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Enzymatic Modification of Starch: A Review

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Abstract

Starch is the most abundant naturally occurring carbohydrate reserve in plants and is found in cereals, roots, tubers, legumes and some immature fruits like bananas or mangos. Starch is usually employed as a food additive, such as a thickening, stabilizer, or texture enhancer to improve some of the products quality characteristics, pharmaceutical and among other. The application of native starch is often restricted owing to its constricted solubility, weak functional attributes and limited tolerance to a wide array of processing conditions. Its low resistance to shear, high retrogradation, and poor freeze-thaw stability, limit the use of starch in industrial applications. These natural shortcomings can be overcome by different methods of modification. In recent decades, enzymatic modifications have been adopted, partly replacing the chemical and physical methods for the preparation of modified starch, as enzymes are safer and healthier than chemical method for both the environment and food consumers. Several enzymes viz., α-amylase, β-amylase, glucose isomerase, pullulanase, xylanase, among others are use in modification of starch. The enzymatic modification of starch molecules directly affected properties of the modified starch especially in freeze-thaw stability of gels and retardation of retrogradation during storage. Combined enzymatic modification resulted in a marked increase in resistant starch and enzyme modified starch can be well utilized as a fat replacer. It is environment-friendly method and can provide desired functional characteristics.

Key words: Starch, Stabilizer, Retrogradation, Enzymes, Modification, Modified starch.

INTRODUCTION

Starch is the most abundant naturally occurring, inexpensive and biodegradable polysaccharide and carbohydrate reserve found in leaves, flowers, fruits, seeds, different types of stems and roots of plants. Major starch synthesis and storage in cereals (40 to 90%) mainly endosperm, roots (30 to 70%), tubers (65 to 85%), legumes (25 to 50%) and some immature fruits like bananas or mangos, which contain approximately 70% of starch by dry weight. Stach is composed of glucose molecules and a mixture of two polymers called as Amylose and Amylopectin (Krithika and Ratnamala, 2019). Amylose is a linear polymer with α-1,4 glycosidic bonds, while amylopectin molecules is a branched-chain of glucose units linked by α-1,4 and α-1,6 glycosidic bonds Figure 1 (Park and Kim, 2021). Starch is a major energy source on earth, providing up to 80% of the calories by releasing glucose during cellular respiration. Starch is one of the most common biodegradable polymers found in nature with wide application including food and beverage, bioplastic industry, paper industry, textile, and biofuel industries. Starch has received significant attention due to its easy fabrication, relative abundance, non-toxicity and biodegradability. However, native starch application is often restricted owing to its poor thermo-mechanical properties, higher water absorptivity, constricted solubility, weak functional attributes and limited tolerance to a wide array of processing conditions. Therefore, native starch needs to be modified before its use (Amaraweera *et al.*, 2021, Yu *et al.*, 2021). Starch plays an essential role in developing food products as a food additive such as a thickening, stabilizer, or texture enhancer to improve some of the products quality characteristics including moisture retention, the potential to be employed as a delivery vehicle for chemicals of interest to the food and pharmaceutical sectors such as antioxidants, colorants, flavors and pharmaceutically active proteins among other things (Abegunde *et al.*, 2013). However, its low resistance to shear, high retrogradation, and poor freeze-thaw stability limit the use of starch in industrial applications and these natural shortcomings can be

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overcome by different methods of modification as chemical, physical, enzymatic and genetic techniques (Tang *et al.*, 2020, Bensaad *et al.*,2022). The enzymatic modification method has recently attracted much attention from academia and the corporate sector since it is environment-friendly method (Zhang and Bao, 2021) considered “Clean LabelTM ingredients” as they are free from artificial and synthetic ingredients (Park and Kim, 2021) and can provide desired functional characteristics.

