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The Use of Starch Processing Enzymes in the Food Industry

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Abstract:

Man's usage of enzymes dates back to the earliest times of civilization. Significant human activities in primeval communities such as the production of certain kinds of foods and beverages, and the tanning of hides and skins to produce leather for garments, involved the application of enzyme activities, albeit unintentionally. Though, not until the 19th century with the development of biochemistry and the pioneering work of a number of well-known scientists did the nature of enzymes and how they work initiate to be explained. The nineteen-sixties observed two major revolutions that had a major effect on the enzyme industry: the commercialisation of glucoamylase which catalyses the manufacture of glucose from starch with much greater efficacy than that of the chemical process of acid hydrolysis, and the launch of the first enzyme-containing detergents. In this paper we are going to see starch treating enzymes and their usage in the food production in detail.

Keywords — **Starch, Enzyme, Food Industry, Amylase, Isomerisation.**

1. INTRODUCTION

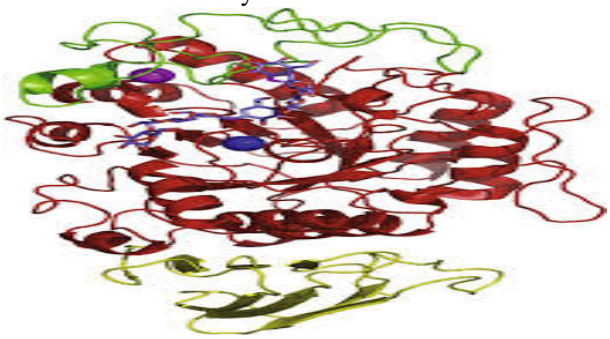
Starch, the main constituent of numerous agricultural products, e.g. corn (maize), potatoes, rice and wheat, is deposited in plant cells as reserve material for the organism in the form of granules which are insoluble in cold water. This carbohydrate is the main component of food products such as bread and other bakery goods or is added to numerous foods for its functionality as a thickener, water binder, emulsion stabilizer, gelling agent and fat substitute. Starch granules contain of two kinds of molecules collected of -D-glucose units called amylose and amylopectin. In amylose almost all the glucose remains are linked by -1,4-glycosidic bonds, whereas in amylopectin about 5 % of the carbohydrate units are also linked by -1,6-linkages creating branch points. The relative substances of amylose and amylopectin rest on on the plant species. For instance, wheat starch comprises about 25% amylose while waxy corn starch is more than 97–99% amylopectin. Starch origin also makes changes to the size, shape and structure of the polysaccharide granules, their swelling power, and gelatinisation temperature, range of esterification with phosphoric acid, and the amounts of lipids and other mixtures which are reserved inside the hydrophobic inner surface of the amylose helices. Increasing starch functionality can be attained through chemical or enzymatic modifications. The most significant approaches of enzymatic starch processing are the production of cyclodextrins and the hydrolysis of starch into a combination of simpler carbohydrates for the production of syrups having dissimilar compositions and properties. These products are used in an extensive diversity of foodstuffs: soft drinks, confectionery, meats, packed products, ice cream, sauces, baby food, canned fruit, preserves, etc. Additionally, glucose formed during starch hydrolysis can be transformed to fuel alcohol and other bio–products by yeast or bacterial fermentation, or isomerised to fructose in a reaction catalysed by glucose isomerase. High fructose syrup is used as a sweetener in dissimilar food products and is more apt for diabetics than usual household sugar.

2. ENZYMES USED FOR STARCH HYDROLYSIS:

2.1. α-Amylases

The industrial degradation of starch is regularly originated by -amylases (α -1, 4- glucanohydrolases) a very communal enzyme in micro-organisms. Composed with other starch-degrading enzymes (eg. pullulanases), α -amylases are comprised in family 13 of glycosyl hydrolases (Henrissat and Bairoch, 1996) described by a $(\alpha / \beta)_8$ -barrel conformation. The structural and functional features of α -amylases have been studied by Nielsen and Borchert (2000) and MacGregor et al. (2001). The enzyme comprises a characteristic substrate binding cleft that can accommodate amongst four to ten glucose units of the substrate molecule. Each binding site has comparison to only one glucose unit of the carbohydrate chain. However, the interactions of

