Enzyme as Processing Aids

The food industry is using a wide variety of crop plants and animal products as basis for their manufacturing processes, leading to an even wider variety of consumer products. Biotechnology, which has been used to manufacture food products for more than 8000 years, offers ways to improve the processing of raw materials into final products. Bread, alcoholic beverages, vinegar, cheese and yogurt and many other foods have been made using enzymes which were found in various microorganisms. Today, biotechnology is still affecting the food industry by providing new products, lowering costs and improving processes on which food producers have long relied. Without a doubt, this will continue into the future.

Using biotechnology, improvements in functionality, nutritional value, sensoric properties, like flavour and texture have been achieved as well as improvements in the processing itself, using new tools, such as enzymes, emulsifiers and improved starter cultures. Biotechnology also offers improved ways to deal with waste problems, food safety problems, packaging issues, etc.

Enzymes can modify and improve the functional, nutritional and sensoric properties of ingredients and products, and therefore enzymes have found widespread applications in processing and production of all kinds of food products. In food production, enzymes have a number of advantages. First and most important is that enzymes are used as alternatives to traditional chemical-based technology. Enzymes can thus replace synthetic chemicals in a wide range of processes. This allows advantages in environmental performance of processes by lowering energy consumption levels and biodegradability of products. Furthermore, since enzymes are more specific in their action than chemical reactants, enzyme-catalyzed processes have fewer side reactions and byproducts (waste products). The result is higher quality products and less pollution. Enzymes can catalyze reactions under very mild conditions, allowing mild processing conditions which do not destroy valuable attributes of foods and food components. Finally, enzymes allow processes to be carried out which would be otherwise impossible.

Role of Enzymes in Cheese Making

Enzymes used in cheese making are described as under:

i. Rennet

The use of rennet in cheese manufacture was among the earliest applications of exogenous enzymes in food processing, dating back to approximately 6000 B C. The use of rennet, as an exogenous enzyme, in cheese manufacture is the largest single application of enzymes in food processing. Animal rennet (bovine chymosin) is conventionally used as a milk-clotting agent in dairy industry for the manufacture of quality cheeses with good flavor and texture.

Milk contains proteins, specifically caseins that maintain its liquid form. Rennet and rennin are general terms for any enzyme used to coagulate milk. Technically rennet is also the term for the lining of a calf's fourth stomach. Rennet acts on the milk protein in two stages, by enzymatic and by nonenzymatic action, resulting in coagulation of milk, which is the first step in cheese manufacture. The most common enzyme isolated from rennet is chymosin which carries out an extremely specific and limited proteolysis, making the casein micelle metastable. In the second step, non-enzymatic phase, the resultant milk becomes a gel due to the influence of calcium ions and the temperature used in the process. Chymosin can also be obtained from several other animal, microbial or vegetable sources, but indigenous microbial chymosin that are obtained from fungi or bacteria is ineffective for making cheddar and other hard cheese varieties.

ii. Proteases

Proteases are proteolytic enzymes that are added to milk during cheese production, to hydrolyze caseins, specifically kappa casein, which stabilizes micelle formation preventing coagulation. Milk contains a number of different kinds of proteins in addition to the caseins. Neutral proteases have been found to accelerate cheese ripening by increasing the development of flavor, wheareas acid and alkaline proteases caused bitter tastes. Cow milk also contains whey proteins such as lactalbumin and lactoglobulin. The denaturation of these whey proteins, using proteases, results in a creamier yogurt product. Destruction of whey proteins is also essential for cheese production.

During production of soft cheeses, whey is separated from the milk after curdling, and may be sold as a nutrient supplement for body building, weight loss, and for lowering the blood pressure. Recently, reports on dietary whey for cancer therapies are also available, and having a role in the induction of insulin production for those with diabetes mellitus type II disorder. Proteases are used to produce hydrolyzed whey protein, which is less likely to cause allergic reactions and is used to prepare supplements for infant formulas and medical uses.