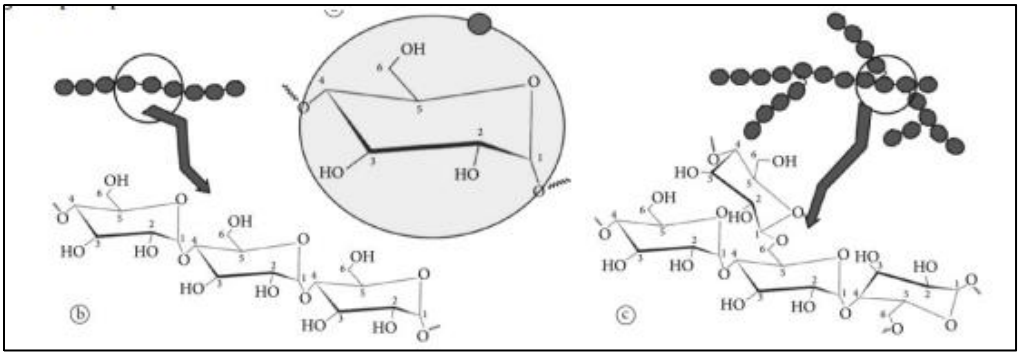

Figure 1: **Structure of Amylose and amylopectin (Krithika and Ratnamala, 2019)**

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, 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).

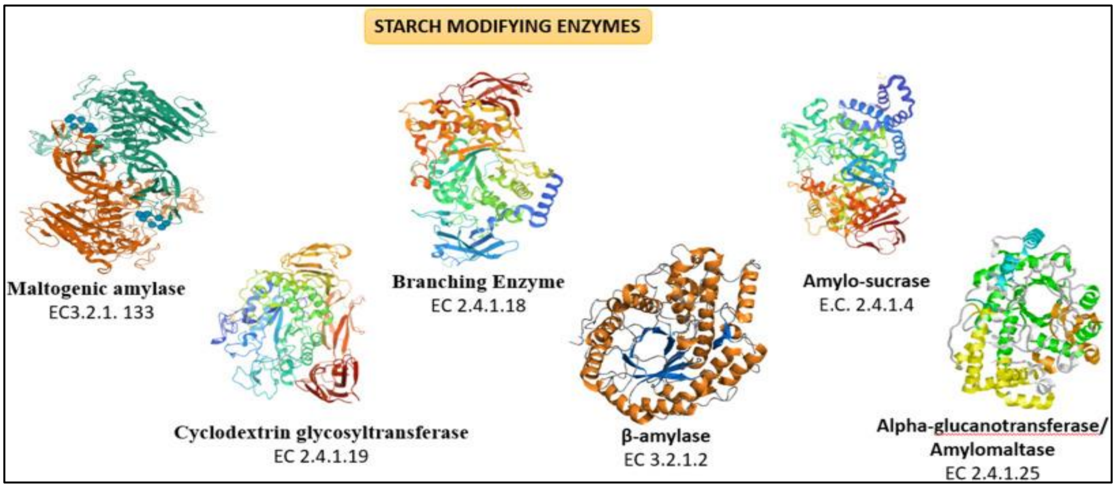

Figure 2: Various starch modifying enzymes (Bangar *et al.*, 2022)

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)

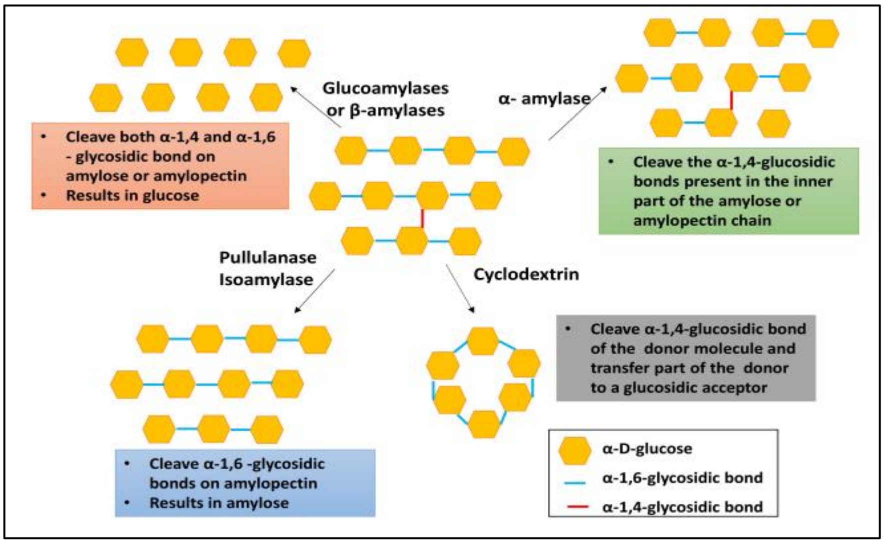

Figure 3. Enzymatic hydrolysis of starch. (Amaraweera *et al.*, 2021)

