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An Overview Enzymatic Modification of Starch by Different Starch Hydrolyzing Enzymes

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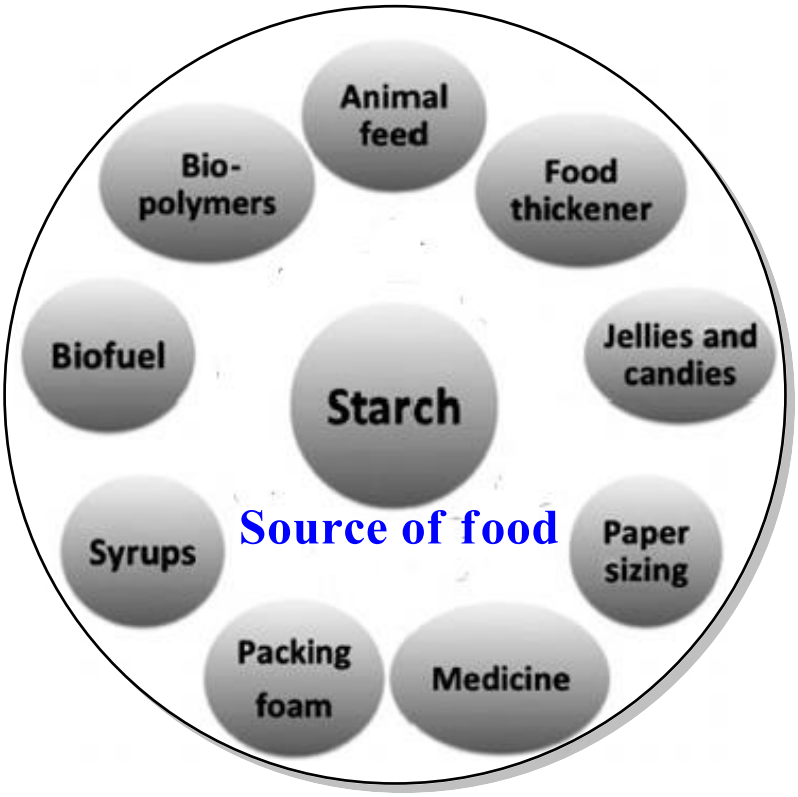
Graphical Abstract



Various Applications of Starch

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ABSTRACT:

The most common naturally occurring carbohydrate reserve in plants is called starch, and it can be found in grains, legumes, tubers, roots, and even young fruits like mangos and bananas. Usually used as a food addition, starch improves a product's quality in several ways, including pharmaceuticals, thickening, stabilizing, and texture enhancement. Microorganisms are useful to man for their natural roles in the biosphere and because they can be deliberately exploited in several ways. This exploitation is increasing rapidly and it forms the basis of the current revolution in biotechnological industries. Enzymes in industrial processes have been used for several decades. The use of enzymes of animal origin like papain in the tenderization of meat has been practiced for a long back. However, the large-scale extraction of enzymes of animal and plant origins has been difficult and costly. Therefore, efforts were made to use microorganisms as an alternate source of industrial enzymes. Microbes as the source of enzymes have the advantage of large-scale fermentation, ease in isolation and purification of target enzymes, and easy genetic manipulation to enhance enzyme yields. Amylases are starch hydrolyzing enzymes that break down starch into small molecular weight products like dextrin, malt oligosaccharides, maltose, and glucose. Depending on the type of sugar linkages they hydrolyze and the products formed, starch-hydrolyzing enzymes are of different categories like α-amylase, β-amylase, glucoamylase, and debranching enzymes. Even though amylases can be found in a variety of creatures, including plants, animals, and microbes, the enzymes derived from microbial sources typically have potential uses in several commercial processes, including those in the food, pharmaceutical, textile, and paper industries. In the starch processing sectors, microbial amylases have effectively taken the place of chemical hydrolysis of starch.

Keywords: Starch, Degradation, Enzymes, Modification, Starch.

1. Introduction

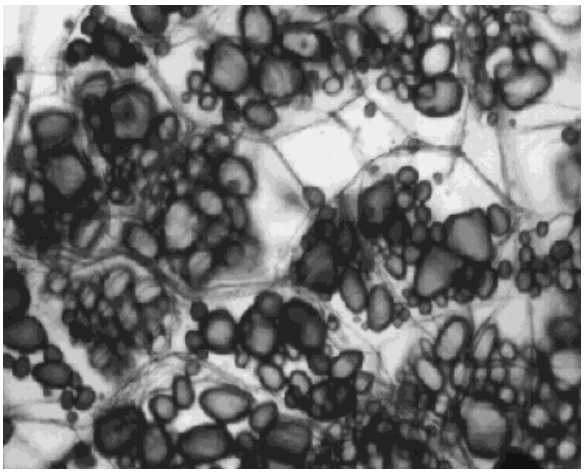
Natural starch is rarely frequently employed due to its limited solubility, poor functional quality, and low resistance to a wide range of processing conditions. The rapid retrogradation, low shear resistance, and weak freeze-thaw stability of starch limit its use in industrial environments. Different modification strategies can be used to deal with these innate issues. Enzymatic modifications, which partially replace chemical and physical processes for the synthesis of modified starch, have gained favor in recent decades since they are safer, better for the environment, and better for food consumers than chemical procedures. A variety of enzymes, including pullulanase, xylanase, glucose isomerase, α-amylase, and β-amylase, are employed in the modification of starch. The features of modified starch, particularly the freeze-thaw stability of gels and the delay of retrogradation during storage, were directly impacted by the enzymatic modification of the starch molecules. The combination of enzymatic modification produced a significant rise in resistant starch, and starch that has been changed by enzymes is a useful fat substitute. It can offer the necessary functional qualities and is environmentally friendly. Biopolymers, which include cellulose, starch, pectin, xylan, and lignin, are widely found in nature and make up a significant portion of living organisms. Starch is the main carbohydrate that is photosynthesized by plants using sun energy, after cellulose. In human diets, this polysaccharide is easily absorbed. Cereal grains, legumes, and tubers are the main sources of starch (Guilboat and Mercier, 1985). According to Guzman-Maldonado and Paredes-Lopez (1995), starches can assist food products to perform better and are a source of various oligo, di, and monosaccharides.

Starch is the most abundant, least expensive, and most biodegradable of all the naturally occurring polysaccharide and carbohydrate reserves found in leaves, flowers, fruits, seeds, different kinds of stems, and roots of plants. The main locations of starch production and storage in cereals (40–90%) are endosperm, roots (30–70%), tubers (65–85%), legumes (25–50%), and some immature fruits, like bananas or mangos, which contain roughly 70% of starch by dry weight. According to Kritika and Ratnamala (2019), starch is made up of glucose molecules combined with a combination of two polymers known as amylose and amylopectin (Park and Kim, 2021) shows that amylopectin molecules are a branching chain of glucose units connected by α-1, 4 and α-1, 6 glycosidic links, whereas amylose is a linear polymer with α-1, 4 glycosidic bonds (Figure 1). During cellular respiration, starch releases glucose, accounting for up to 80% of Earth's energy. One important energy source is starch. Starch is one of the most widely distributed biodegradable polymers in nature and is used in many different industries, including food and beverage, bioplastic, paper, textile, and biofuel. Starch has attracted a lot of attention because of its simplicity of manufacturing, relative abundance, non-toxicity, and biodegradability. However, native starch utilization is often limited due to its limited tolerance to a wide variety of processing conditions, constricted solubility, weak functional features, poor thermo-mechanical properties, and enhanced water absorptivity. Therefore, native starch needs to be modified before usage (Amaraweera et al., 2021, Yu et al., 2021). To improve certain aspects of the product's quality, such as moisture retention, starch is crucial for development. It can be used as a vehicle for the delivery of chemicals that are useful to the food and pharmaceutical industries, such as flavors, colorants, antioxidants, and pharmaceutically active proteins, among other things (Abegunde et al., 2013). The use of starch in industrial applications is limited by its poor freeze-thaw stability, high retrogradation, and low shear resistance. These inherent drawbacks can be addressed through a variety of modification techniques, including chemical, physical, enzymatic, and genetic methods (Tang et al., 2020, Bensaad et al., 2022). Since the enzymatic modification method is considered "Clean Label TM ingredients" because it is free of artificial and synthetic ingredients and can provide desired functional characteristics, it has garnered significant attention from academia

