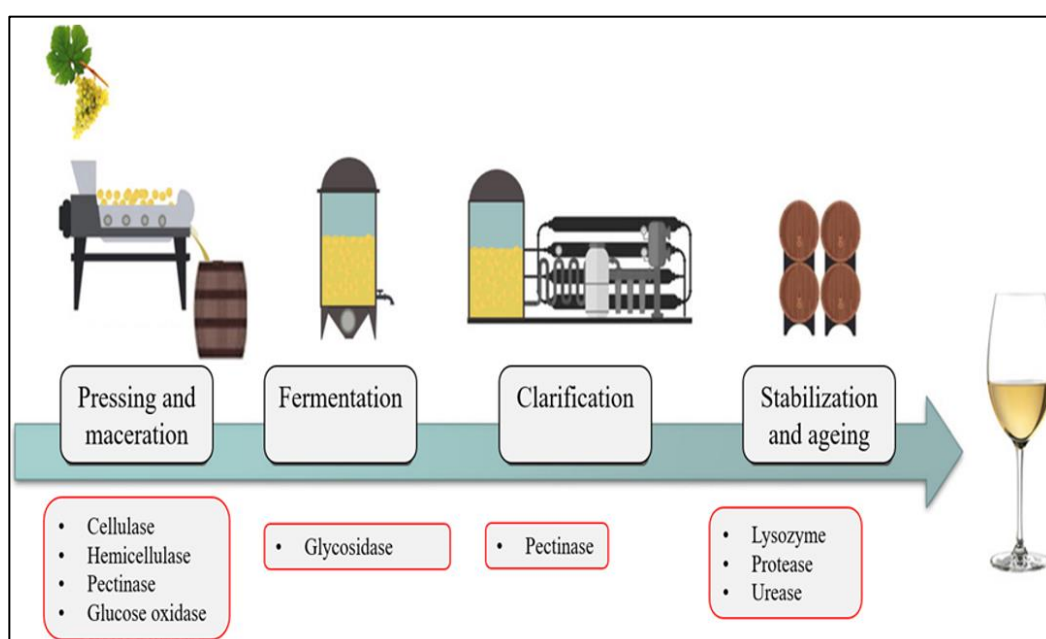


Figure 5.4: Commercial wine making process

5.8 Starch Industry:

Starch is a widely used renewable resource. It is present as a storage compound in the leaves, tubers, seeds and roots of many plants. Unprocessed native starches are structurally too weak and functionally too restricted for application in pharmaceutical, food and non-food technologies. Modifications are necessary to create a range of functionality (Berski *et al.*, 2011). Raw starch properties should be improved using modification. Starch modification can be done by modifying the structure including the hydrogen bonding in a controlled manner to enhance and extend their application in industrial prospective. Starch is composed of two fractions, they are amylose and amylopectin. The respective ratio of amylose and amylopectin, significantly affects the physicochemical properties of starch which in turn, influences its functionality and eventual applications. Modification can be chemical, physical and enzymatic. Enzymes are ideal catalysts for the conversion of starch into products like high fructose corn syrup, maltodextrin, maltose syrup etc. (Esmaeili and Noorolah, 2017). Applications of different enzymes in starch industry are detailed in Table 5.4.

Table 5.4: Applications of different enzymes in starch industry

Application	Enzymes	References
Starch liquefaction	α -amylase	Kammoun <i>et al.</i> (2008)
Starch saccharification	α -amylase Glucoamylase Pullulanase Isoamylase Maltogenic amylase	Hii <i>et al.</i> (2012); Zareian <i>et al.</i> (2010)
Anti-staling	α -amylase, β -amylase, pullulanase, debranched enzymes, branching enzymes, maltogenic amylase, glucoamylase, cyclodextrin glucanotransferases	Van der Maarel and Leemhuis (2013); Esmaeili and Noorolahi (2017)

There are basically four groups of starch-converting enzymes. They are endoamylases, exoamylases, debranching enzymes and transferases. Endoamylases are able to cleave α ,1-4 glycosidic bonds present in the inner part (endo) of the amylose or amylopectin chain. α -amylase is a well-known endoamylase. Exoamylases cleave α ,1-4 glycosidic bonds α ,1-6 glycosidic bonds. Exoamylases act on the external glucose residues of amylose or amylopectin and thus produce only glucose (glucoamylase and α -glucosidase), or maltose and β -limit dextrin (β -amylase).

