

# MODIFICATION OF STARCH: A REVIEW OF VARIOUS TECHNIQUES

P. LAKSHMI KRITHIKA<sup>1</sup> & Dr. K.V. RATNAMALA<sup>2</sup>

<sup>1</sup>Department of Pharmaceutics, RBVRR Womens college of Pharmacy,

Affiliated to Osmania University, Barkhatpura, Hyderabad, Telangana- 500027

<sup>2</sup>Associate Professor, Department of Pharmaceutics, RBVRR Womens College of Pharmacy,

Affiliated to Osmania university, Barkhatpura, Hyderabad, Telangana-500027

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**ABSTRACT:** Starch is an important polysaccharide extracted from various natural sources. Other than being a major food product, it is used in coatings and sizings of papers, textiles and carpets. As an adhesive, binder, encapsulants, adsorbants and bone replacement implants, drug delivery systems and tissue engineering scaffolds. In pure form it exhibits low stability, decomposition, processing and poor solubility in common organic solvents. Therefore to overcome these limitations, Starch is processed by various methods. The main aim of starch modification is to overcome any one of the above limitations and enhance its versatility and usage. Methods of starch modification are classified as physical, chemical, and enzymatic methods. This review article summarizes the various starches, their properties and the various methods of starch modification.

**Key Words :** Starch, modification, uses.

## INTRODUCTION

Starch is the most abundant naturally occurring, biodegradable, inexpensive and abundantly available polysaccharide and carbohydrate reserve in plants and is found in leaves, flowers, fruits, seeds, different types of stems and roots. Starch is used by plants as source of carbon and energy. The main location of starch synthesis and storage in cereals is the endosperm. Major starch sources are cereals (40 to 90%), 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<sup>1</sup>. The accumulation pattern of starch granules in each plant tissue, shape, size, structure and composition is unique to each botanical species<sup>2</sup>. Potato starch granules are large, oval in shape, 15-100 ~m in diameter, with pronounced oyster-shell-like striations. Corn starch granules are medium sized, round or polygonal in shape, and 15 ~ in diameter. Rice starch granules are small, polygonal, and 3-8 ~m in diameter.<sup>3</sup>

## COMPOSITION

Starch is composed of glucose molecules. Starch is composed of a mixture of two polymers called as Amylose and Amylopectin. Amylose is a linear polymer with molecular weight which is less than 0.5 million Dalton, depending on its biological source. Amylose Macromolecules consist of  $\alpha$ -D- glucopyranose units joined by  $\alpha$ -1,4 acetal linkages. Amylopectin molecules are much larger and highly branched with molecular weight of 50-100 million Dalton. The molecules contain  $\alpha$ -1,4 linear bonds and is branched through a  $\alpha$ -1,6 linkages at 10 nm along the molecule's axis<sup>4</sup>.

The polymodal distribution of  $\alpha$ -glucans chains of different sizes and the grouping of branch points in the amylopectin molecule allow the formation of double helical chains. Amylose and amylopectin can be arranged in a semicrystalline structure forming a matrix of starch granules with alternating amorphous (amylose) and crystalline (amylopectin) material, which is known as the growth rings in superior plant starch<sup>4</sup>

Several types of starches are known as "waxy" starches due to the waxy appearance of the endosperm tissue from which they are derived; these tissues contain a minimal amount of amylose in their granule composition (<15%). Waxy starch requires high energy for gelatinization due to its high crystallinity. Other starches have a high content of amylose (>30%); these starches can also contain other polysaccharide molecules and exhibit a slight deformation in granule appearance.<sup>5</sup>

Cereal starches contain lipid molecules in their structures in the form of phospholipids and free fatty acids; they are associated with the amylose fraction<sup>6</sup>. The presence of lipid complexes in starch granules is observed as a hydrophobic nucleus situated within helices formed by amylose chains. The lipid complexes vary between 0.15 to 0.55% of the amylose fraction in cereal starches. Lipids in starch granules, despite representing a small fraction, can significantly reduce the swelling capacity of the starch paste. Starch

contains approximately 0.6% of protein associated with the molecule. The origins of protein and lipids on starch are situated on the granule surface. Lipids and proteins in starch granules can raise its functionality. In wheat for example, the associated protein in the starch granules receives a lot attention due to its association with grain hardness. Starch also contains a relatively small quantity (<0.4%) of minerals (calcium, magnesium, phosphorus, potassium and sodium). Among these, phosphorus is of primary importance and is present in starch in three main forms: monophosphate esters, phospholipids and inorganic phosphate.