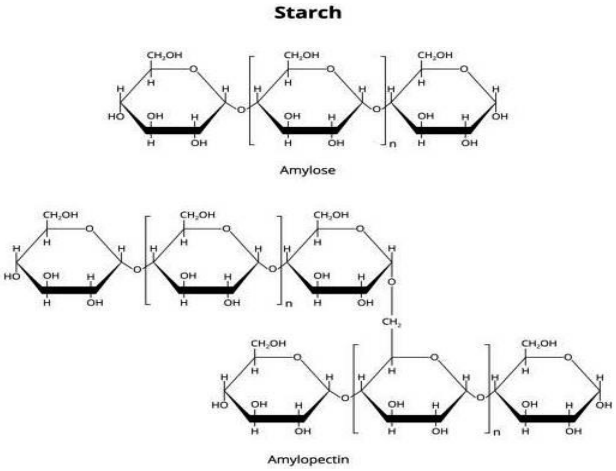
and the corporate sector recently (Zhang and Bao, 2021). Additionally, the method is environmentally friendly (Park and Kim, 2021).

Starch

Starch, a glucose polymer, is made up of two high molecular weight compounds, namely amylose (15-25 %) and amylopectin (75-85 %). Due to differences in the structure of amylose and amylopectin they show significant differences in their physical properties (Antranikian, 1992). Amylose consist of a linear chain in which the glucose residues are linked between C1 and C4 in the alpha orientation. The chain length varies from a few hundred to 6000 residues (Swinklers, 1985). Amylopectin on the other hand is a branched polymer containing α-1-4–linked glucan with α-1-6-linked branched points at every 17-26 glucose residues. It, therefore, contains around 5 % α-1-6-glycosidic bonds. This molecule is one of the largest molecules in nature and may have a molecular weight of more than one million (Swinklers, 1985) (figure 1).



Stained starch granules



Starch molecule Figure 1.

Hydrolysis of Starch

Historically, starch was hydrolyzed by mineral acid (Guzman-Maldonado and Paredes-Lopez, 1995). The major disadvantages of acid hydrolysis were the low glucose yields the formation of large amounts of salt and the need to use corrosion-resistant equipment (Clazer and Nikaido, 1995). The activities of microbial enzymes indirectly in the form of microbial cells have been observed and even utilized for centuries. However, it has been only in recent times that the use of microbial enzymes has been produced on a large scale and commercialized. Thus, although ancient civilizations are known to have employed crude fungal preparation to bring about starch hydrolysis for brewing, the first commercial use of fungal enzyme was made at the end of the nineteenth century. Takadiastase was the first enzyme that was produced industrially and used as a pharmaceutical agent in the United States in 1894 to cure digestive disorders (Cruger and Cruger, 1985). Since that time microbial amylases have found application in several areas and their commercial production has increased enormously. Amylases are one of the largest volume industrial enzymes on the market (Crueger and Crueger, 1985) and are used in numerous and various processes (Fogarty *et al*., 1974; Rajanikanth and Ravi, 1998).

Steps Involved in Starch Hydrolysis

(1) Liquefaction,

Liquefaction of starch is necessary to allow the efficient production of dextrose by saccharifying enzymes (Ingle and Erickson, 1978). It was done by acid treatment earlier but

due to various disadvantages, it has been replaced by enzymatic treatment (Crueger and Crueger, 1985). The α-amylases are used with continuous starch cooking by direct steam contact. This process is called 'jet cooking' in commercial terms. This type of process provides improved process control, which produces a consistent control of saccharides (Ingle and Erickson, 1978). The liquefaction is carried out at pH 6.0-6.5 by a thermostable α-amylase from *B. licheniformis*. This process is first conducted at 105 ^{0}C for 5 min and then at 95 ^{0}C for 2 h. Ca^{2+} ions are required for the activity and stability of the enzyme. This step causes the thinning of starch by breaking down amylose and amylopectin. The products formed are dextrins and small amounts of oligosaccharides (Jensen and Norman, 1984).

Saccharification

Saccharification is the process of production of free sugar (glucose) from the starch or its liquefaction products like dextrin, maltose, and malto-oligo-saccharides. For saccharification usually a glucoamylase from *Aspergillus niger* is used at pH 4.5-5.0 and temperature 60 ^{0}C. This enzyme converts various polymers to glucose. If maltose is desired β-amylase from fungi or plants has to be added. To obtain a higher dextrose concentration or maltose syrup, pullulanase from *B. acido pullulyticus* is added (Jensen and Norman 1984). Due to the absence of more suitable enzymes, the process conditions have to be varied in the second step. The pH and temperature have to be lowered to 4.5 and 60 ^{0}C, respectively. Furthermore, longer incubation times (48-72 h) are needed for completion of the reaction.

Isomerization,

The production of high fructose syrup, which is an excellent alternative to sucrose, can be achieved by the conversion of glucose by glucose isomerase obtained from *B. coagulans* (Coker and Vankatasubramanian, 1985) (Fig.2).

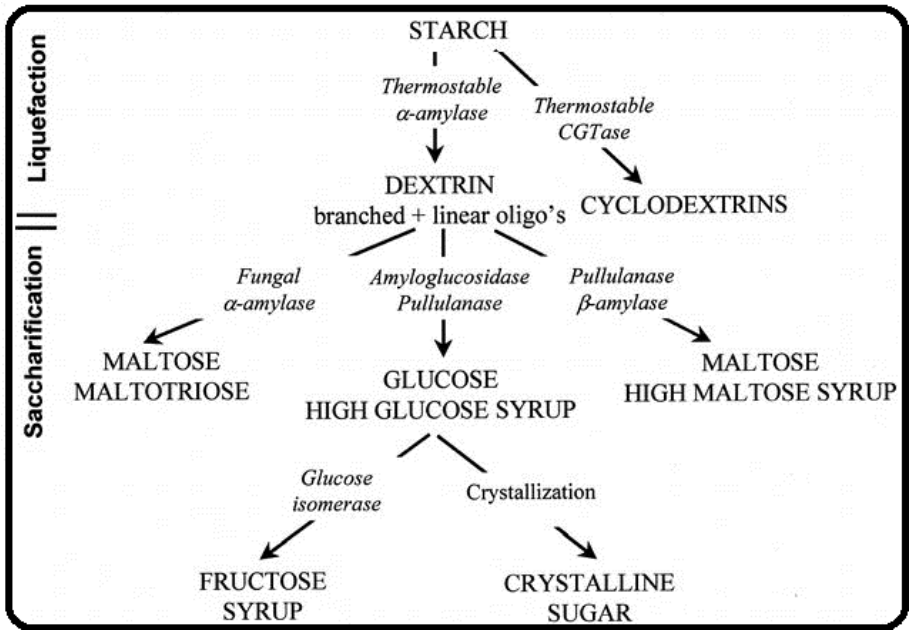


Figure 2. Bioconversion process of starch to various sugars.