Lactic acid bacteria have a complex proteolytic system capable of converting milk casein to the free amino acids and peptides necessary for their growth. These proteinases include extracellular proteinases, endopeptidases, aminopeptidases, tripeptidases, and proline-specific peptidases, all of these enzymes are serine proteases in nature. The proteolytic system of lactic acid bacteria is essential for their growth in milk, and contributes significantly to flavor development in the fermented milk products. Aminopeptidases are important for the development of flavor in fermented milk products, since they are capable of releasing single amino acid residues from oligopeptides formed by extracellular proteinase activity.

In recent years, proteinases have found additional applications in dairy technology, for example in acceleration of cheese ripening, modification of functional properties and preparation of dietary products.

iii. Lipases

Lipases are used to break down milk fats and give characteristic flavours to cheeses. Strongly flavoured cheeses, for example, the Italian cheese, Romano, are prepared using lipases. The flavour comes from the free fatty acids produced when milk fats are hydrolyzed. Various animal or microbial lipases gave pronounced cheese flavor, low bitterness and strong rancidity, while lipases in combination with proteinases and/or peptidases give good cheese flavor with low levels of bitterness. In a more balanced approach to the acceleration of cheese ripening, mixtures of proteinases and peptidases, attenuated starter cells or cell-free extracts (CFE) are being used.

Animal lipases are obtained from kid, calf and lamb, while microbial lipase is derived by fermentation with the fungal species *Mucor meihei*. Although microbial lipases are available for cheese-making, they are less specific in fats which they hydrolyze, while the animal enzymes are more partial to short and medium-length fats. Hydrolysis of the shorter fats is preferred because it results in the desirable taste and flavor of many cheeses. Hydrolysis of the longer chain fatty acids can result in either soapiness, or produce no flavour at all.

Lipolytic enzymes extracted from microbial sources enhance cheese flavor results in rancidity and soapy flavours. Despite these limitations, these lipolytic enzymes provide a balance between flavour enhancement and cost and are suitable for use.

Role of Enzymes in Fruit Juice Processing

Enzymes break down specific components within fruit such as pectin, starch, proteins and cellulose which results in improved extraction and increased yields, shortening of processing time and improving sensory characteristics. They can also be used to decrease the viscosity of nectars, and to provide ‘cloud stability’ and texture in juices.

Enzymes are used in the processing of fruit juices to maximize the production of clear or cloudy juice. Nearly all fruits contain pectin. The presence of soluble pectin in squeezed juice causes cloudiness. The addition of pectin degrading enzymes such as pectin methyl esterase which cleaves pectin at the pressing stage increases the amount of juice produced and at the same time can reduce cloudiness. The desired flavor and colour of citrus juices depends on the insoluble, cloudy materials of the pressed juice. The pectin component is manipulated requiring a balance between pectin methyl esterase, to promote cloudiness by increasing the pectin/calcium complex formation and polygalacturonase to break cloudiness by depolymerisation of the pectin. Pectin degrading enzymes or pectinases are used to disintegrate the fruit and to clarify the resulting juices to give a clear sparkling liquid after the filtration of the debris. The advantages of using enzymes in the fruit juice industry allow it to be more economic and have consistent quality.

Naringinase is used for removing the bitter tasting substance from citrus fruits, especially grape fruits. Naringin is a flavonoid found in grapefruits giving them their characteristic bitter flavor. Flavonoids are a group of polyphenolic secondary metabolites secreted by plants and found widely among plants. Although naringin is supposed to have some beneficial effects such as stimulating our perception of taste by stimulating the taste buds, the bitter taste is undesirable in fruit juices.

Role of Enzymes in Baking Industry

Traditional method of baking is based on the presence of endogenous enzymes which catalyse natural changes during growth, ripening and storage. They also carry out the saccharification of starch prior to fermentation and the degradation of gluten which is a very important determinant of the rheological properties of the dough.

i. Amylases

Alpha amylases have significant effects on baked goods. If the amylase activity is low, this leads to low dextrin production and poor gas production. This in turn results in inferior quality bread with reduced size and poor crust color. To compensate for the deficiencies of the grain, it is necessary to add either sugar or alpha amylase. The addition of enzymes offers certain advantages over sugar. At a flour mill, it is possible to standardize the enzyme content of the flour so that a uniform commodity can be supplied. Furthermore, enzymes bring about a gradual formation of sugar, which matches the needs of the yeast. When the dough is placed in the oven, the steadily increasing temperature leads to an increase in the enzymes' rate of reaction and more sugar is produced. Malt flour and malt extract can be used as enzyme supplements as malt is rich in alpha amylases.