oligosaccharides with numerous binding sites form a multipoint association which results in the correct planning of long substrate molecules near the catalytic site. Variances in the number of substrate binding sites and the location of catalytic regions decide substrate specificity, the length of the oligosaccharide fragments released after hydrolysis and the carbohydrate profile of the final product. Substrate binding is not adequate for catalysis when all the glucose residues of the involved oligosaccharide chain fall outside the catalytic region. This phenomenon happens only in cases of advanced hydrolysis creating oligosaccharide molecules which are too short to lodge all the substrate binding sites. The likelihood of inappropriate binding donates to a quick decline in the reaction rate during the final stages of reaction and also describes variances in the carbohydrate profiles of the final products produced by -amylases creating from numerous sources. Other domains in the -amylase molecule preserve the structure of the protein. One of these called "the starch binding domain" has affinity for starch granules in those enzymes which can degrade starch without the necessity for its gelatinisation. All structural alterations result in a great diversity in enzyme activity, stability, reaction conditions and substrate specificity, which differ both in preference for chain length and the capability to cleave the α-1, 4-bonds close to the α-1, 6-branch point in amylopectin molecules. For instance, the temperature-activity optima of microbial -amylases range from around 25 C to 95 C. Calcium ions play a important role in continuing the structural integrity of the catalytic and/or substrate binding sites in α -amylases, amylopullulanases and numerous other glycosyl hydrolases. Thus the addition of calcium salts to the reaction mixture fundamentally increases enzyme activity and stability. However, extreme amounts of Ca2+ induce inhibitory effects and decrease the reaction yield.



Structure of α -amylases

2.2. Debranching Enzymes

There are two key groups of endo-acting debranching enzymes which can cut the -1, 6-glycosidic linkages present at the branch points of amylose, glycogen, pullulan and related oligosaccharides. The first group are pullulanases that specifically attack α -1, 6- linkages, delivering linear oligosaccharides of glucose remains related by α-1, 4- bonds. The second group of debranching enzymes are neopullulanases and amylopullulanases, which are active toward both α-1, 6- and α-1, 4- linkages. Pullulanases are usually produced by plants, e.g.rice, barley, oat and bean, as well as by mesophilic micro-organisms such as: Klebsiella, Escherichia, Streptococcus, Bacillus and Streptomyces. These enzymes are somewhat heat-sensitive, and commercially existing preparations attained from Klebsiella pneumoniae or Bacillus acidopullulyticus must be used at temperatures not surpassing 50–60 C. However, the search for effectual sources of thermostable debranching enzymesis underway for the reason that the enzymatic conversion of starch is generally carried out at elevated temperatures. Pullulanases are seldom produced by thermophiles. Though, a recent study shows that a good source of heat-resistant pullulanase is the aerobic, thermophilic bacterium Thermus caldophilus which syntheses an enzyme that is optimally active at 75 C and pH 5.5 and keeps activity up to 90 C (Kim et al., 1996).

2.3. Exo-acting Amylases:

Two kinds of exo-acting hydrolases are normally used for starch saccharification: -amylases and glucoamylases. Both act on glycosidic linkages at the non-reducing ends of amylose, amylopectin and glycogen molecules, creating low-molecular weight carbohydrates in the β-anomeric form. The main end-product of hydrolysis catalysed by β-amylases is maltose, while glucoamylase (amyloglucosidase) generates glucose. Fundamentally, β-amylases and glucoamylases are comprised in families 14 and 15 of the grouping of Henrissat and Bairoch (1996), correspondingly. While β -amylases present an $(\alpha / \beta)_8$ fold comparable to -amylases, glucoamylases are categorized by an $(\alpha / \beta)_6$ structure.