Starch Modifying Enzymes

Enzymes are versatile catalysts largely used in industry. The starch industry – considered in terms of either starch extraction from raw material or starch modification – uses huge amounts of enzymes (glucoamylase, glucose isomerase, α-amylase, β-amylase, pullulanase, xylanase, fungal acid protease and so on) (Vitolo, 2020). Enzymes are biocatalysts

obtainable from plants, animals, or microorganisms. They are widely used to improve the various aspects of food processing and are generally recognized as safe (GRAS) (Obadi and Xu, 2021). Several enzymes, including α-amylase, β amylase, glucoamylase, debranching enzymes, and cyclodextrin glycosyltransferase, among others are used in enzymatic alteration to starch (Wang *et al.,* 2020) and various enzymes employed for starch modification have been shown in Figure 2 (Bangar *et al.,* 2022). Alpha-amylase is an enzyme that can hydrolyze the bonds of α-1,4-glycosidic randomly at the end of the non-reduction site and can reduce the size of starch molecules and the viscosity of starch paste solution. Beta-amylase is an enzyme that can hydrolyze α-1,4-glycosidic bonds regularly at the end of the non-reduction site to remove maltose residue, shorten the external branch chain, as well as increase branch density. Glucoamylase is an enzyme that can cut the bonds of α-1,4-glycosidic at the end of the non-reducing site and slowly hydrolyze the bonds of α-1,6-glycosidic. Pullulanase is an enzyme that catalyzes the hydrolysis of 1,6-bonds at pullulan, amylopectin, and oligosaccharides. Usually, pullulanase is combined with glucoamylase or β-amylase to break down starch during the saccharification process. Like pullulanase, isoamylase (glycogen-6-glukanohydrolase) can hydrolyze bonds α-1,6- glycosidic in amylopectin to produce amylose and oligosaccharides. The branching enzyme cuts down the α-1,4 bonds on the linear chains of amylose and amylopectin followed by a transglycosil reaction to create a new bond with the α-1,6 bond (Li *et al.,* 2017, Hutabarat and Stevensen, 2023). Figure 3, depicted the enzymatic hydrolysis of starch pullulanase, isoamylase, and oligo-1,6-glucosidase are mainly used to debranch the α-1,6-glycosidic bonds in starch, giving linear amylose whereas amylosucrase, α-amylase, and amylomaltase are capable of debranching the 1,4 and 1,6 glycosidic bonds, resulting in small amylose or amylopectin chains (Amaraweera *et al.,* 2021).

Enzymatic Modification of Starch

Enzymatic modifications are adopted partly replacing the physical and chemical methods for the formation of modified starch as enzymes are safer and healthier than chemical methods for both the consumers and the environment (Park *et al.,* 2018; Harder *et al*., 2015). Enzymatic modification is the exact way to alter starch digestibility, including α-amylase, pullulanase, and branching enzymes. The enhanced ordered arrangement of structure and the higher proportion of linear short chains in enzyme-modified starch inhibit the digestive enzymatic hydrolysis resulting in higher resistant starch (Zhang and Bao, 2023). Various enzymes have been added to starches to increase the content of resistant starch (Sorndech *et al.,* 2016; Kim *et al*., 2015), to inspect the impact of starch structure/type on the degradation of starch (Naguleswaran *et al.,* 2014) and for malt production (Chu *et al.,* 2014). Starch is modified by hydrolysis and broken down into smaller units by cutting the glycosidic bonds. Glycosidic bonds of the starch are hydrolyzed or cut by hydrolyzing enzymes to form polymeric or oligomeric compounds and these enzymes are divided into three as endo-acting enzyme α-amylase which breaks α-1,4 bonds in amylose and amylopectin. Exo-acting enzymes act by breaking the glycosidic bonds of the nonreducing ends successively of the substrate producing monomeric or oligomeric compounds. Exo-acting enzyme β-amylase works to break α-1,4 bonds successively on amylose and amylopectin while pullulanase a debranched enzyme works in breaking α-1,6 glycosidic bonds (Bertoft, 2013, Hutabarat and Stevensen, 2022) Enzymatically modified starch was used in the microencapsulation of ascorbic acid (AA) by prepared microcapsules using an enzyme (α-amylase and glucoamylase) -treated corn starch with 16h (ETCS 16h-GA) and 20 h (ETCS 20h-GA) of hydrolysis time and coated with gum (GA). The study suggests that ETCS 16h-GA microcapsules lost less AA during storage time, show greater stability in the protection of AA allow for a controlled release in the gastrointestinal tract (GIT), and are an efficient way of encapsulating functional food ingredients (Leyva-Lopez *et al.,* 2019). Enzymatic hydrolysis modifications of Potato Starch

Granules using Pullulanase enzyme followed by nanoprecipitation of the hydrolysates obtain very tiny starch nanoparticles sized between 20 and 50 nm, much smaller than the native starch granules of average size 10 μm. The most promising results were obtained with a Pullulanase enzyme concentration of 160 npun/g of starch, at a temperature of 60°C in a pH 4 phosphate buffer solution resulting in the production of hydrolysates containing starch polymers with low molecular weights (Chorfa *et al.,* 2022). Dual-enzymatic modification of maize starch using b-amylase and transglucosidase decreased the molecule weight while the amount of short chains and a-1,6 linkages increased. A maximum SDS content (33.5%) was obtained using double enzyme hydrolysis for 6 hr compared to native starch. The results suggested that starches using combined b-amylase and transglucosidase treatment not only increased the amount of short chains accompanied by shortening long chains but also produced an increase of a-1,6 linkages, which led to a slower digestion property (Miao *et al.*, 2014).

Nanostarch production by enzymatic hydrolysis of cereal (maize) and tuber (potato and cassava) crops, and conventional acid hydrolysis process. The nanostarch produced by the enzymatic process from maize is smaller than that of potato and cassava i.e., 18, 29, and 41 wt% for maize, potato, and cassava starches, respectively and the melting enthalpies of nanostarches were lower than their bulk counterparts. The acid hydrolysis resulted in a much smaller size of 18±3 nm of nanostarch as compared to that of enzyme hydrolysis162 ± 23 nm however, the eco-friendly enzymatic process will add to its value for diversified applications (Dukare *et al.,* 2021).

Enzymatic modification of cassava starch (*Corpoica M-Tai*) around the pasting temperature with a commercial α-amylase, presented dextrose equivalents (DE) ranging between 13 and 92%, yield (Y) between 0 and 45.5%. The Scanning electron microscope (SEM) analysis showed that the granules suffered exo-corrosion in agreement with the degree of hydrolysis and the crystallinity degree increased following modification. The native starch showed greater setback and amylose values than hydrolyzed starches, which shows that it is more susceptible to retrogradation or water loss (syneresis) (Jairo Salcedo-Mendoza *et al.,* 2018). Modified taro starch is prepared by enzyme modification (α-amylase) and used as a stabilizer in ice cream with effective overrun, foam stability, and viscosity. EMTS prepared by treating the taro starch with α-amylase resulted in the improvement of functional properties Viz. Swelling power (18.28 g/g) and enzyme digestibility (62.7%) which were higher than that of extracted starch Swelling power (17.43 g/g) and enzyme digestibility (59.32%). It was also noticed that the 0.5% incorporation of EMTS resulted in an improvement in the ice cream stability and overall quality with improved viscosity (110.21Cp) and overrun (65.67%) which might be due to high swelling or gas retention capacity of α-amylase modified taro starch (Choton, *et al.,* 2024).