Enzymatic Modification of Starch

Recently, enzymatic modifications have been adopted partly replacing the physical and chemical methods for the preparation of modified starch as enzymes are safer and healthier than chemical method for both the consumers and environment (Park *et al.*, 2018). Enzymatic modification is a clean and accurate way to modify starch digestibility, including pullulanase, *α*-amylase and branching enzymes. The enhanced ordered arrangement of semi-crystalline structure and the higher proportion of linear short chains in enzyme modified starch resist the digestive enzymatic hydrolysis resulting in higher resistant starch contents (Zhang and Bao, 2023). Numerous enzymes have been added to starches to increase the content of resistant starch (Sorndech *et al.*, 2016; Harder *et al*., 2015; Kim *et al*., 2015), to inspect the impact of starch structure/type on degradation of starch (Naguleswaran *et al.*, 2014) and for malt production (Chu *et al.*, 2014). Starch are 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 by forming 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 acts by breaking the glycosidic bonds of the nonreducing ends successively of the substrate producing monomeric or oligomeric compounds. Exo-acting enzyme β-amylase work to break α-1,4 bonds successively on amylose and amylopectin while pullulanase a debranched enzymes work in breaking α-1,6 glycosidic bonds. ((Bertoft, 2013, Hutabarat and Stevensen, 2022)

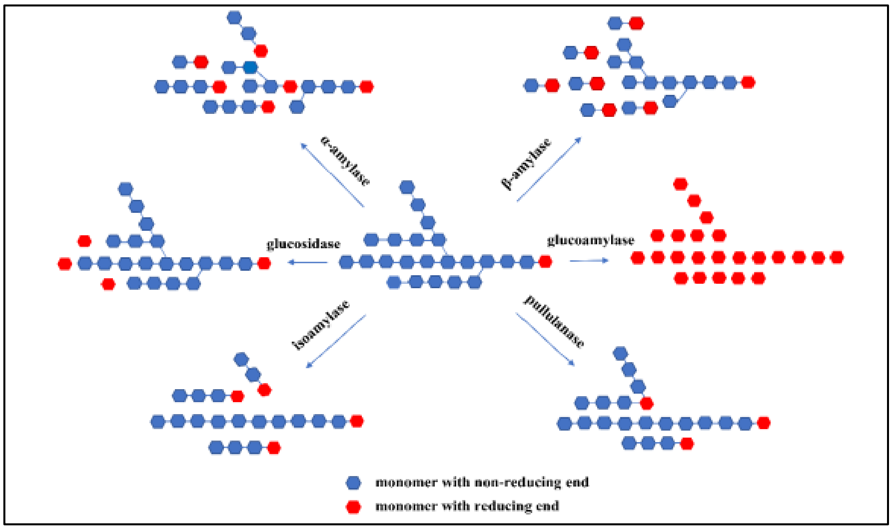

Figure 4: Enzymatic Conversion of starch (Hutabarat and Stevensen, 2022)

Enzymatically modified starch was used in the microencapsulation of ascorbic acid (AA) by prepared microcapsules using 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 suggest that ETCS 16h-GA microcapsules lost less AA during storage time, shows greater stability in the protection of AA and allows for a controlled release in the gastrointestinal tract (GIT) and is 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 weigh while the amount of short chains and a-1, 6 linkages increased. A maximum SDS content (33.5%) was obtained using double enzymes hydrolysis for 6 h compared to native starch. The results suggested that starches using combined b-amylase and transglucosidase treatment not only increased the amount of short chains accompanying with shortening long chains, but also produced an increase of a-1,6 linkages, which led to a more slow digestion property (Miao *et al.*, 2014).