3. ENZYMATIC PROCESSING OF STARCH AND STARCH-CONTAINING FOOD

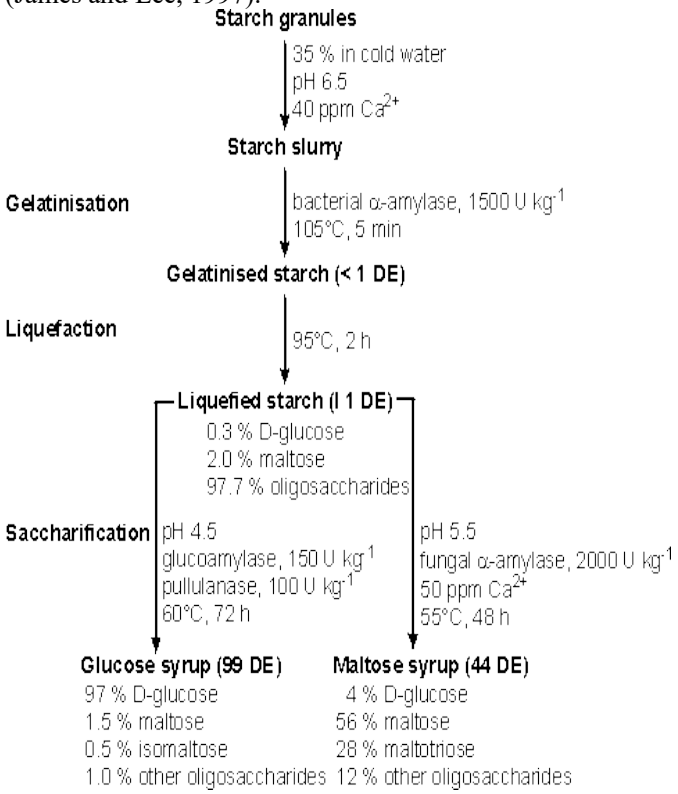
3.1. Products Obtained During Starch Hydrolysis

Starch hydrolases are significant industrial enzymes which are used as additives in detergents, for the deletion of starch sizing from textiles, the liquefaction of starch and the suitable creation of dextrins in baking. They are also added to break down the starch that supplements saccharose in sugar cane juice and interferes with filtration. The discovery and application of enzymes displaying dissimilar activities

and substrate specificities secluded from a variation of microbial sources or attained by gene cloning or protein engineering has occasioned in the development of numerous starch products of diverse carbohydrate profiles and functional properties. The hydrolysis products achieved are commonly separated in two key groups categorized by low- or high-degrees of starch conversion. In the first group are those maltodextrins organized by limited hydrolysis (DE 10–20) of gelatinised starch in reactions usually catalysed by heat-resistant -amylases, without successive saccharification. Maltodextrins provided for some applications are furthermore handled by debranching enzymes to eliminate the side chains of amylopectin molecules thus creating linear oligosaccharides. The key constituents of these products, found in amounts stretching 75–96 % of dry weight, are oligosaccharides comprising more than four glucose residues. Advanced starch hydrolysis which hints to products comprising important amounts of maltose and glucose can be attained during prolonged (48–96 h) times of saccharification. Maltose is the key constituent of the hydrolysates called high-maltose-, enormously high-maltose- and high-conversion syrups, comprising on a dry basis 35–40 %, 70–85 % and 30–47 % of this carbohydrate, correspondingly. Maltulose is collected in the product for the reason that glucoamylases do not cleave the glycosidic bonds amongst glucose and fructose residues. Unwanted maltulose fusion can be eradicated when saccharification is catalysed at pH below 6.0.

3.2. Production of starch hydrolysates:

There are two basic steps in the enzymatic conversion of starch (see Fig. 2): liquefaction and saccharification. In liquefaction the concentrated slurry of starch granules (30–40 %, w/v) is gelatinised at an prominent temperature (90–110C). The addition of thermostable endoamylase at this stage of the procedure protects against a rapid upsurge in starch solution viscosity instigated by the release of amylose from swelling starch granules (Guzman-Maldonato and ParedesLopez, 1995). The saccharification step is carried out at a lower temperature and tips to the hydrolysis of the oligosaccharides acquired into glucose or maltose in reactions catalysed by glucoamylase or β-amylase correspondingly. The yield of starch hydrolysis might be improved by using glucoamylase or β-amylase in mixture with pullulanase or other debranching enzymes. In common the use of pullulanase upsurges the glucose yield up to 94 % (Crabb and Mitchinson, 1997). Since the gelatinisation of starch granules is finalized near 100C in the majority of industrial procedures, thermostable -amylases are used. These enzymes are extensive among thermophilic bacteria and archea, and the genes encoding a few of them have been cloned and stated in mesophilic hosts (Frillingos et al., 2000; Grzybowska et al., 2004). Termamyl initiates from Bacillus licheniformis, and other -amylase preparations used for starch liquefaction typically show highest activity at temperatures above 90 C and at pH 5.5 to 6.0. These circumstances are not though well-matched with those of the glucoamylases or α-amylases used in the next step which are more sensitive to heat and are deactivated above 60 C. An alternate to starch treating using thermostable α-amylase is the application of endo-glucanase which has activity towards native starch granules, as for instance glucoamylase from Rhizopus sp. (James and Lee, 1997).