Enzyme-modified sweet potato starch was used with khoa viz., 0:100, 10:90, 20:80, 30:70, and 40:60 respectively to replace fat in the formulation of ice cream. It was noticed that there was a remarkable decrease in fat content from 9.85 to 5.79 percent with increased enzyme-modified starch level. It concluded that the use of enzyme-modified sweet potato starch can be well utilized as a fat replacer in the preparation of ice cream (Kale *et al.,* 2020). The combined enzymatic modification of corn starch with thermostable α-amylase and pullulanase resulted in a marked increase in resistant starch. Corn starch was dually modified using thermostable α-amylase and pullulanase to prepare resistant starch (RS) and a maximum resistant starch content of 10.75% was obtained using 15% starch liquid, 3 U/g thermostable α-amylase, 35 min of enzymatic hydrolysis and 8 U/g pullulanase significantly higher than that of native and single enzyme-treated starch (Liu *et al.,*2022). Enzymatically modified potato starch with the help of amylosubtilin and amylase from Bacillus licheniformis is used as the fat replacer. The features of yogurt formulated with modified potato starch of amylosubtilin and amylase are similar to those of native potato starch-formulated yogurt, and can be used as an alternative in a low-fat yogurt (Nikitina *et al.,* 2019).

Sopawong *et al. (*2022) modified native lotus seed flour (N-LSF) by different methods, namely, partial gelatinization (PG), heat–moisture treatment (HMT), or pullulanase treatment (EP). PG increased rapid digestible starch (RDS) and decreased resistant starch (RS) while HMT and EP increased amylose and RS contents to 34.57–39.23% and 86.99–92.52% of total starch, respectively. Pullulanase-treated enzyme-modified flour showed more rapid pasting and much larger changes in viscosity during the pasting cycle than other modified flours and improved the thickening effect of lotus seed flour while maintaining the low glycemic value. Ulbrich *et al. (*2021) modified granular potato starch using the debranching enzyme isoamylase in aqueous suspension (40% w/w) at 35 °C by grading the volume (100, 250, and 400 μL/50 g of starch, 200 U·mL−1) of enzyme solution added. A slight reduction of the weight average molar mass of the amylose fraction and partial molecular degradation of the starch polysaccharides showed that debranching enzymes like ISO and PUL could be an alternative to acid-involved processes. Zhang *et al.* sequential changes of amylopectin by amylosucrase and pullulanase in which elongation of side chains of amylopectin by amylosucrase, followed by debranching with pullulanase produces linear chains that self-assembled to form microparticles containing aggregated nanoparticles 200-400 nm that were suspendible and lost gelation properties and increased the content of resistant starch from 9.1% of native amylopectin to be as much as 67.2% of microparticles (Zhang *et al.* 2019). The enzymatic treatments changed the structures of modified starch to produce functional starch microparticles with reduced digestibility providing an effective to manufacture new modified starches.

Amylases in Starch Hydrolysis

A family of enzymes called 'amylases' brings about the above process of starch liquefaction and saccharification. Broadly speaking, amylases are extracellular enzymes that hydrolyze starch molecules to give such diverse products as dextrin and progressively smaller polymers composed of glucose units. This family of enzymes has been well characterized through the study of various microorganisms. The amylases are classified in various ways depending on how they act on the starch molecules (figure 3).

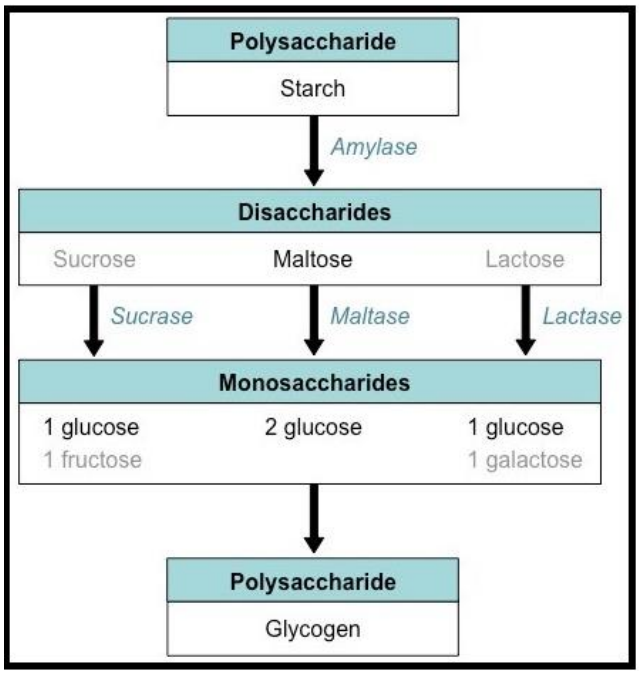


Figure 3. Degradation of starch by different types of amylases the various categories of enzymes are

α- amylases,

α-amylases (endo 1, 4-α-D-Glucan Glucanohydrolase, EC 3.2.1.1) are extracellular enzymes which randomly hydrolyze α-1-4– glycoside bonds of amylose or amylopectins. These enzymes are endoenzymes, splitting the substrate in the interior of the molecule. Their action is not inhibited by α-1-6–glycosidic bonds present in amylopectin, this enzyme is capable of bypassing the branching points (Selvakumar *et al*., 1996). Its action on starch, therefore, causes the formation of linear and branched oligosaccharides of varying lengths (fig. 3). Such branched oligosaccharides are also called α-limit dextrins. The end products have α configuration at C1. α-Amylase action is accompanied by a rapid loss of viscosity. Prolonged hydrolysis also produces a mixture of glucose, maltose, and maltotriose (Antranikian, 1992).

β- amylases

β-amylases (1, 4-α-D-Glucan Maltohydrolase, EC 3.2.1.2) are exo-acting enzymes that occur usually in plants and a few microbes. They hydrolyze α-1-4– linkages from the non-reducing end of the polysaccharide (fig. 3). It causes the inversion of the anomeric configuration of the liberated maltose to beta. These breaks alternate bonds in the polymer, that is, they cleave two glucose molecules at a time thus producing maltose. These enzymes are incapable of acting at branching points (α-1-6-linkages) of amylopectin, hence producing a high molecular weight product called β-limit dextrin. In β-limit dextrins, all of the original branch points are left unattacked (Robyt and Ackermann, 1971; Kainuma *et al*., 1972; Sakano *et al*., 1982).

Glucoamylases

Glucoamylases (1, 4-α-D-Glucan Glucanohydrolase, EC 3.2.1.3) like β-amylases are exoenzymes that hydrolyze single glucose residue from the non-reducing terminals of amylose and amylopectin in a stepwise manner (fig. 3). These enzymes are also referred to as amyloglucosidases, glucogenic enzymes, starch glucogenases, gamma-amylases (Salvakumar *et al*., 1996). Unlike the α-amylases, most glucoamylases are also able to hydrolyze the α-linkages at the branching points of amylopectins, although at a lower rate than the 1,4-linkages. The action of this enzyme liberates one molecule of β- D-glucose at a time causing the complete conversion of polysaccharides to glucose. Prolonged incubation time and high concentration of starch usually cause reversion of the reaction catalyzed by glucoamylase. This results in the formation of maltose, isomaltose, and some oligosaccharides (Antranikian, 1992).