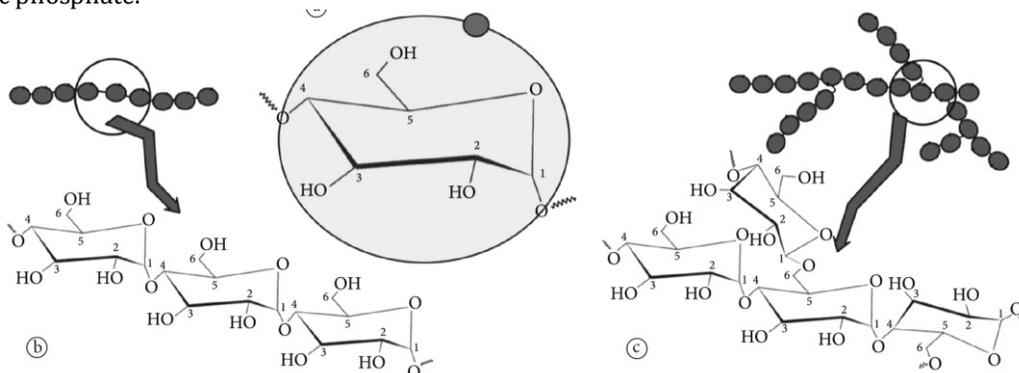


Figure1: Structure of Amylose and amylopectin

## PHYSIOCHEMICAL PROPERTIES OF STARCHES

### 1. MORPHOLOGY, SIZE AND COMPOSITION

Species	Granule shape	Diameter ( $\mu\text{m}$ )	Lipid (%w/w)	Protein (%w/w)	Amylose (%w/w)
Wheat	Spherical and lenticular	<30	0.08-0.12	0.2-0.3	25.6 $\pm$ 1.0
Barley	Disc shape	10-25	0.7-1.2	0.2-0.4	19-22.1
Sorghum	Polygonal, Dented and round	8-14.5 Polygonal, 8-10 round	0.8	2.3	23.7-27.6
Rice	Angular and polygonal	<20	0.6-1.4	0.1	21-25
Potato	Smooth surfaced, Oval and irregular	<110	0.1	0.1	29.3 $\pm$ 0.2
Sweet Potato	Polygonal	5-25	-	-	28.9 $\pm$ 0.23
Cassava	Flake shaped and irregular	5-25	0.2	0.3	23.7 $\pm$ 0.1
Turmeric	Polygonal and angular	5-70	-	0.6 $\pm$ 0.1	-
Ginger	Polygonal and angular	5-40	-	0.53 $\pm$ 0.1	-
Dioscorea	Polygonal and angular	3-22	-	-	48.5 $\pm$ 0.1

Table 1: Morphology, size and composition of various starches.

The amount of amylose present in the granule significantly affects the physicochemical and functional properties of starch. The amylose content can vary within the same botanical variety because of differences in geographic origin and culture conditions<sup>7</sup>. Researchers have highlighted the role of amylose in the initial resistance of granules to swelling and solubility, as swelling proceeds rapidly after leaching of amylose molecules. The capacity of amylose molecules of form lipid complexes prevents their leaching and consequently the swelling capacity<sup>8</sup>. Amylose is anhydrous and can form excellent films, which are important characteristics for industrial applications. Films formed by amylose are very strong, colorless, odorless and tasteless<sup>9</sup>.

The phospholipid content in starch granules is proportionally related to amylose. Phospholipids tend to form complexes with amylose and long branches of amylopectin, resulting in starch granules with limited solubility<sup>10</sup>. Phosphorus in starch granules has an important influence on the transmittance of the paste. Starches such as wheat and rice with high phospholipid contents produce pastes with low transmittance power compared to potato or corn starch pastes because the latter starches contain less phospholipids. Potato starch demonstrates high transmittance due to its phosphate monoester content.<sup>11</sup>