3.3. Glucose Isomerisation:

The isomerisation of starch-derived glucose to fructose tips to greater sweetness of the acquired syrup which is usually used in numerous food and beverage products, e.g. as a sweetener and a garnish of citrus flavour. Fructose is the sweetened tasting of all the carbohydrates and is apt for the formulation of low-calorie products having abridged sucrose content, or as a sweetener for diabetics for the reason that it can be metabolised without insulin. The use of fructose syrup as an

preservative to some baked products outcomes in necessary browning developed as a result of Maillard reactions. In adding, fructose acts as a crystallization inhibitor which preserves sucrose in solution thus producing a cookie that retains its soft texture during storage. Fructose syrups are generally made in a incessant procedure catalysed by restrained glucose (xylose) isomerase at temperatures of 55–60 C. Under these circumstances only 40-42 % of the glucose is changed to fructose.

3.4. Trehalose:

Production Starch or maltose syrups can be efficaciously treated into trehalose in reactions catalysed by enzymes isolated from mesophilic or thermophilic micro-organisms. Trehalose (-D-glucopyranosyl -D-glucopyranoside) is a stable, non-reducing disaccharide containing 1, 1 glycosidic linkages amongst the glucose moieties. This carbohydrate is intricate in protection of biological edifices during freezing, desiccation or heating (Richards et al., 2002). The mild sweetness of trehalose, its low carcinogenicity, good solubility in water, stability under low pH conditions, reduction of water activity, low hygroscopicity, depression of freezing point, high glass transition temperature and protein protection assets make it a valued food ingredient. Trehalose can be created from starch by using two novel enzymes derived from definite mesophiles, e.g. Arthrobacter, Brevibacterium, Micrococcus and Rhizobium, as well as from the hyperthermophilic archaeon Sulfolobus shibatae (Lama et al., 1990; Nakada et al., 1996; Di Lernia et al., 1998). These enzymes are nominated as maltooligosyl-trehalose synthase and maltooligosyl-trehalose trehalohydrolase. Though, the thermostable enzyme, e.g. from Thermus caldophilus with optimum activity at 65 C, seems to be more suitable for the reason that the higher conversion temperature avoids contamination of the reaction combination by micro-organisms.

3.5. Cyclodextrin Synthesis:

Starch debasing enzymes are also used for the manufacture of cyclodextrins. In the first stage of this procedure both -amylases and pullulanases are intricate in forming unbranched oligosaccharides. Consequently, the resulting linear molecules are hewed by cyclomaltodextrin glucanotransferase, and enzyme first isolated from Bacillus macerans, to produce oligosaccharides of 6-8 units. As a value of the helical structure of these oligosaccharides, the two ends of each molecule are in close vicinity to each other, hence they are easily combined together to form the ring organization characteristic of cyclodextrins. The final product is a mixture of α - , β- and γ-cyclodextrins, poised of six, seven or eight α -1, 4-linked glucose residues. The hydroxyl groups of a cyclodextrin molecule are situated on the surface of the oligosaccharide ring; however its interior is apolar and can effortlessly form insertion complexes with hydrophobic compounds of sufficient size and structure. This assets makes cyclodextrins apt for numerous applications in the food, cosmetics and pharmaceutical businesses, since they can detention unwanted tastes or odours, steady unstable compounds and upsurge the solubility of hydrophobic materials in water. For instance, cyclodextrins are used for the debittering of citrus juices, shielding lipids against oxidation or for the deduction of cholesterol from eggs (Shaw et al., 1984; Szejtli, 1982).

3.6. Significance of Amylolytic Enzymes in Food Processing:

Starch hydrolysing enzymes play a important role in the treating of some raw food materials, particularly in the baking and brewing industries as well as in the manufacture of soft and alcoholic drinks. The enzymes essential for these dedications are frequently natural constituents of raw food materials, e.g. α- and β-amylases in flour, or are obtained from malt or other preparations attained from higher plants and micro-organisms.