α- Glucosidase

α-Glucosidase (α-D-Glucosidase Glucohydrolase, EC 3.2.1.20) attacks the α-1,4 and/or α-1,6 linkages from the non-reducing ends in short saccharides which are usually formed by the action of other amylolytic enzymes. Unlike the glucoamylase action, the α-glucosidase action liberates glucose with an α-configuration. α-Glucosidase has a very low affinity to polysaccharides and therefore attack starch at a very slow rate. Since most enzymes show high activites towards maltose they were also referred to as maltases. α-Glucosidase appears to be the final enzyme involved in the metabolism of starch. It was shown that α-glucosidases are highly specific for the glucose residues but specificity for the aglycone portion of the substrate is not distinct. Therefore, maltose derivatives can also act as substrates (Gottschalk, 1950).

Isoamylase

Isoamylase (Glycogen 6-Glucanohydrolase) is specific for α-1,6-linkages in polysaccharides like amylopectin, glycogen, and branched dextrins. Isoamylase has a higher affinity to large-branched polysaccharides rather than short-branched substrates (Marshall, 1975).

Distribution and Properties of Microbial Amylases

Amylases have been found to occur in various groups of aerobic and anaerobic microorganisms.

α- amylase

This endoacting enzyme is widespread among aerobic as well as among anaerobic microbes. Most enzymes were found to require Ca^{2+} ions for activity and stability. The most thermostable α-amylase known so far has temperature optima of above 100 ^{0}C (Brown *et al*., 1990; Koch *et al*., 1990).

Occurrence among Aerobes

A large number of aerobic bacteria are capable of growing on starch between 30 - 70 ^{0}C. The majority of enzyme producers are mesophiles. A large number of these organisms belong to the genus *Bacillus*. The temperature optimum of α-amylase of the mesophilic bacterium *Bacillus licheniformis* which grows at 30 ^{0}C, is exceptionally high and it is optimally active at 90 ^{0}C (Morgan and Priest, 1981). The α-amylases produced by *B. stearothermophilus* and *B. acidocaldarius,* which grow at 55 and 50 ^{0}C are optimally active at 80 and 70 ^{0}C, respectively (Kanno, 1986). Amylases with extreme pH optima like 3.5 or 10.5 are produced by *B. alcalophilus* which grow optimally at extreme pH values, pH 2-4.5 and 9-10.5, respectively (Kanno, 1986). Calderon *et al*. (2003) isolated *Lactobacillus fermentum* Ogi E1, from benin maize sourdough. It has a pH optima of 5.0 but it is acid tolerant. Other α-amylase-producing bacteria include *Pseudomonas, Arthrobacter, Escherichia, Proteus, and Serratia* (Crueger and Crueger, 1985). Amylolytic activity was detected in two Sulfolobales (*S. acidocaldarius* and *S. solfataricus*) with an optimal growth temperature of 88 ^{0}C (Haseltine *et al*., 1996; Kengen *et al*., 1996). Commercially two types of bacterial enzymes have been produced and used. One is an amylase derived from *Bacillus subtilis* (variant *amyloliquefaciens*) which has been used for many years. This enzyme has an optimum temperature in the presence of Ca^{2+} ions of around 65–75 ^{0}C but is active at 82 ^{0}C (MacAllister, 1979; Fullbrook, 1984).

The other enzyme derived from *Bacillus licheniformis* was first introduced around 1973 (Madsen *et al*., 1973). It is active and stable in starch solution at temperature >90 ^{0}C, relatively independent of Ca^{2+} ions for stabilization, and is more active over a wide pH range (Slott *et al*., 1974; Rosendal *et al*., 1979). Few halophiles such as *Acinetobacter* sp., *Halobacterium halobium, and Micrococcus halobius* produce enzyme that requires Na^+, K^+, and Ca^{2+} ions for activity and stability (Onishi, 1972; Onishi and Hidaka, 1978). Extracellular amylase production by the moderate halophile *Halomonas meridiana* was studied by Coronado *et al*. (2000). The amylase exhibited maximal activity at pH 7.0, being relatively stable in alkaline conditions. The optimal temperature and salinity for activity were 37 ^{0}C and 10 % NaCl, respectively. Few lactic acid bacteria like *Streptococcus* sp., *Micrococcus halobius,* and *Lactobacillus cellobiosus* produce α-amylases (Hobson and Macpherson, 1952; Onishi, 1972; Sen and Chakrabarty, 1984).

Some actinomycetes also elaborate α-amylases. α-amylase-producing actinomycetes belong to the genera *Streptomyces, Thermoactinomyces*, and *Thermomonospora*. α-Amylase produced by these actinomycetes is optimally active between 35 ^{0}C to 65 ^{0}C and pH 5.0-7.0 (Fairbairn, 1986). *Thermomonospora curvata* and *Thermoactinomyces vulgaris* have been reported to produce α-amylase (Ingle and Erickson, 1978) which had temperature optimum of 60 ^{0}C and 70 ^{0}C, respectively. Uguru *et al*. (1997) isolated a strain of *Thermoactinomyces thalophilus* from flour mill wastes, which was capable of growing on sorghum and producing an extracellular amylase, in shaken flask cultures. The enzyme was thermostable with optimum activity at a temperature of 90 ^{0}C and pH 5.0. The α-amylases produced by the fungi

Aspergillus, Fusarium, Mucor, Humicola, and *Trichoderma* are optimally active from 22 ^{0}C to 55 ^{0}C, and their pH optimum is slightly acidic (Bhumibhamon, 1983; Chary, 1985). Generally, yeast does not utilize starch, as they are not able to synthesize and secrete extracellular amylases (Casida, 1996). However, in recent years several amylolytic species, belonging to genera *Endomyces, Endomycopsis, Saccharomycopsis, Schwanniomyces, Lipomyces, Trichosporon, Candida,* and *Filobasidiomyces* have been reported to utilize starch (Clementi *et al*., 1980; Clementi and Rossi, 1986; Abouzied and Reddy, 1987; Gogoi *et al.*, 1998). Amylases have been reported from *Lipomyces starkeyi* (Mouldin and Galzy, 1979) and many species of *Schwanniomyces* including *S. castelli, S. alluvius* and *S. occidentalis* (Clementi *et al*., 1980; Dhawale and Ingledew, 1983; Sills *et al*., 1983, 1984; Clementi and Rossi, 1986).

Occurrence in Anaerobes

Unlike aerobic microorganisms less extensive research has been carried out concerning the production of polysaccharides degrading enzymes. Recent studies have shown that anaerobic organisms produce various starch hydrolyzing enzymes (Antranikian, 1990). These organisms belong to the genera *Clostridium, Thermoanaerobacter, Thermoanaerobium,* and *Thermobacteroides* and their optimal growth temperature is between 60 ^{0}C and 65 ^{0}C. *Dictyoglomus thermophilum* on the other hand requires higher temperature (i.e. 78 ^{0}C) for growth (Saiki *et al*., 1985). Interestingly, most of the anaerobes thermophiles investigated secrete amylases that are optimally active above their growth temperature, the highest temperature optimum being 90 ^{0}C. All α-amylases from anaerobic bacteria are optimally active at slightly acidic pH values which range between 4.5 and 5.5. Compared to aerobes the level of extracellular enzymes from anaerobes is low. A significant increase in the level of extracellular α-amylase is achieved after the optimization of cultivation conditions (Antranikian *et al*., 1987).