Starch granules have very complex structures. The complexity is built around variations in their composition ( $\alpha$ -glucans, moisture, lipids, proteins and phosphorylation), component structure and variation between amorphous and crystalline regions. Amylose associated with large branches of amylopectin molecules comprise the amorphous region of granules, and amylopectin molecules with short branches comprise the crystalline region; therefore, a higher proportion of amylopectin in starch granules results in greater crystallinity<sup>12</sup>. There are three types of crystalline structures: A-type characteristics from cereal starches, B-type found in tubers and C-type present in legumes<sup>13</sup>. Crystalline structures are based on the double helix formed by the amylopectin molecule. In A-type structures, the amylopectin branches are short (polymerization degree of 6-15) and linked by  $\alpha$ -1,6 bonds. A-type is characteristic of amylopectin ramifications. In B-type, the glucose chains are more polymerized and can act as bases where the branches are A-type or form branched amylopectin molecules. B-type chains are subdivided into B1, B2, B3 and B4. B1 chains have a polymerization degree between 15 and 25, and B2 chains are typically between 40 and 50; B3 and B4 are the highest. C-type crystallinity is a combination of the A- and B-types and consists of amylopectin molecules with non-reduced ends.<sup>12</sup>

## 2. BIREFRENGENCE OF STARCH MOLECULES

Birefringence is formally defined as the double refraction of light in a transparent, molecularly ordered material, which is manifested by the existence of orientation-dependent differences in refractive index. Many transparent solids are optically isotropic, meaning that the index of refraction is equal in all directions throughout the crystalline lattice. Crystals are classified as being either isotropic or anisotropic depending upon their optical behavior and whether or not their crystallographic axes are equivalent. All isotropic crystals have equivalent axes that interact with light in a similar manner, regardless of the crystal orientation with respect to incident light waves. Light entering an isotropic crystal is refracted at a constant angle and passes through the crystal at a single velocity without being polarized by interaction with the electronic components of the crystalline lattice. The term **anisotropy** refers to a non-uniform spatial distribution of properties, which results in different values being obtained when specimens are probed from several directions within the same material. On the other hand, as mentioned above, isotropic properties remain symmetrical, regardless of the direction of measurement, with each type of probe reporting identical results. When light enters the **optical axis** of anisotropic crystals, it behaves in a manner similar to the interaction with isotropic crystals, and passes through at a single velocity. However, when light enters a non-equivalent axis, it is refracted into two rays, each polarized with the vibration directions oriented at right angles (mutually perpendicular) to one another and traveling at different velocities. This phenomenon is termed as double refraction or birefringence and is exhibited to a greater or lesser degree in all anisotropic crystals.<sup>14</sup> All starch granules in their native form exhibit birefringence that is proportional to their crystalline structure. Birefringence patterns in starch granules represent the radial arrangement of amylopectin molecules, and their chains form 90° angles with the reduced ends in the direction of the hilum or starch granule center. Weak birefringence patterns are indicative of disorganization of the crystalline region<sup>15</sup>. Loss of birefringence in starch granules is associated with deformation due to its modification<sup>16</sup>.  $T_g$  describes the induction temperature of the progressive transition from an amorphous state to a rubbery state as the material is heated, generally in the presence of a solvent or plasticizer when referring to polysaccharides<sup>17</sup>. Because starch consists of an amorphous and a crystalline region, the exact  $T_g$  is detected with difficulty. The glass transition temperature ( $T(g)$ ) of potato and wheat starches, stored for several periods after gelatinization, was measured by differential scanning calorimetry (DSC), and the relative crystallinity of the starches was measured by X-ray diffractometry.  $T(g)$  of stored starches was higher than that of starches without storage, and the  $T(g)$  increment of starches gelatinized at 120 degrees C was higher than that of starches gelatinized at 60 degrees C. The water content at which the glass transition of a starch occurs at 25 degrees C was estimated from DSC data, and it increased linearly with relative crystallinity in two groups that differed in the gelatinization method.<sup>18</sup>

## 3. SWELLING PROPERTY

One of the most important structural characteristics of starch is that it passes through several different stages from water absorption to granule disintegration. Water absorption and consequent swelling of the starch granule contribute to amylopectin-amylose phase separation and crystallinity loss, which in turn promotes the leaching of amylose to the inter-granular space<sup>19</sup>. When starch molecules are heated in water excess, the semi-crystalline structure is broken, and water molecules associate by hydrogen bonding to hydroxyl groups exposed on the amylose and amylopectin molecules. This association causes swelling and increases granule size and solubility. The swelling capacity and solubility of starch illustrate the interactions

of the polymeric chains comprising the amorphous and crystalline granule fractions<sup>20</sup>. The extent of this interaction is influenced by the amylose-amylopectin proportion and is characteristic of each molecule depending on the polymerization degree, length and grade of chain branching, molecular weight and molecular conformation (<sup>21;22</sup>). The swelling capacity of starch is directly associated with the amylopectin content because the amylose acts as a diluent and inhibitor of swelling. Some species of starch that contain amylose-lipid complexes exhibit swelling capacity and solubility restrictions<sup>23</sup>.