In the baking industry, α - and β -amylases of the cereal grain play a vital role. Though, their content in flour be contingent on the climatic situations during ripening and harvesting. When the weather is very humid the grain starts to grow and the content of amylolytic enzymes is too high for the preparation of good quality bakery merchandises. In compare, the flour attained from cereals refined in a hot and dry climate frequently has a very low α -amylase content and its deficit need to be accompanied. α - and β-amylases have dissimilar but opposite functions during the bread making procedure (Martin and Hoseney, 1991). The α-amylases break down starch into low-molecular weight dextrins. β-amylase changes these oligosaccharides into maltose which is essential for yeast growth. Inadequate amounts of fermentable sugars diminish the secretion of carbon dioxide leading to partial dough rise and decreased crumb volume. Fitting levels of amylolytic enzymes are particularly vital during bread making for the formation of dextrins which subsidize to the browning of the crust; add flavour to the bread as well as influencing the degree of staling retardation. Staling is chiefly initiated by starch retrogradation leading to limited water holding capability and abridged crumb elasticity. The contrivance of starch retrogradation is still not well

understood. Though, it is known that vulnerability to retrogradation hinge on on the amount of linear amylose existing in starch and can be reduced when the side chains of branched amylopectin molecules are reduced by the action of maltogenic α-amylases (Christophersen and Otzen, 1998).

In the baking manufacturing glucoamylases are also used to assist the conversion of starch into fermentable sugars. They are particularly required to increase bread crust colour which is the result of Maillard reactions and exaggerated by released glucose. Glucoamylases are also added in mixture with fungal α - amylases to chilled or frozen dough for the reason that they guarantee the existence of adequate quantities of fermentable sugars for yeast when it is time for baking. Amylolytic enzymes are also used for the creation of the low-molecular weight carbohydrates exploited by yeast increasing on starch-containing materials during brewing or the production of alcoholic drinks. In the traditional brewing procedures α - and β -amylases as well as proteinases initiate from barley grains sprouted for a period of about seven days. This is tailed by a procedure of kilning in which the grain is heated in order to develop colour and flavour. During the mashing stage the enzymes degrade the starch and proteins present in the malt and the additives organized from crushed starchy cereals such as maize, sorghum, rice or barley. The combination is then strained and the clear liquid is boiled in order to deactivate the enzymes. The yields of the enzymatic degradation of the malt and additives i.e. simple sugars, amino acids and oligopeptides are exploited by yeast, e.g. Saccharomyces cerevisiae for the manufacture of alcohol and carbon dioxide, new yeast cells and flavouring constituents.

Substantial savings can be attained by substituting some of the malt by unmalted cereals and commercial α -amylases, β -amylases, glucoamylases, β -glucanases and proteinases. This allows better control of the procedure for the reason that the content and activity of the enzymes in the malt are highly inconstant. Native starch from cereals is resistant to enzyme action and wants to be gelatinized at a raised temperature. Though, gelatinized cereals are very viscous and difficult to handle. Application of thermostable α - amylase at this stage of the procedure prevents an unwanted rise in viscosity.

Immobilized glucoamylases are used in the recent technologies of low-calorie beer production. Under traditional brewing situations a large amount of starch is changed into non-fermentable dextrins which are conceded through to the final product. Passing the agitating beer through a reactor containing immobilized glucosidase leads to the break-down of these dextrins to glucose which is then almost entirely transformed into alcohol. Moreover, none of enzyme pollutes the final product.

CONCLUSION

The incorporation of enzymes in food and feed procedures is a well-established method, but indication evidently displays that devoted research efforts are constantly being made as to make this application of biological agents more operative and/or diversified. The usage of α-amylase in starch based productions has been widespread for several decades and a number of microbial sources are existent for the effectual production of this enzyme, but only limited selected strains of fungi and bacteria meet the benchmarks for commercial production. More recently, several authors have presented good results in increasing α-amylase purification methods, which support applications in food industry which need high purity amylases. In this paper we saw the α-amylase structure, Debranching enzymes, and other types of amylases, then enzymatic processing of starch and starch-containing food (Starch hydrolysates production) and finally saw the Significance of Amylolytic Enzymes in Food Processing in detail.

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