Extremely thermo-active α-amylases have been characterized by hyperthermophilic archaebacteria. Amylolytic activity was detected in *Thermoproteales (Thermoproteus tenax), Desulfurococcales (Desulfurococcus mobilis, Desulfurococcus mucosus, Staphylothermus marinus*), and *Thermococcales (P. furiosus, P. woesei)* (Bragger *et al*., 1989; Canganella *et al*., 1994). *P. woesei* and *P. furiosus* secrete the most thermo-active and thermostable enzymes known so far. These microbes grow optimally at temperatures above the boiling point of water (Zilling *et al*., 1987; Fiala and Statter, 1986). The enzyme from *P. woesei* has been purified and its physiochemical properties were determined (Antranikian *et al*., 1990). The enzyme is active between 50 ^{0}C and 140 ^{0}C. Optimal activity is measured at 100 ^{0}C and pH 5.5. Ca^{2+} ions are not required for catalytic activity. This enzyme was still active after autoclaving for 4 h at 120 ^{0}C (Antranikian, 1992).

β- amylase

Unlike α-amylase, this enzyme is rare among microbes and is commonly present in plants. Most β-amylases are active in a neutral pH range and are thermolabile.

Occurrence in Aerobes

Few aerobic bacteria such as the mesophilic bacilli, *Bacillus megaterium,* and *Bacillus polymyxa* produce β-amylase (Thomas *et al*., 1980; Nanmori *et al*., 1985; Kawaza *et al*., 1987). Other amylase producers reported are *B. cereus, B. stearothermophilus, B. subtilis* IMD 198, and *Pseudomonas* sp. BQ06 (Fogarty and Bourke, 1983). Very few actinomycetes and fungi were reported to produce this enzyme.

Occurrence in Anaerobes

The capability of anaerobic bacteria to produce β-amylase seems to be very restricted. *Clostridium thermosulfurogenes* ATCC 33743 grows optimally at 60 ^{0}C and secretes a thermoactive β-amylase; its temperature optimum being 75 ^{0}C (Hyun and Zeikus, 1985; Shen *et al*., 1988). Bacterial amylases have greater heat resistance (> 70 ^{0}C) than plant β-amylases and the pH optimum is also higher. In contrast to α-amylases, calcium is not necessary for stabilization of bacterial β-amylases.

Glucoamylase

Glucoamylases are rare in prokaryotes. It is a typical enzyme of fungi and is also produced by yeasts. Most enzymes are thermolabile and are active in the acidic range (Saha and Zeikus, 1989). Glucoamylases are among the most important industrial enzymes, which are used for the production of glucose syrup.

Occurrence in Aerobes

Bacteria such as *Bacillus stearothermophilus, Flavobacterium* sp., and *Halobacterium sodomese* (Chang *et al*., 1992) were reported to produce glucoamylase. Major sources among various microorganisms are filamentous fungi (Fogarty and Kelly, 1980). Strains of two genera viz, *Aspergillus* and *Rhizopus* are mainly used including *Aspergillus niger, A. oryzae, A. awamori, Rhizopus niveus, R. delemer, R. formasaensis,* and *R. javanicus. Aspergillus niger* is the most commonly used organism (Crueger and Crueger, 1985; Selvakumar *et al*., 1996). In the past *Endomycopsis capsularis* and *Endomyces* sp. have been reported to produce glucomyces. Some more yeast has been reported to produce glucoamylases and these include *Saccharomyces diastaticus* (Yamashita *et al*., 1985).

Occurrence among Anaerobes

Glucoamylases were reported to be produced by *Clostridium acetobutylicum, C. thermoamylolyticum* and *C. thermohydrosulfuricum* (Hyun and Zeikus, 1985; Katkocin *et al*., 1985). Recently a novel thermostable glucoamylase was purified from *C. thermosaccharolyticum*. The enzyme is optimally active at 75 ^{0}C and pH 5.0 (Specka *et al*., 1990).

α- Glucosidase

Unlike the above-mentioned enzymes, α-glucosidase is also present as an intracellular enzyme.

Occurrence in Aerobes

Various bacilli such as *Bacillus subtilis* (Wang and Hartman, 1976), *B. amyloliquefaciens* (Urlaub and Wober, 1978), *B. amylolyticus, B. cereus* (Yamasaki and Suzuki, 1974), as well as *Pseudomonas amylodermosa* and *P. fluorescens* W (Amemura *et al*., 1974; Guffanti and Corpe, 1975; Guffanti and Corpe, 1976), produce α-glucosidases that are active optimally at slight acidic and neutral pH values at temperature between 40 ^{0}C and 55 ^{0}C. In *B. thermoglucosidus* KP 1006 and *Bacillus* sp. KP1035 the enzyme is found intracellularly (Suzuki *et al*., 1976). It can be released at the end of the exponential growth phase by the cell lysis. Some lactic acid bacteria such as *Lactobacillus acidophilus a*nd *Streptococcus pyogenes* (Doolin and Panos, 1969; Kelly and Fogarty, 1983) also form this enzyme. α-Glucosidases that hydrolyze maltose and isomaltose have been detected in fungi such as *A. niger* (Rudick and Elbein, 1974). Some α-glucosidases from *Mucor javanicus* (Yamasaki *et al*., 1973), *M. recemosus* (Yamasaki *et al*., 1977), *M. rouxii* (Flores-Carreon *et al*., 1970), *Penicillium oxalicum* and *P. purpurogenum* (Yamasaki *et al*., 1977) were reported. Yeasts such as

Saccharomyces cerevisiae (Matsusaka *et al*., 1977), *and S. italicus* (Halvorson and Ellias, 1958) synthesize this enzyme. Interestingly, these enzymes have higher activity toward sucrose than towards maltose, a phenomenon that has not been observed with bacterial and fungal enzymes.

Occurrence in Anaerobes

In the culture supernatants of anaerobic bacteria such as *Clostridium thermosulfurogenes* EM1, *C. thermosaccharolyticum, C. thermohydrosulfuricum, C. acetobutylicum, Thermoanaerobium* TOK 6-B1, and *Fervidobacterium* sp., α- glucosidase activity was detected (Antranikian, 1990; Plant *et al*., 1987). The optimum temperature ranges from 65 ^{0}C to 90 ^{0}C. The pH optimum ranges from 4.5 to 5.5. Extremely thermoactive α-glucosidases have been identified in archaebacteria such as *Pyrococcus woesei* and *P. furiosus* (Costantino *et al*., 1990).

Isoamylase

Isoamylases are very rare among microbes. *E. coli* possesses an intracellular isoamylase and is capable of hydrolyzing amylopectin completely. This can be accomplished only if additional amylolytic enzymes are also formed by this bacterium. Among these isoamylase producers, *Pseudomonas amyloderamosa* is the most important strain. In addition, other organisms belonging to genera *Agrobacterium, Erwinia, Staphylococcus, Serratia, Nocardia, Bacillus, Pediococcus, Lactobacillus,* and *Leuconostoc* have also been reported to produce isoamylases (Crueger and Crueger, 1985). Yeast like *Lipomyces kononenkoae* and *Saccharomyces cerevisiae* also produce this enzyme (Spencer and Martins, 1982; Gunja *et al*., 1961). All enzymes are thermolabile and do not require metal ions for their activity. The temperature range for these enzymes is between 25 ^{0}C and 52 ^{0}C.