Amylases can be derived from bacteria and fungi. They play a major role in the food and beverages (baking), brewing, and starch and sugar industries. This enzyme is used extensively in drink industry for example the production of *High Fructose Syrup* (HFS). Amylases can be made from various microorganisms especially from *Bacillus*, *Pseudomonas* and *Clostridium* species. Potential bacteria that are recently used to produce amylases in industrial scale are *Bacillus licheniformis* and *Bacillus stearothermophillus.* It is preferable to use *Bacillus stearothermophillus* because it can produce enzyme that is thermo stable so that can reduce production cost.

However, it is better to use a fungal alpha amylase. The alpha-amylases degrade the damaged starch in wheat flour into small dextrins, thus allows the yeast to work continuously during dough fermentation, proofing and the early stage of baking. This results in improved bread volume and crumb texture. In addition, the small oligosaccharides and sugars such as glucose and maltose produced by these enzymes enhance the reactions for the browning of the crust and baked flavour.

During starch processing, enzymes help to separate raw milled cereals into polysaccharides, gluten and fibres. Enzymes are also used to further break down the long chains of sugars (polysaccharides) into multiple sugars (e.g. glucose, fructose, sucrose, maltose, maltotriose, raffinose).

ii. Proteases

Proteases are used to modify and optimize dough strength for a particular product, and serve to reduce mixing time to obtain the proper dough viscoelasticity. Proteases can also improve the performance of flours with damaged gluten that confer less elastic and stiffer doughs. Exogenous proteases are added to affect controlled hydrolysis of the gluten during the dough conditioning stage, although proteases may continue to act during baking until thermally deactivated. Hydrolysis of gluten weakens the gluten network, resulting in enhanced extensibility and viscoelasticity of the developed dough, and these properties are associated with increased bread volume, uniform crumb development, and tenderness of the final product. Controlled hydrolysis is obtained by making use of a protease with moderate peptide bond selectivity (to guard against exhaustive hydrolysis), and dosing at a rate that provides the desired degree of hydrolysis prior to deactivation during the baking cycle. Excessive proteolysis will yield low product volume and textural defects. Use of less specific proteases (or mixtures of proteases) is appropriate when weaker doughs are required for forming into shapes, such as pizza crust, wafers, or biscuits. Choice of protease can be critical to product quality since proteases have different specificities of reaction with the major gluten proteins, either gliadin or glutenin. Such differences can account for different degrees of performance enhancement of doughs by choice of exogenous protease.

Role of Enzymes in Meat Processing

Papain and other sulfhydryl endopeptidases (bromelain and ficin) are applied to muscle or meats that do not become sufficiently tenderized during postmortem aging. These enzymes are effective in this application because they can hydrolyze collagen and elastin, connective tissue proteins that cause toughness in meat. However, the two drawbacks of tenderization by exogenous endopeptidases are that they can be “overdosed” and the pattern of tenderization is not the same as that which occurs in naturally aged/tenderized meat (proteolysis selectivity patterns are different). Enzyme (usually papain) in a powdered form (using salt or other innocuous material as carrier) can be applied directly to the surface of meats, or the enzyme in dilute saline can be injected or applied as a dip. Antemortem application of enzyme is possible, as a fairly pure solution in saline injected intravenously into animals 2–10 min before slaughter, sometimes after stunning; this helps distribute the enzyme throughout the muscle tissues. Injection of inactivated papain (disulfide form) obviates any discomfort among animals, since the enzyme becomes activated by the reducing conditions that soon prevail postmortem. In many cases, owing to the relative thermal stability of these endopeptidases, perhaps as much tenderizing effect occurs during the cooking phase of meat preparation as it does during chilled handling and storage of meat.

Role of Enzymes in Egg Processing

Several enzymes have been used in egg processing such as phospholipases, proteases, glucose oxidase and catalase. Phospholipases are used to enhance the emulsifying properties of egg yolk. Specific proteases are used to improve the gelation temperature of egg white. Glucose oxidase/catalase system is used to remove glucose from egg white before drying and thus helps to prevent discoloration (browning) of the dried product.