The swelling stage of starch granules is the initial step of all other paste characteristics. Initially, granule swelling is reversible, increasing its volume up to 30% <sup>24</sup>. Water absorption and heating of the starch dispersion breaks the hydrogen bonds responsible for granule cohesion, partially solubilizing the starch. Water penetrates the interior of the starch granule, hydrating the linear fragments of amylopectin<sup>25</sup>. This process leads to irreversible swelling, increasing the granule size several fold and the paste viscosity. Paste viscosity is essentially the principal measure of the potential application of starch in industry.

#### 4. GELATANISATION AND RETROGRADATION

Starch, when heated in the presence of excess water, undergoes a transition phase known as gelatinization, and there is a characteristic temperature interval for gelatinization corresponding to each starch species. Gelatinization occurs when water diffuses into the granule, which then swells substantially due to hydration of the amorphous phase causing loss of crystallinity and molecular order <sup>26, 27, 28</sup>. Gelatinization occurs initially in the amorphous region, favored by the weak hydrogen bonds present in this area. The process then extends to the crystalline region. Amylose presence reduces the fusion point in the crystalline region and the amount of energy necessary to initiate gelatinization <sup>29</sup>. Gelatinization of starch granules is associated with a loss of birefringence and crystalline order due to the breaking of the double helix in the crystalline region and the leaching of amylose<sup>26, 29</sup>. This transitions starch from a semi-crystalline form (relatively indigestible) to an amorphous form that is easily digestible<sup>30</sup>. Similar to water, other solvents are also used to promote gelatinization. The principal consideration with solvents is their capacity to form hydrogen bonds with the molecules in the starch granules (i.e., liquid ammonia, formamide, formic acid, chloroacetic acid and dimethyl sulfoxide). The gelatinization process is affected by solvent type and starch/solvent proportions<sup>31</sup>. Gelatinization is necessary for particular processes, e.g., textile and hydrolyzed starch industries. Gelatinization affects the rheological properties and viscosity of the paste, making the starch granule more accessible to enzymatic action. When starch granules swell and its components are in solution, the medium properties change from a simple starch granules suspension to a starch paste. Amylose and amylopectin form separate phases because of thermodynamic immiscibility.

The molecular interaction produced after gelatinization and cooling of the paste is known as retrogradation<sup>32</sup>. During retrogradation, amylose molecules associate with other glucose units to form a double helix, while amylopectin molecules re-crystallize through association of its small chains. After retrogradation, starch exhibits lower gelatinization and enthalpy compared to native starch because its crystalline structure has been weakened. Initially, the amylose content exercises a strong influence over the retrogradation process; a large amount of amylose is associated with a strong tendency for retrogradation. Amylopectin and intermediate materials influence the retrogradation process during storage under refrigeration; each polymer has a different recrystallization rate. Starch retrogradation is intensified by repeated freezing and thawing of paste<sup>33</sup>.

#### 5. RHEOLOGICAL PROPERTIES

Starch paste forms immediately after gelatinization, and starch granules are increasingly susceptible to disintegration by shearing because they are swollen. The paste obtained is a viscous mass consisting of one continuous phase of solubilized amylose and/or amylopectin and one discontinuous phase of the remaining starch granule<sup>34</sup>. Starch granules are insoluble in cold water due to the hydrogen bonds and crystallinity of the molecule. When starch is dispersed in hot water below its  $T_g$ , the starch granules swell and increase several times in size, breaking the molecules and consequently leaching amylose to form a three-dimensional network and increase the paste's viscosity. Starch paste can contain un-swollen granules, partially swollen granules, aggregates of swollen starch granules, fragments and molecules of retrograded starch and starch that has dissolved or precipitated<sup>35</sup>.

