Enzyme application in food industry

1-Amylases

Enzymes that cleave polysaccharides such as α and β amylases are of interest for plant food processing. Few examples include processes that occur in the ripening of fruit, in the processing of potatoes and flour to cakes and pastries, and in the degradation of cereals in preparation for alcoholic fermentation. α-Amylase hydrolyzes starch, glycogen, and other 1,4-α-glucans and is a calcium dependent endoenzyme. The viscosity of a starch solution rapidly decreases on hydrolysis by α-amylase (starch liquefaction). Starch hydrolysis by the use of this enzyme also increases reducing sugar content of processed foods. The activity of the enzyme decreases rapidly with decreasing degree of polymerization of the substrate. β-amylase catalyzes the hydrolysis of 1,4-α-D-glycosidic bonds in polysaccharides, effecting successive removals of maltose units from the nonreducing end. It is an exoenzyme and commonly found in vegetable tissues. This enzyme is known as saccharifying enzyme since maltose production increases the sweetness of the starch solution. Due to its high affect on starch, the amylase activity must in many cases be monitored. The reason behind monitoring varies between ingredients; in some cases a high amylase activity is desired but in most cases a high amylase activity cause problems.

Depending upon their origin, α- and β-amylases show differences in pH and temperature optima, thermostability, and other chemical stability. They do not require coenzymes for activity, although α-amylase activity is enhanced by the presence of calcium. The pH optimum for α-amylase is 4.5 and it is inactivated at a pH of 3.3 to 4.0. This pH dependence decreases the efficacy of this enzyme in sourdoughs. β-Amylase is active across a much broader pH range, 4.5-9.2, with a pH optimum of 5.3. α-Amylase is relatively thermostable up to 70°C, whereas β-

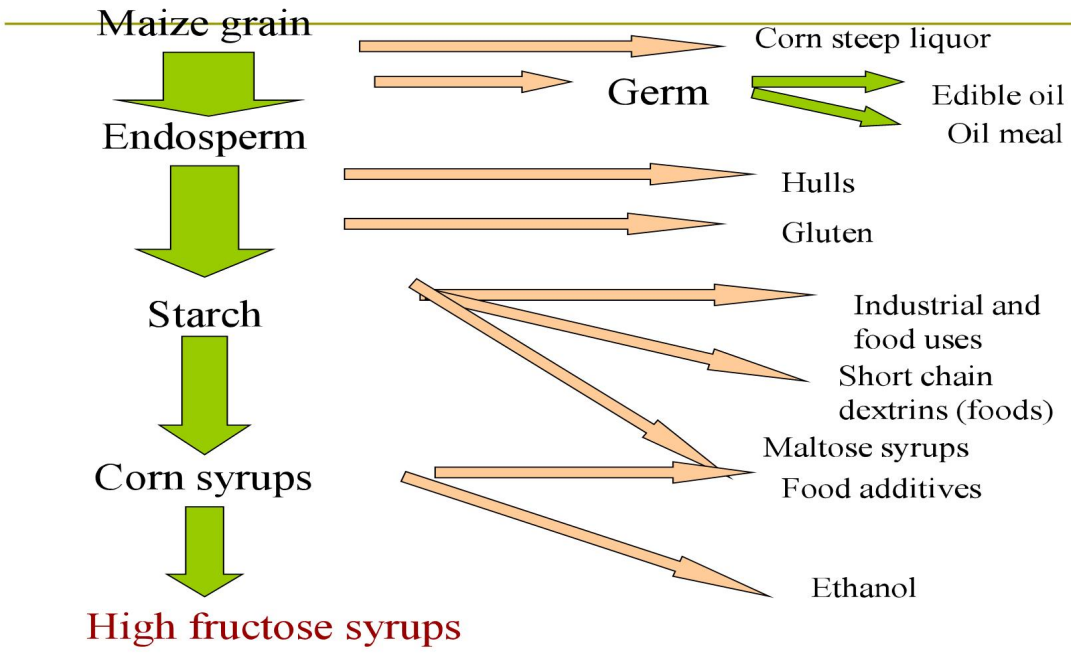
amylase loses about half of its activity at this temperature. Fungal amylase is the least temperature stable, followed by cereal amylase, while bacterial amylase is stable at higher temperatures.