The third group of starch-converting enzymes are the debranching enzymes that exclusively hydrolyse α ,1-6 glycosidic bonds. Most commonly using debranching enzymes are isoamylase and pullulanase type I. The fourth group of starch converting enzymes are transferases that cleave an α ,1-4 glycosidic bond of the donor molecule and transfer part of the donor to a glycosidic acceptor with the formation of a new glycosidic bond. Enzymes such as amylomaltase and cyclodextrin glycosyltransferase are commonly used in food industry (Van der Maarel *et al.*, 2002).

Enzymatic conversion of starch involves three basic steps (Figure 5.5). They are liquefaction, saccharification and isomerization. The raw material is milled to separate the starch from the oil, protein and fibers. Enzymes ease this process and the starch is usually present in a water slurry which is passed on to the next stage, known as liquefaction. The enzyme breaks down the large starch molecules into maltodextrins. In the saccharification stage, enzymes break the maltodextrins into glucose molecules. During isomerization, the glucose is converted into fructose which in turn enables the production of high fructose syrup and crystalline fructose used commonly in the food and beverage industries (Li *et al.*, 2016).

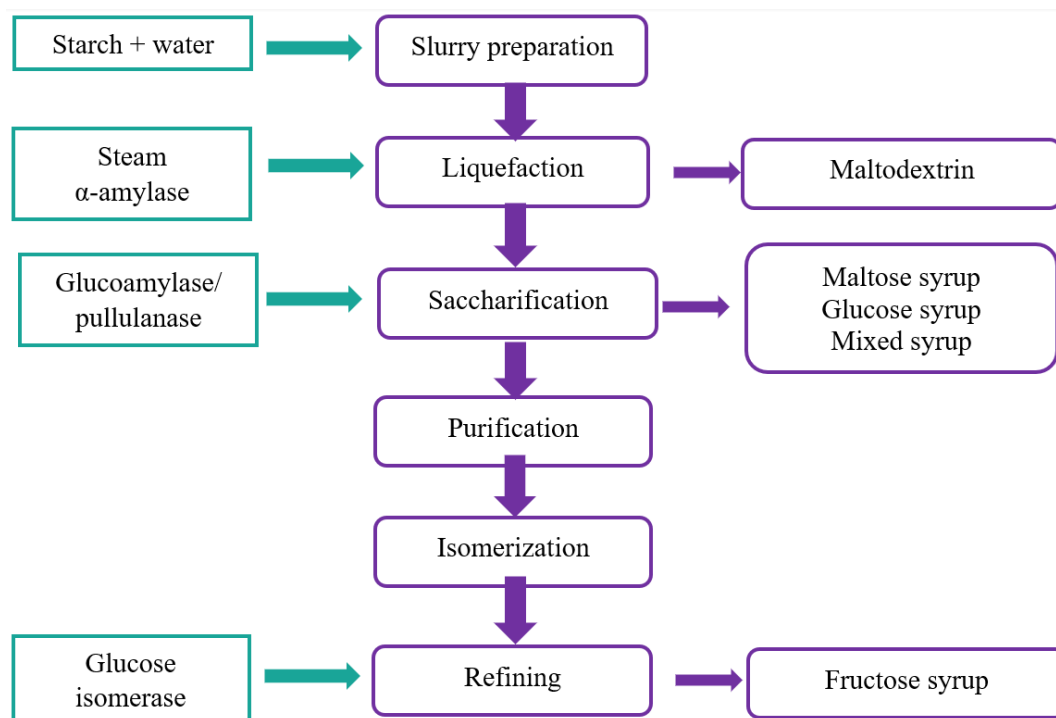


Figure 5.5: Enzymatic conversion of starch

5.9 Baking Industry:

Baked goods are prepared from flours such as wheat flour, which has starch as its main constituent. Amylolysis enzymes break down flour starch into small dextrin that become better substrates for yeast to act upon in the bread making process. The use of enzymes in the baking industry is expanding to replace the use of chemicals in making high-quality products, in terms of better dough handling, anti-staling properties, as well as texture, colour, taste, and volume. Baking comprises the use of enzymes from three sources, they are endogenous enzymes in flour, enzymes associated with the metabolic activity of the dominant microorganisms and exogenous enzymes which are added in the dough (Di Cagno *et al.*, 2003). The supplementation of flour and dough with enzyme improvers is a usual practice for flour standardization and also as baking aids. Enzymes are usually added to modify dough rheology, gas retention and crumb softness in bread manufacture, to modify dough rheology in the manufacture of pastry and biscuits, to change product softness in cake making and to reduce acrylamide formation in bakery products. The enzymes can be added individually or in complex mixtures, which may act in a synergistic way in the production of baked goods and their levels are usually very low (Penella *et al.*, 2008). The baking industry predominantly makes use of different types of enzymes (Table 5.5). Amylases are used to convert starch to sugar and to produce dextrans. For strengthening and bleaching of the dough, oxidases are used. Hemicellulases and proteases are the enzymes which have an effect on wheat gluten. While hemicellulases improve gluten strength and proteases reduce gluten elasticity. Xylanases are most often combined with amylases, lipases and many oxidoreductases to attain specific effects on the rheological properties of dough and organoleptic properties of bread.

Xylanases have also been used to improve the quality of biscuits, cakes and other baked products. Glucose oxidase can be used as alternative oxidizing agent instead of potassium bromate in breadmaking.