Nanostarch production by enzymatic hydrolysis of cereal (maize) and tuber (potato and cassava) crops and the conventional acid hydrolysis process. The nanostarch produced by 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 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 a greater setback and amylose values than hydrolyzed starches, which shows that it is more susceptible to retrogradation or water lost (syneresis) (Jairo Salcedo-Mendoza *et al.*, 2018).

Modified taro starch is prepared by enzyme modification (α-amylase) and use as stabilizer in ice-cream with effective overrun, foam stability, and viscosity. EMTS prepared by treating the taro starch by α-amylase resulted in 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 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 (More *et al.*, 2017).

Enzyme modified sweet potato starch use with khoa viz., 0:100, 10:90, 20:80, 30:70 and 40:60 respectively to replace fat in formulation of ice cream. It was noticed that there was remarkable decrease in fat content from 9.85 to 5.79 percent with increased enzyme modified starch level. It concluded that use of enzyme modified sweet potato starch can be well utilized as a fat replacer in the formulation 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 use as the fat replacer. The results showed that the characteristics of yogurt formulated with modified potato starch of amylosubtilin and amylase are similar to those of native potato starch formulated yogurt; therefore 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 exhibited more rapidly pasting and much larger changes in viscosity during pasting cycle than both the native and other modified flours and improve 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 and shows that debranching enzymes like ISO and PUL could be an alternative to acid-involved processes.

Zhang *et al. (*2019) sequential modification of amylopectin by amylosucrase and pullulanase in which elongation of side chains of amylopectin by amylosucrase, followed by debranching with pullulanase produce 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. The enzymatic treatments remarkably altered the molecular and crystalline structures of modified starch to produce functional starch microparticles with reduced digestibility provide an effective and environmentally friendly technology to manufacture novel modified starches, Figure 5.

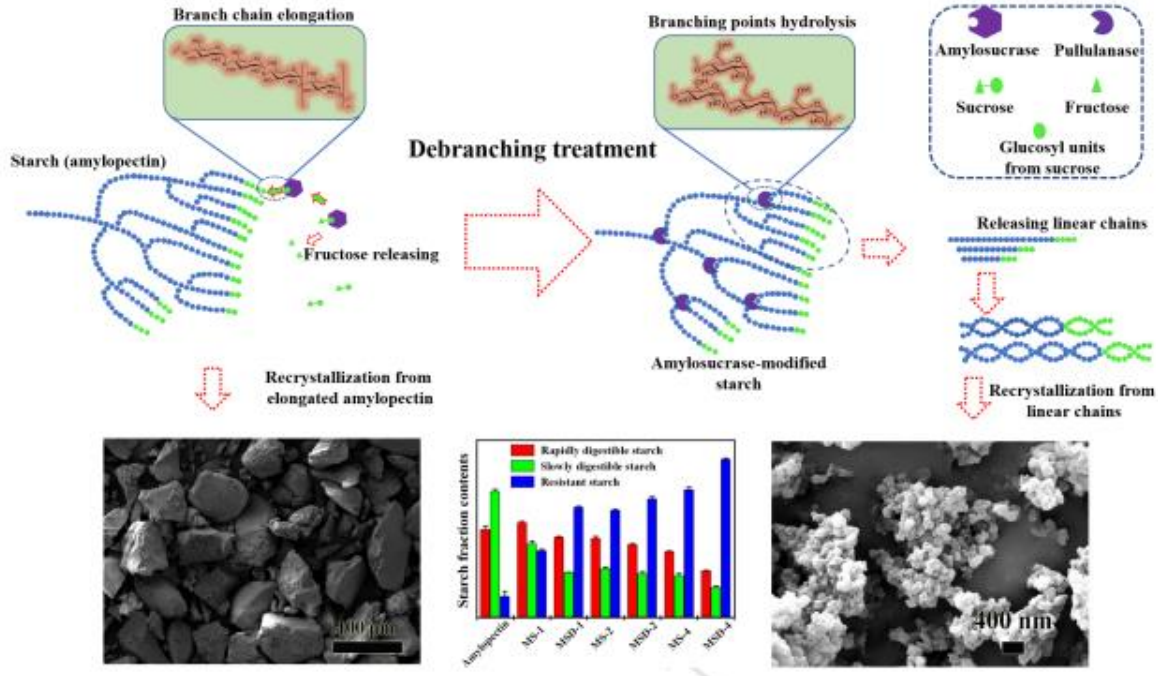

Figure 5: Enzymatically modified starch from amylopectin by sequential amylosucrase and pullulanase treatments (Zang *et al.*, 2019)