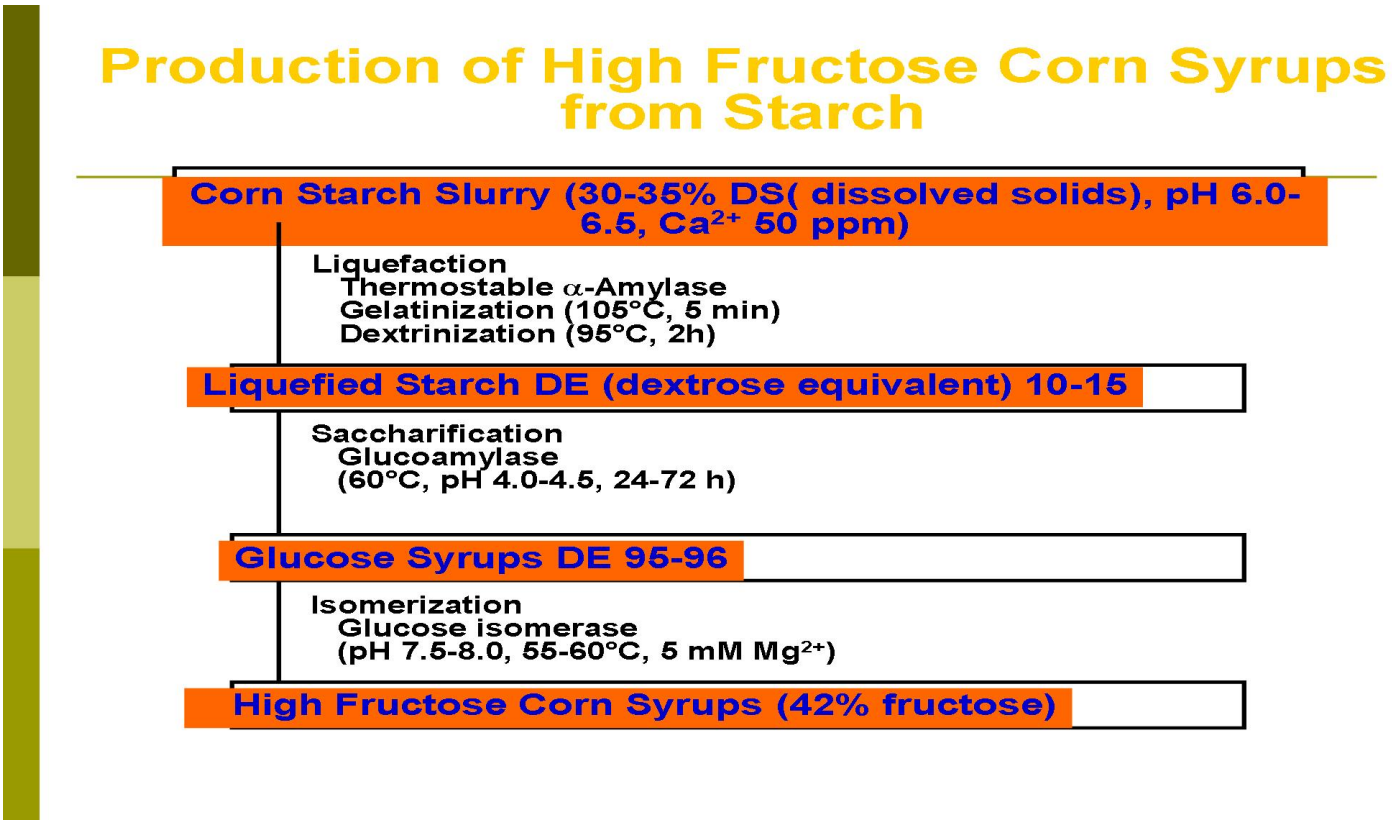
High Fructose Corn Syrup:-

Amylases

- Alpha – amylase –
  - cuts 1,4 bonds
  - Yields dextrins and oligosaccharides
- Beta-amylase – maltose units only
- Combo - produces almost all maltose
- Gluco-amylase – glucose
- Pullunase –
  - Cuts beta - 1,6 linkages

Corn starch processing 1





High Fructose Corn Syrup:-

1-The soaked corn is milled to release the oil-containing germ.

2-The resulting starch, hull and protein components are then finely ground and screened to remove the hull. The resulting slurry is passed to a continuous centrifuge to separate the starch and protein components.