Potassium bromate is an oxidizing agent that was traditionally used in baking, and its use was prohibited in many countries after it was recognized as carcinogenic. All these enzymes together play an important role in maintaining volume, crumb softness, crust crispiness, crust colouring or browning and in maintaining freshness (Leman *et al.*, 2005).

Table 5.5: Different Enzymes Used in Baking Industry and Their Functions

Enzyme	Functions
α -amylase	<ul style="list-style-type: none"> • Degrading starch in flours • Controlling the volume and crumb structure
β -xylanases	<ul style="list-style-type: none"> • Improving dough handling and dough stability
Maltogenic α -amylases	<ul style="list-style-type: none"> • Improves shelf-life of bread and cake • Anti-staling effect
Hemicellulose	<ul style="list-style-type: none"> • Improve gluten strength
Glucose oxidase	<ul style="list-style-type: none"> • Make weak doughs into stronger • Make elastic
Lipoxygenase	<ul style="list-style-type: none"> • Bleaching and strengthening dough

5.10 Juice Industry:

Enzymes are processing aids used worldwide for fruit processing, particularly for the production of clear fruit juice and concentrate. Enzymes can increase the yield of solid recovery during pulp washing, facilitate the production of highly concentrated citrus bases, improve essential oil recovery from peel, debitter juice, clarify lemon juice or increase the worth of waste products (Grassin and Fauquembergue, 1996).

Pectinases are one of the important upcoming enzymes of the commercial sector especially for fruit juice industry as prerequisites for obtaining well clarified and stable juices with higher yields.

Other enzymes used in the juice industry are amylases, glucoamylases, cellulases, hemicellulose, laccase, naringinase and limoninase (Table 5.6).

Vegetable juice processing therefore requires more cellulases in addition to pectinases to reduce viscosity sufficiently for juice extraction using a decanter (Sandri *et al.*, 2012).

Table 5.6. Different enzymes used in juice industry and their functions

Enzyme	Functions
Carbohydrase	<ul style="list-style-type: none"> • Increase juice yield • Increase sugar and acid extraction
Amylase, glucoamylase	<ul style="list-style-type: none"> • Breaking down starch into glucose • Clarifying cloudy juice
Pectinases	<ul style="list-style-type: none"> • Degrading pectin • Increase overall juice production
Cellulases, hemicellulases	<ul style="list-style-type: none"> • Maintenance of texture • Lowering viscosity
Naringinase	<ul style="list-style-type: none"> • Remove bitterness of citrus juice • Remove haze

5.11 Meat Industry:

Tenderness of meat is considered as the most important quality of meat (Zor *et al.*, 2009). Tenderness in meat results from a combination of breakdown within muscle fibres, primarily because of the activity of enzymes and loosening of connective tissue, in particular collagen.

Various pre-slaughter and post-slaughter factors and their mutual effect influence tenderness of meat. In meat industry and catering predominantly protein-degrading enzymes have been used.

Enzymes such as papain, bromelain, ficin, proteases and actinidin breakdown the muscle fibres in the meat and helps to tenderise meat. Widely using enzymes, sources and their functions are described in Table 5.7.