The presence of relatively short chains of amylose and amylopectin adds opacity to starch suspensions. Rheological starch properties are studied through the behavior of viscosity curves, which are influenced by temperature, concentration and shear stress. "Paste properties" is the term used to describe the changes that occur in starch after gelatinization in excess water. Instruments like the Rapid Visco Analyzer (RVA)

describe the viscosity parameter as functions of temperature and time. The RVA describes paste behavior in three periods: (i) a controlled heating period, increasing the temperature of the suspension from room temperature to a maximum that is generally determined at 95 °C; (ii) an isothermal period, maintaining the suspension at the maximum temperature for analysis; and (iii) a cooling period, decreasing the temperature to approximately 50 °C. Throughout the analysis, the suspension is subjected to shear forces. Suspensions typically exhibit a peak in viscosity that starts after gelatinization and increases as the granules swell, followed by a decrease in viscosity due to granule disintegration and polymer realignment. A "Breakdown" is defined by a difference between the viscosity peak and the minimum viscosity at the maximum analysis temperature. During the cooling period, amylose leaching forms a gel or three-dimensional network. Gel formation further increases the viscosity, called the "cold paste viscosity". The difference between the paste viscosity at the end of the cooling period and the minimum viscosity at 95 °C is termed the "setback"<sup>36,37</sup>

### **METHODS OF STARCH MODIFICATION**

Starch is rarely consumed in its intact form and frequently used by industry in its native form. Most native starches are limited in their direct application because they are unstable with respect to changes in temperature, pH and shear forces. Native starches show a strong tendency for decomposition and retrogradation. Additionally, some starch granules are inert, insoluble in water at room temperature, highly resistant to enzymatic hydrolysis and consequently lacking in functional properties. Native starches are often modified to develop specific properties such as solubility, texture, adhesion and tolerance to the heating temperatures used in industrial processes.<sup>38</sup>

Several methods have been developed to produce modified starches with a variety of characteristics and applications. All of these techniques alter the starch polymer, making it highly flexible and changing its physicochemical properties and structural attributes to increase its value for food and non-food industries.<sup>39</sup> The starch modification industry is constantly evolving. Modifications of starch include physical, chemical and enzymatic methods.<sup>40</sup>

### **PHYSICAL METHODS**

#### **1. Heat-moisture treatment**

Heat-moisture treatment (HMT) of pulse starches at restricted moisture levels (22-27%) and high temperature (100-120°C) for 16 h has been shown to alter the structure and physicochemical properties of smooth pea, wrinkled pea, navy bean, lentil and pigeon pea starches<sup>41</sup>. HMT of pulse starches has been shown by the above authors to decrease amylose leaching, granular swelling and peak viscosity, and to increase thermal stability, gelatinization temperatures and susceptibility towards  $\alpha$ -amylase and acid hydrolysis. These changes were attributed to an interplay of factors such as:

- (1) amylose content,
- (2) interactions between starch chains,
- (3) arrangement of amylose chains within the amorphous domains and
- (4) lipid-amylose complexes.

#### **2. Annealing**

Annealing refers to treatment of starch in excess water (<65% w/w) or at intermediate water contents (40-50% w/w) at temperatures below the onset temperature of gelatinization. The physical aim of annealing is to approach the glass transition temperature, which enhances molecular mobility without triggering gelatinization. Annealing of lentil, smooth pea and wrinkled pea starch<sup>41,42,46,47</sup> has also been shown to decrease granular swelling and amylose leaching and to increase gelatinization temperatures, thermal stability, and susceptibility towards  $\alpha$ -amylase. These changes were attributed by the above authors to an increase in crystalline perfection and increased interaction between amylose-amylose and amylose-amylopectin chains.

#### **3. Retrogradation**

The retrogradation process is defined as the linking of starch chains into ordered crystalline structures<sup>18</sup>. The linear fraction of starch is particularly susceptible to retrogradation. Amylose crystals are characterized by high thermostability (dissolution in water at 120-150°C) and resistance to the activity of amylase. Amylopectin, being the branched fraction of starch is, however, slowly retrogradable; whilst crystalline forms appearing only on the outside of the globule are characterized by a significantly lower temperature (40-70°C) of re-pasting and an increased susceptibility to amylase activity than the retrograded amylose<sup>49</sup>. The productivity and properties of the retrograded starch obtained have been reported to be largely affected by: origin of starch<sup>50-51</sup>, amylose content<sup>52-53</sup>, length of the amylopectin end chains<sup>54-55</sup>, density of the paste<sup>56-57</sup>, physical<sup>58-59</sup> or chemical<sup>60-61</sup> modifications, the presence of other compounds<sup>62-64</sup> well as by paste storage conditions<sup>65</sup>. Recrystallization of starch occurs most easily at a temperature approximating 0°C, but also at temperatures over 100°C, yet then it applies to amylase only. The retrogradation is intensified

by the application of repeated freezing and defrosting of the starch paste. The resulting starch thus produced is known as resistant starch that demonstrates resistance to digestibility by amylase enzymes and thus can be as an alternative nutrient aid for diabetic patients and as a rate controlling polymer coat in controlled drug delivery systems.