Induction and Catabolite Repression of Amylase Production

The carbon sources greatly influence the synthesis of amylases. Both substrate induction and catabolite repression control the rate of bacterial amylase biosynthesis (Ingle and Erickson, 1978). Amylase synthesis in some organisms appears to be at least partially inducible while in others it has been reported to be completely constitutive. Since starch is a macromolecule, it can not be taken up by the cells and hence cannot act as an inducer of amylase synthesis. Therefore, it is generally accepted that a small amount of enzyme is produced in the medium where it degrades the exogenous substrate and the resultant low molecular weight products enter the cell which further induces the enzyme synthesis (Crueger and Crueger, 1985). The evidence for substrate induction (e.g. Starch or α-1,4-linked oligosaccharides) has been obtained in studies with *B. stearothermophilus* and *B. licheniformis* (Saito and Yamamoto, 1975). The studies with *B. stearothermophilus* demonstrated that although the strain was partially constitutive, there was an instantaneous increase in the rate of α-amylase synthesis after the addition of a series of oligosaccharides (e.g. maltose through maltohexoses). Maltotetraose was the most efficient inducer. Almost similar results were observed in *B. licheniformis*. Maltotriose and the higher maltooligosaccharides (G5, G6, and G7) are less efficient than maltotraose and cellobiose is a very poor inducer. It has been long known that higher yields of α-amylase are obtained from bacteria grown on a medium containing complex starch materials than on artificially defined media (Srivastava and Baruah, 1986; Fogarty *et al*., 1992; McTigue *et al*., 1994). Not only do these carbon sources fail to exert catabolic repression, but also it would seem that they also provide maximal induction of α-amylase. Some bacilli, however, appear to synthesize α-amylases constitutively. Thus *B. amyloliquefaciens* (Coleman, 1967), *B. subtilis*-168 (Sekiguchi and Okada, 1972), and *B.*

licheniformis (Meers, 1972) have been reported to synthesize α-amylase in the absence of α-1,4- linked oligosaccharides.

The synthesis of β-amylase by *B. polymyxa* occurs only in the presence of starch or starch-like products (Griffin and Fogarty, 1973). Maltose, the major end product of the exo-attack on amylose by β-amylase yields only 50% of the enzyme activity produced when starch is supplied as the carbon source. Like β-amylases the cyclodextrin glucosyl transferase of *Bacillus marcerans* is produced maximally in starch-containing media and the enzyme accumulates as the concentration of the carbon source declines (Lane and Pirt, 1973). Catabolite repression is significant in the production of most extracellular enzymes and amylases are no exception. Amylase synthesis has been reported to be subjected to catabolite repression. Glucose promotes the best growth compared to other substrates, but it is least favorable in terms of amylase production. It has been reported that an inverse ratio exists between the growth and total amount of amylase production. Studies with *B. subtilis* (Sekiguchi and Okada, 1972), *B. stearothermophilus, B. amyloliquefaciens* (Coleman and Grant, 1966), and *B. licheniformis* have all indicated that carbohydrate sources or concentrations that tend to increase the growth rate will inhibit the formation of amylase.

In continuous fermentation with carbohydrates as a limiting factor, growth is inversely proportional to amylase production. Most studies indicate that the *Bacillus* amylase biosynthetic system is under catabolite repression and that even glycerol and acetate at a level of 0.5 % can completely repress amylase synthesis (Saito and Yamamoto, 1975). When nitrogen is used as a limiting factor with excess glucose, only traces of amylases are produced (Crueger and Crueger, 1985). The addition of glucose to *B. subtilis* cells synthesizing α-amylase results in the repression of the enzyme synthesis. *Bacillus amyloliquefaciens* had doubling times of 2.2, 1.7, and 1.6 h when grown in media containing starch, glycerol, and glucose, respectively, and the rate of α-amylase synthesis is inversely related (Coleman and Grant, 1966). Almost similar results were observed in *B. stearothermophilus* with different carbon sources (Srivastava and Baruah, 1986). More recently Bajpai *et al.* (1991) have observed in the case of *Bacillus* sp. TCRDC-M1 that although the highest growth was observed when grown in glucose, fructose, and sucrose there was no detectable α-amylase production. The α-amylase of *Streptomyces* sp. IMD 2679 was subjected to catabolite repression. Four different growth rates were achieved when the organism was grown at 40 ^{0}C and 50 ^{0}C in the presence and absence of cobalt, with an inverse relationship between amylase production and growth rate. Highest α-amylase yields (520 units/ml) were obtained at the lowest growth rate (0.062/h), at 40 ^{0}C in the absence of cobalt, while at the highest growth rate (0.35/h), at 55 ^{0}C in the presence of cobalt, α-amylase production was decreased to 150 unit/ml (McMohan *et al*., 1977). During α-amylase production in batch and continuous cultures of *A. oryzae,* induction, and repression have been reported by Morkeberg *et al.* (1995). At high glucose concentrations, there was a low constitutive synthesis of α-amylase, whereas at low glucose concentrations depression resulted in an increased production rate. Shift from glucose- to a maltose-limited chemostat showed that maltose induces both the production and secretion of α-amylase.

Benefits of Enzyme Modification of Starch

Physical techniques are inexpensive and environmentally friendly, but they disrupt the starch structure, which limits their industrial use. Applications for physically altered starch can be found in both the food and non-food industries, including packaging and 3D printing. Chemical modifications of starch and its derivatives can be achieved via grafting, dual modifications, etherification, esterification, alkali treatment, cross-linking, and grafting. These modifications can yield chemically modified starch and derivatives with the appropriate product attributes. Chemically modified starch finds extensive application in the industry and

holds potential applications in the fields of superabsorbent, pharmaceuticals, bio-based adhesives, packaging, agriculture, and wastewater treatment (Amaraweera et al., 2021). But because of safety issues (Onyango, 2016) and the copious amounts of chemical acids, acetates, hypochlorides, phosphates, and other waste products that are released into the environment, requiring recycling, consumers do not approve of the inexpensive, easily modified starch. Treating carbohydrates with enzymes results in fewer byproducts than physical or chemical modification because enzyme reactions are gentle and target-specific (Park et al., 2018). According to the clean label, there are other benefits of enzymatic starch modification, including enhanced purity, consistent high-quality products free of unwanted compounds, reduced cost, and desirable functional properties (Park and Kim, 2021). When starch is enzymatically transformed, low-molecular-weight, linear short-chain fractions of amylopectin are generated. This encourages freeze-thaw and raises the content of resistant starch (Liu et al., 2017; Woo et al., 2021; Zhang and Bao, 2021). Additionally, it has distinct rheological characteristics, lowers paste viscosity, and enhances the digestibility and elastic behavior of starch (Kim et al., 2017; Singla et al., 2020).