3-The enzyme alpha-amylase is added to slurry of starch and water to liquefy or reduce the particle size of the starch to produce glucose polymers. This step is followed by saccharification with the enzyme glucoamylase, which breaks the glucose polymers down to their basic building blocks. The resulting glucose mixture is filtered and clarified by centrifugation, carbon filtration and ion exchange.

4- Glucose isomerase, an enzyme, is used to convert a portion of the glucose to

fructose. The resulting mixture is 42% fructose, 53% glucose and 5% higher sugars. The mixture is refined with carbon filters and ion exchange. The fructose content of the resulting syrup is enriched by chromotographic separation, by passing the syrup through a column of adsorbent containing calcium or other cation that attracts the fructose portion of the syrup. This step produces syrup that is about 90% fructose, which is then blended with the 42% fructose syrup to produce one that is 55% fructose, 42% glucose and 3% higher sugars.

5-The final syrup is refined by carbon filtration and ion exchange and then evaporated to 77% solids.

2-Amylyses in Baking

The most widely used enzyme in bread baking in terms of amount dosed and value is fungal α-amylase (Taka-amylase) from *Aspergillums oryzae* (E.C. 3.2.1.1). This is supplemented to flours to optimize their α-amylase activity with regard to final volume and crumb structure of the baked product. The primary effect of α- amylase supplementation was before believed to be securing enough gassing power by degradation of damaged starch granules in the dough, which facilitates maltose production by endogenous β-amylase. The resulting maltose can serve as fermentable sugar for yeast fermentation. This mechanism may be important in some very lean dough systems without added sugars, but generally the primary effect is reduction of dough viscosity during initial starch gelatinization, which provides a prolonged oven spring leading to an increased volume and a more homogenous crumb structure.

Antistaling Amylases in baking:

The second important goal for use of amylases in baking is antistaling, i.e., improving the fresh keeping of baked goods. Staling is a highly complex

phenomenon , but it is generally accepted that retrogradation of amylopectin is the main contributor to bread staling .Upon cooling in the first hours after baking the initial crumb structure is set by amylose gelation, which creates a network in which the gelatinized starch granules are embedded. Recrystallization of amylopectin side chains leads to rigidification of the starch granules and to an overall strengthening of the crumb structure, measured as an increase in crumb firmness. Different types of amylases used for antistaling provide different functionalities . First, a limited effect of fungal α-amylase is caused indirectly by volume increase. Secondly, endo-amylases weaken primarily the intergranular amylose network, leading to reduced crumb firmness immediately after baking and during storage. This effect is more evident when endo-amylases with high thermostability are used. An example of the most effective type is the B. amyloliquefaciens α-amylase. This amylase survives baking and is probably active during cooling of the bread and during storage. Consequently, only a very narrow window of optimal dosage exists, and even moderate overdosing can lead to gummy crumb structure with strongly reduced elasticity. As an alternative, intermediately thermostable amylases have been developed. They have lower risks of overdosing, but may also be limited in their antistaling effects. Compared to classical endo-amylases, exo-amylases such as G4-amylase (E.C.3.2.1.60) and maltogenic amylase (E.C. 3.2.1.133) offer clear improvements for antistaling applications. By shortening amylopectin side chains and releasing maltooligosaccharides,they efficiently reduce the rate of amylopectin retrogradation, leading to significant crumb softening and improved elasticity without risk of excessive weakening of the amylose network.

Glucoamylases are also used in commercial baking to improve flour quality, retard dough staling and improve dough, giving more efficient machinability. They have also been used to enhance bread crust color, bleach flour, improve the quality of

high-fiber baked products. Fungal and bacterial enzymes which are commercially available for use in bakery processing.

24.2. Triacylglycerol hydrolases (Lipases)

Lipases(E.C. 3.1.1.3) hydrolyze only emulsified acyl lipids at water/lipid interface. Lipase activity is detected, for example, in milk, oilseeds (soybean, peanut), cereals (oats, wheat), fruits and vegetables and in the digestive tract of mammals and even in microorganisms. The enzyme cleaves the following types of acyl glycerols with a decreasing rate of hydrolysis: triacyl- >diacyl->>monoacylglycerols. Lipase-catalyzed reactions are accelerated by Ca2+ ions since the liberated fatty acids are precipitated as insoluble Ca-salts.