#### 4. Freezing

Freezing is a physical treatment widely applied for preservation, drying and lyophilisation of starchy food<sup>66-67</sup>. It is also used for sample preparation in granule structural investigations by means of many physical methods, for instance in scanning electron microscopy (SEM) or transmission electron microscopy (TEM)<sup>68-71</sup>. It was reported that freezing influenced textural and gelatinization characteristics of starch<sup>72-73</sup>. It was also considered to cause some changes in the nutritional properties of starch<sup>74</sup>. Freezing of starch sols resulted in their coacervation and increasing retrogradation, while pregelatinised starch became less sensitive to retrogradation and stayed smooth after the process<sup>73,75</sup> as well as Perry and Donald (2001) reported that some reversible structural disorder of starch granules occurred at sub-zero temperatures. Freezing water inside or outside of the starch granule seems to be a particularly effective way for modification for potato starch. This is because the starch B granules contain a significant amount of structural water, which determines the granular inner structure.

#### 5. Ultra High Pressure Treatment

UHP treatment of the starch-water suspension reversibly hydrates amorphous regions within granules, leading to the granule swelling and crystalline region distortion, and thus, the crystalline regions become more accessible to water<sup>76-80</sup>. Finally, starch granules are non-thermally gelatinized over a critical pressure level which varied depending on botanical sources and crystal packing arrangements of starch<sup>80-82</sup>. Further, UHP-treated starch that is little or partially gelatinized maintains its granular structure<sup>83</sup> and restricts amylose leaching from granules and granule swelling capacity<sup>82-83</sup>. Moreover, UHP treatment has been shown to transit A-type crystallite pattern to B-type crystallite pattern<sup>84-85</sup>. These noted physical and structural characteristics of UHP-treated starch granules are anticipated to impact its reactivity in starch chemical modification and modified starch property.

#### 6. Glow Discharge Plasma Treatment

A glow discharge plasma is used which can produce high energy electrons and other highly active species at room temperature. This approach has been extensively used in material modification<sup>86</sup>. When applied to starch, the highly active species can excite chemical groups in starch, inducing modification process without the assistance of other chemicals. Thermal degradation is minimized. Starch is highly cross linked by glow discharge plasma, without the assistance of conventional chemical agents, thus avoiding any environmental concerns<sup>87</sup>.

#### 7. Osmotic-Pressure Treatment

"Osmotic-pressure treatment" (OPT) was carried out in the presence of high salt solutions<sup>88</sup>. Starch solution was suspended in sodium sulphate to obtain a uniform starch suspension and heat distribution. The potato-starch treated changed from a B to an A type after treatment with the gelatinization temperatures increasing significantly. A uniform heat distribution is provided for using this method as compared to heat-moisture treatment and modified starch is able to be produced in a large scale.

#### 8. Thermal Inhibition

Thermal inhibition of starch is done by dehydrating starch until it is anhydrous (<1% moisture) and treating it to a temperature of 100°C or greater for a period of time enough to inhibit starch. An alkaline condition enhanced the effect of heating. Pastes formed from these starches had increased resistance to viscosity breakdown and a non-cohesive texture<sup>89</sup>. Thermal inhibition with ionic gums had sodium alginate, CMC and xanthan behaving as crosslinking agents and were able to form graft copolymers through ester formation<sup>90</sup>.