Applications of Enzymatic Modified Starch

In addition to being used for starch hydrolysis, enzymes have also been thoroughly studied for possible environmental and consumer benefits, including the production of safer and healthier starch foods than those resulting from chemical procedures (Obadi and Xu, 2021). Two of the main objectives of enzymatic starch modification include reducing starch molecules to different oligosaccharides and improving the products' functional attributes and nutritional value for broader industrial uses (Wang et al., 2015). Carbohydrate enzymes are essential for the production of food products, improving product quality, and increasing food processing efficiency in the food and noodle sectors (Obadi and Xu, 2021). Among other things, the food sector employs them as emulsifiers, thickeners, and binders for chemically dual-modified starches. However, as heavy metal absorbents, they have uses in industries other than food (Bensaad et al., 2022). These modified starches can exhibit fat-like carbohydrates, form thermos reversible gels that can effectively replace one or more fats in dishes, and provide a range of fat-like mouth feel characteristics and textures from fatty to velvety to tacky. They can also be used in low-calorie foods, noodles, and baked goods, as an alternative to gelatin, as an emulsion stabilizer in ice cream, and water oil-water emulsions (Liu et al., 2017, Bangar et al., 2022). Enzyme-modified sweet potato starch can efficiently replace fat in low-calorie ice cream recipes by up to 20% (Kale et al., 2020). The disproportionation enzyme-transferred glucan fraction of amylose to amylopectin resulted in soft bread with a high specific volume. This also decreased the amylose content, which is directly connected with hardness (Le Loan et al., 2021). The enzyme modifies taro starch at a concentration of 0.5%, resulting in ice cream manufactured with guar gum—a conventional stabilizer—having less viscosity and overrun than ice cream created with the enzyme. A few advantages over other popular food additives are their ideal texture, low melting points for longer storage durability, and affordability (More et al., 2017). Low-fat yogurt's viscosity and texture can be maintained by using potato starch that has been modified by amylosubtilin and amylase (Nikitina et al., 2019).

Li and colleagues (2019) isolated mesophilic and thermostable α-amylase from extruded noodles, thereby modifying wheat starch. After enzymes were introduced to the dough and starch was extracted, extruded noodles displayed starch gelatinization and a well-developed porosity structure. In comparison to noodles prepared without enzymes, this makes extruded noodles more appetizing and makes rehydration easier. Tablets with a smooth texture and high gloss can be bound with pullulanase or isoamylase-prepared debranched starches (DBS), which is an efficient direct compression tablet binding agent. They also possess outstanding elastic modulus properties. They could be used to create tablet and capsule

formulations for pharmaceutical drugs that have an immediate or delayed release of the active components (Liu et al., 2017). The microcapsules containing modified maize starch and gum Arabic released ascorbic acid over a longer period in the digestive system, and there was noteworthy stability. Following the enzymatic treatment of starch, which left holes on their surface where ascorbic acid is trapped, gum arabic was added to the microcapsules (Levya-Lopez et al., 2019). Among the most extensively used enzymes available, amylases are employed in a wide range of sectors, such as milling, bread, body tools, detergents, and medications (Crueger & Cruger, 1985). Here are a few instances of how they are used.

Starch Industry

Glucose, maltose, and fructose are produced by hydrolysis of corn starch by a multi-step process. The first stage involves the liquefaction of starch which is brought about by thermostable alpha-amylase from bacteria. Alpha amylase acts on starch-producing dextrin, malto oligosaccharides, and maltose. In the second stage, glucoamylase from fungi is used to produce free glucose from the above-mentioned liquefaction products. Amylases are also employed in the production of maltodextrins (Guzman-Maldonado and Paredes-Lopez, 1995).

Alcohol and Brewing Industry

In the alcohol industry, starch-containing substrates are used for the production of alcohol. For the preparation of mash for alcohol fermentation, the starch is hydrolyzed enzymatically to fermentable sugars at 85 ^{0}C with alpha-amylase (liquefaction) and then at 55 ^{0}C with glucoamylases (saccharification). In brewing, alpha-amylases are used to produce dextrins of low fermentability and to remove starch hazes from beer and also the production of beer from dextrins, the so-called low calories beers (Ingle and Erickson, 1978; Gayal *et al*., 1997).

Baby Foods

Fungal amylases are used extensively in the preparation of dried baby foods and cereal products. Partial treatments of starch with fungal amylases increase the digestibility thus making them suitable for very young children. The cereal to be treated is heated to 80-85 ^{0}C then it is subjected to fungal amylase digestion for four minutes with constant agitation. The cereals are then dried at 100 ^{0}C, which dries the products as well as inactivates the enzyme thus imparting malt flavor to the product (Windish and Mhatre, 1965).

Milling and Bread Making.

In milling, amylases from *Aspergillus* sp. are used for modification of alpha-amylase deficient flour. In bread making the amount of fermentable sugars is increased by the addition of some amount of amylase to flour. This aids in beavering, improves dough consistency, and enhances gas reiteration thus resulting in a lighter loaf (Crueger and Crueger, 1985).

Preparation of Sugars and Jellies

Amylases are used to remove haze from fruit juices used in the preparation of jellies. Jellies made from quince and apple juices are hazy in appearance due to high starch contents. Treating the juice with amylase for h at 40-50 ^{0}C produces a clear juice suitable for jelly making (Windish and Mhatre, 1965). In the sugar industry amylases are used to improve the filterability of sugar cane juice via the breakdown of starch in juice.

Production of Chocolate Syrup

Cocoa slurries are treated with alpha-amylases, as a result, chocolate syrups are produced which do not 'layer' in storage. This treatment also removes stiffening and a product with better flavour and solubility is produced.

Feed Industry

Bacterial amylases are used for the treatment of barely that is used as feed for poultry and calf raising.

Medicine and Pharmaceuticals

Microbial amylases are used as digestive aids in persons with defects in starch-digesting enzymes. Acid-resistant amylases from *Aspergillus oryzae, A. niger*, and α-amylase from *Bacillus subtilis* are used as digestive aids. One such enzyme is a product with the trade name Taka-diastase (Windish and Mhatre, 1965).

Paper Industry

In the paper industry amylases are used for selective liquefaction of starch. The starch is liquefied in such a way that no free sugar is produced. This starch slurry is used for sizing of paper (Gayal and Khandeparker, 1991)

Textile industry

Bacterial amylases because of their more thermo stability are used in the textile industry. During weaving, the wrap is subjected to considerable strain which may result in the breaking of the wrap. Strength to the yarn is given by coating the threads with gelatinized starch by passing them through the starch solution. After weaving the starch is removed by washing the cloth with dilute solutions of amylases at 50 ^{0}C. This treatment is called de-sizing. This process is used for cotton, woolen, and rayon goods (Windish and Mhatre, 1965).

Biological Detergents

Detergent formulations containing amylases along with proteases have been prepared and used in domestic laundries and dry-cleaning industry (Casida, 1996). These detergents are also incorporated in drain cleaners and used as septic tank rejuvenates (Windish and Mhatre, 1965).

Effluent Treatment

Khire and Pant (1992) have reported a salt-tolerant amylase from *Bacillus* sp-64., which may find application in treatment of effluents containing starch residues.

2. Conclusion

Although starch is the most prevalent naturally occurring carbohydrate, its application is frequently limited due to its poor solubility, weak functional characteristics, and limited ability to withstand a broad range of processing conditions. The limits of natural starches are addressed through the application of physical, chemical, and enzymatic changes. Modifications in morphology, crystal structure, and amorphous areas can result from enzymatic starch modification. The swelling power and starch solubility can be influenced by enzymatic modification, which can also raise the pasting temperature and lower peak viscosity. Enzymatically modified starch has been shown to improve the content of resistant starch, be environmentally benign, and give desired functional qualities. Among the most significant enzymes, amylases have a major role in modern biotechnology. The enzymes from microbial sources typically satisfy industrial needs, even though they can be obtained from a variety of

sources, including plants, animals, and microorganisms. If appropriate enzymes could be produced, microbial amylases would prove beneficial for the fine-chemical and pharmaceutical sectors. The range of applications for amylase has expanded with the discovery of new biotechnological frontiers. These include clinical, medicinal, and analytical chemistry, in addition to their common use in starch saccharification and the food, beverage, textile, and distilling industries.

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