Advantages of Enzyme Modification of starch

Physical methods being eco-friendly and low cost they destroy starch structure limiting their industrial applications. Physically modified starch have application in food and non-food sector such as packaging and 3D printing. Chemical methods such as crosslinking, alkali treatment, etherification, esterification, acid hydrolyzing, grafting, and dual modifications can be used to alter the structural properties of starch to prepare chemically modified starch and its derivatives to achieve the desired product attributes. Chemically modified starch are widely used in the industry and show potential in packaging, bio-based adhesives, pharmaceutical, agriculture, superabsorbent and wastewater treatment applications (Amaraweera *et al.*, 2021). However, modification of starch by means of chemical being low cost, ease modification is not acceptable to consumers because of safety concerns (Onyango, 2016) and huge effluents of chemical acids, acetates, hypochlorides, phosphates, etc., cause detrimental to the environment and requires recycling. Starches treated with enzymes result in producing fewer byproducts compared to physical and chemical modification as enzyme reactions are target specific and mild (Park *et al.*, 2018). Enzymatically modification of starches being clean label offer various other benefits such as improved purity, consistency of high-quality products, free from unwanted products, less expensive and desirable functional properties (Park and Kim, 2021). Enzymatic modification of starch results in the emergence of low-molecular-weight, linear short chains fractions of amylopectin, enhances the amount of resistant starch content, promoting freeze-thaw (Liu *et al.*, 2017; Woo *et al.*, 2021; Zhang and Bao, 2021) exhibited distinct rheological properties, reduced the paste viscosity, increases starch elastic behavior and digestibility (Kim *et al.*, 2017; Singla *et al.*, 2020).

Application of Enzymatic Modified starch

Enzymes were widely explored for the environment and consumers in healthier and safer starch foods than those generated by chemical methods and enzyme treatments have been used for starch hydrolysis (Obadi and Xu, 2021). One of the main objectives of enzymatic starch modification is to reduce starch molecules to different oligosaccharides and increase the products' functional characteristics and nutritional value for broader industrial applications (Wang *et al.*, 2015). Carbohydrate enzymes play an essential role in the food and noodle industry in the manufacturing of food products, improving product quality, and increasing food processing performance (Obadi and Xu, 2021). There are several applications in the food industry, such as binders, thickeners and emulsifiers for chemical dual-modified starches. In contrast, these can be used as heavy metal absorbents in the non-food industry (Bensaad *et al.*, 2022).These modified starches can exhibit fat-like carbohydrates, form thermos reversible gels that can efficiently replace one or more fats in dishes and provide a range of fat-like mouth feel characteristics and from like textures from fatty to velvety to tacky and thus can be used in low calorie foods, noodles, baked goods, alternate to gelatin, emulsion stabilizer in ice cream and water oil-water emulsions, Figure 6 (Liu *et al.*, 2017, Bangar *et al.*, 2022).

Enzyme-modified sweet potato starch can be successfully used up to 20% as a fat replacer in low-calorie ice cream formulation. (Kale *et al.*, 2020). Transfer of glucan fraction of amylose to amylopectin by disproportionation enzyme decrease in amylose content which is also directly proportional to hardness thus resulted in soft bread with high specific volume (Le Loan *et al.*, 2021). Enzyme modifies taro starch at 0.5% levels results in effective foam stability, higher viscosity, and overrun in ice cream than the ice cream prepared with conventional stabilizer guar gum. Several advantages over other standard food additives including optimal texture, low price and low melting qualities for greater storage stability (More *et al.*, 2017). The viscosity and texture of low fat yogurt could be maintained by using potato starch modified by amylosubtilin and amylase (Nikitina *et al.*, 2019). Li *et al.* (2019) used thermostable α-amylase and mesophilic α-amylase isolated enzymes from extruded noodles to modify wheat starch. As enzymes were introduced to the dough and subsequently starch was extracted, extruded noodles displayed starch gelatinization and a well-developed porosity structure, which aids in the rehydration and palatability of extruded noodles compared to noodles without enzymes. Debranched starches (DBS) prepared by pullulanase or isoamylase enzymes have excellent elastic modulus properties and are an outstanding direct compression tablet binding agent that gives tablets a high gloss, smooth texture. They could be used to prepare pharmaceutical drug formulations such as capsules and tablets either for instantaneous or delayed release of the active agents (Liu *et al.*, 2017). Microcapsules prepared by using modified corn starch and gum Arabic as wall material exhibited pronounced stability, protection of ascorbic acid and also sustained release in the digestive tract. The enzymatic treatment of starch lead to the formation of voids on the surface of microcapsules in which ascorbic acid trapped and was further coated with gum Arabic (Levya-Lopez *et al.*, 2019).