Baking

The substrate for lipases in wheat flour is the 2–3% of endogenous wheat lipids. Lipids are probably the longest known and used bread improvers, whereas the use of lipase as a bread improver is relatively new.

The desirable effects obtained by lipases are improved dough stability and better dough handling properties, and improved stability of the gas cells in dough, yielding a nice, homogenous crumb structure and increased bread volume. Lipase modifies flour lipids or added lipids, and the beneficial effect of an added lipase is dependent on the effect of the hydrolysis products. Some of these hydrolysis products are quite surface-active or more surface-active than the unmodified lipid and therefore better stabilize the gas cells in the dough. This again leads to better crumb structure, increased dough stability, and increased volume.

Among the potential reaction products, monoglycerides are well-known crumb softeners in baking, as they form a complex with starch and thus decrease retrogradation.

Phospholipases are a class of four enzymes hydrolyzing specific bonds in phospholipids phospholipase A1 (E.C. 3.1.1.32), phospholipase A2 (E.C. 3.1.1.4), phospholipase C (E.C. 3.1.4.3), and phospholipase D (E.C. 3.1.4.4).The release of one fatty acyl chain from phosphatidylcholine yields 1-acylglycerophosphorylcholine,more commonly known as lysolecithin, which is an excellent emulsifier with a very positive effect in baking.

Recently, a new class of lipases acting on polar lipids as well as triglycerides has become commercially available. These new lipases have a higher activity on galactolipids and phospholipids than on triglycerides. By modifying phospholipids and galactolipids to the related lysolipids (e.g., lecithin to lysolecithin and digalactosyl diglyceride to digalactosyl monoglyceride.The new lipases therefore offer the opportunity to generate surface-active materials and possibly to reduce or replace the use of added emulsifiers in bakery products . The ability of the polar lipids to form lipid monolayers at the gas–liquid interface leads to stabilization of the gas cells in the dough and thus to better gas retention.

Furthermore, the interaction between polar flour lipids and gluten proteins may play an important role in gas retention.

Dairy

Lipase from microbial sources (e. g. *Candida lipolytica*) is utilized for enhancement of aromas in cheese making. Limited hydrolysis of milk fat is also of interest in the production of chocolate milk. It enhances the “milk character” of the flavor. The defatting of bones, which has to be carried out under mild conditions in the production of gelatin, is facilitated by using lipase-catalyzed hydrolysis. Action of endogenous lipases on triglycerides can lead to off flavours in foods. The lipase of bovine milk is normally inactive but homogenization and cooling during milk processing activate the enzyme.

Lipolysis plays a significant role in the flavor formation of mold-ripened cheeses, blue cheeses, and some Italian types. In other types of cheese the contribution of lypolysis to flavor formation is still little known. Milk contains the endogenous lipoprotein lipase (LPL, E.C. 3.1.1.34). This LPL is associated with casein, is consequently incorporated in the curd. In milk this lipase is relatively inactive due to its immobilization on the casein micelle and due to the milk fat being present in milk fat globules surrounded by a protective fat globules membrane. Severe agitation or homogenization may damage these structures, after which lypolysis may occur, and the milk becomes rancid.

Pregastric esterases are present in rennet paste and are held responsible for the ‘‘piccante’’ taste of several Italian hard cheeses. Pregastric lipase powders are now used in Italian types of cheese along with a milk coagulant in view of the poor microbiological quality of rennet pastes. Due to pasteurization of raw milk, endogenous lipases are inactivated. Cheese types that, in the past, were made from raw milk are now made from pasteurized milk and added lipases.

Replacement of mammalian lipases by microbial ones is a topic of interest. Lipases from R. miehei and Aspergilli strains are commercially available and useful for the manufacture of Italian cheese types. These enzymes have a preference for hydrolyzing fatty acids located at the 1,3-positions of the glycerol group. The short-chain fatty acids are flavorful and volatile and contribute most to lypolitic flavor development.

Lipases in blue cheeses originate from the P. roqueforti surface mold.This leads to the formation of short and medium length volatile fatty acids and spoilage of milk. Spontaneous lipolysis can be prevented if the formation of the lipase–milk fat globule membrane complex is prevented by delayed cooling of milk or addition of NaCl.