Table 5.7: Enzymes used in meat industry

Enzymes	Source	Functions
Plant source	Papaya	<ul style="list-style-type: none"> • Meat tenderization • Increases protein dispersability • Increase palpability, solubility and digestibility
Papain	Fig	
Ficin	Pineapple	
Bromelain		
Actinidin	Kiwi	<ul style="list-style-type: none"> • Improve tenderness in processed meat

Enzymes	Source	Functions
Microbe source	Aspergillus niger	<ul style="list-style-type: none"> • Improve flavour, nutritional and functional properties of proteins • Converts animal carcasses into flavourous compounds
Acid proteases	A. oryzae	
Lipase	Aspergillus spp. Candida spp.	<ul style="list-style-type: none"> • Hydrolyse triglycerides • Improves flavour in sausages

Papain was obtained through a series of processes. The first phase eliminates small organic and inorganic molecules and other proteins present on the extracted latex by adding ammonium sulphate and EDTA.

The latex was diluted with alcohol using 95°C water as heating medium. The enzyme was ground to get a fine powder (Andrade-Mahecha *et al.*, 2011) (Figure 5.6).

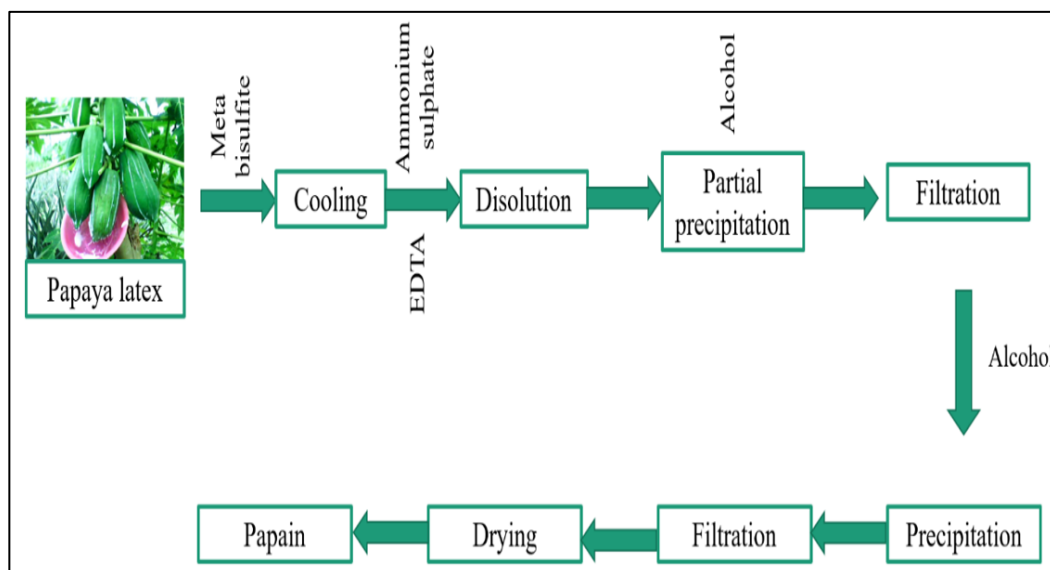


Figure 5.6: Extraction of Papain from Papaya Latex

The use of enzymes in the food industry is a well-established approach, in particular due to the specificity of enzyme action and their green, environmentally friendly nature. As mentioned above, enzymes are currently used in several different food products and processes and new areas of application are constantly being added.

The introduction of enzymes as effective biocatalysts working under mild conditions results in significant saving in resources such as energy and the environment. Evidence clearly shows that dedicated research efforts are consistently being made so as to make this application of biological agents more effective and diversified.

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6. Food Fortification– The Indian Scenario

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Abstract:

WHO and FAO recognized that there are over 2 billion people worldwide, who suffer from a variety of micronutrient deficiencies. It is statistically proved that approximately 1 in 3 people worldwide were at risk for iodine, vitamin A, or iron deficiency. For obtaining compensation of micronutrients in the foods, innovative technologies like food fortification or enrichment of food products were used through which these micronutrients are compensated in the regular diet of the common people. Food fortification is defined as the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food). Fortification is being promoted through both, the open market and the government safety net programmes, such as Integrated Child Development Services (ICDS), Mid-Day Meal Scheme (MDM) and public distribution system. Food safety and Standards Authority of India (FSSAI) operationalised standards for fortification of five staples, namely wheat flour and rice (with iron, vitamin B12 and folic acid), edible oil and milk (with vitamin A and D) and salt (with iron in addition to iodine).