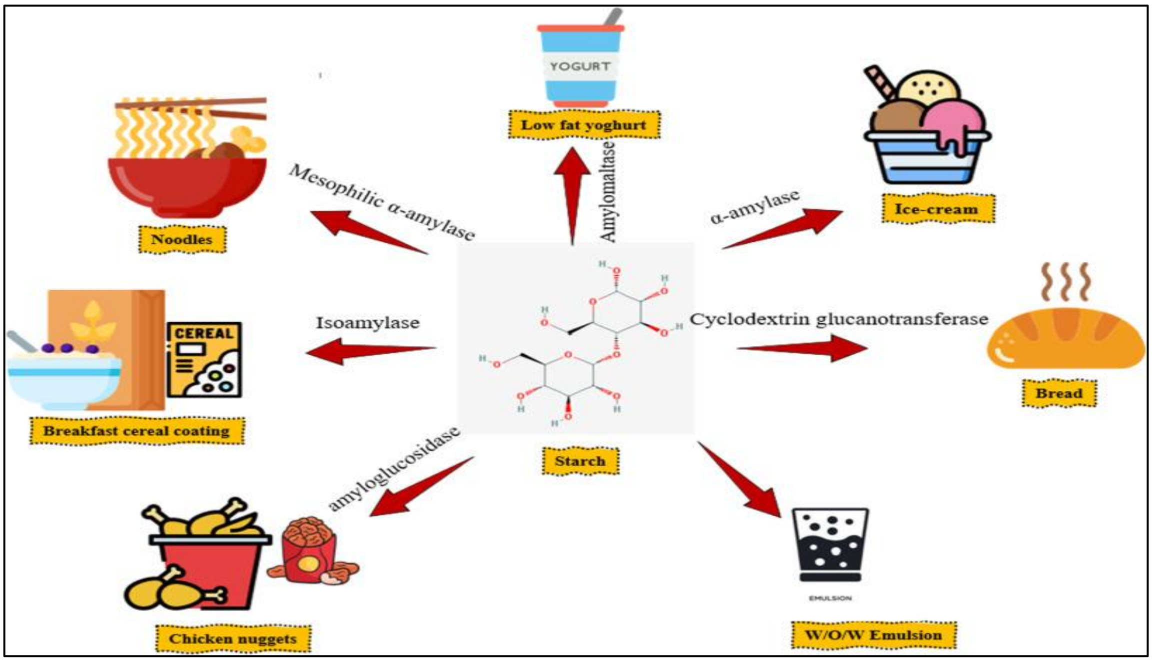

Figure 6: Application of enzyme modified starch (Bangar *et al.*, 2022)

CONCLUSION

Starch is the most abundant naturally occurring carbohydrate however application of native starch is often restricted owing to its constricted solubility, weak functional attributes and limited tolerance to a wide array of processing conditions. Physical, chemical and enzymatic modifications are applied to overcome the limitations of native starches. Enzymatically modified starch can cause changes in morphology, crystal structure and amorphous regions. Enzymatically modified starch can affect the swelling power and increase the starch solubility, affecting the decrease of peak viscosity and increase of pasting temperature. Enzymatically modified starch increases resistant starch content, it is environment-friendly method and can provide desired functional characteristics.

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