#### 9. Gelatinization

The process of gelatinization causes substantial changes in both the chemical and the physical nature of granular starch due to the rearrangement of intra- and intermolecular hydrogen bonding between the water and starch molecules resulting in the collapse or disruption of molecular orders within the starch granule<sup>91</sup>. This results in irreversible changes in the starch properties<sup>92</sup>. Evidence of the loss of an organized structure includes irreversible granule swelling, loss of birefringence and crystallinity<sup>93,94</sup>

### CHEMICAL MODIFICATION

#### Chemical Modifications

Chemical modification involves the introduction of functional groups into the starch molecule, resulting in markedly altered physico-chemical properties. Such modification of native granular starches profoundly alters their gelatinization, pasting and retrogradation behavior. The chemical and functional properties

achieved when modifying starch by chemical substitution depend, inter alia, on starch source, reaction conditions (reactant concentration, reaction time, pH and the presence of catalyst), type of substituent, extent of substitution (degree of substitution, DS1; or molar substitution, MS2), and the distribution of the substituents in the starch molecules.<sup>95,96</sup>

Modification is generally achieved through derivatization such as etherification, esterification, cross-linking and grafting of starch; decomposition (acid or enzymatic hydrolysis and oxidization of starch). These techniques are however limited due to issues concerning costomers and the environment. These techniques include:

### 1. Etherification & Esterification

This method primarily includes substitution of hydrophilic hydroxyl groups of starch by various hydrophobic functional groups resulting into hydroxypropylation, carboxymethylation, acetylation, succinylation, etc.. The carboxymethyl substitution of starch hydroxyl groups gives rise to derivatives that are cold water-soluble. To prevent starch gelatinization, the reaction has to be carried out in an organic medium. Carboxymethyl starch, under the name sodium starch glycolate, is used in the pharmaceutical industry as a disintegrant and as a sizing and printing agent in the textile industry. Highly substituted derivatives are possible. In acetylation, hydrophilic hydroxyl groups are substituted with hydrophobic acetyl groups. Acetylation makes starch more hydrophobic and prevents the formation of hydrogen bonding between hydroxyl groups and water molecules. Since the tendency of an aqueous starch dispersion to increase in viscosity on cooling and finally to gel is related to the association of amylose molecules, a treatment such as acetylation which retards or eliminates this crystallization or retrogradation will effect stabilization of the starch sol. Acetylation also prevents or minimizes association of amylopectin outer branches. This is of practical value in many industrial and food applications because such associations can cause cloudiness and syneresis in aqueous dispersions of starches. Hydroxypropylated starches are generally prepared by etherification of native starch with propylene oxide in the presence of an alkaline catalyst. The hydroxypropyl groups introduced into the starch chains are capable of disrupting the inter- and intra-molecular hydrogen bonds, thereby weakening the granular structure of starch, leading to an increase in motional freedom of starch chains in amorphous regions. Hydroxyethyl starch, prepared by reaction with ethylene oxide, was of considerable biomedical interest as a blood plasma expander and also as a cryoprotective agent for erythrocytes<sup>101</sup>. Starch phosphorylation is the earliest method of starch modification<sup>102</sup>. The reaction gives rise to either monostarch phosphate or distarch phosphate (cross-linked derivative), depending upon the reactants and subsequent reaction conditions. Monoesters, rather than diesters, are produced with a higher level of phosphate substitution on starch. The introduction of phosphate substitution on amylose or outer branches of amylopectin prevents linearity of molecular chains due to steric hindrance. Thus, a situation is achieved where individual chain segments can no longer approach each other closely enough to establish intermolecular or intramolecular association; they, therefore, lead to a better paste clarity. The phosphate diester starches have the phosphate esterified with two hydroxyl groups, very often from two neighboring starch molecules<sup>103</sup>. This leads to the formation of a covalent bridge or cross-linking. Phosphate cross-linked starches show resistance to high temperature, low pH, high shear, and leads to increased stability of the swollen starch granule. They improve viscosity and textural properties of the starch. As a thickener and stabilizer, starch phosphate diesters are superior to unmodified starches. They also provide resistance to gelling and retrogradation, and do not synerese on storage. Derivatization of starch with an ionic substituent group such as succinate at low degree of substitution (DS) converts it into a polyelectrolyte, which makes it acquire typical properties of a polyelectrolyte like increased hydrophilic

character and increased solution viscosity<sup>104</sup>. This modification is also known to weaken the internal bonding that holds the granules together. In addition starch succinate offers very desirable properties such as low-temperature stability, high-thickening power, and low-gelatinization temperature, clarity of cooked food, good film-forming properties and reduced tendency to retrograde. High cationic properties have been observed for starch citrates. The reaction is facilitated thermochemically by the dry heating (~120°C for 24 h) of citric acid to an anhydride, which reacts with starch to form an adduct, followed by further reaction to yield cross-linked starch citrate. The reaction can be controlled to maximize reaction efficiency, minimize cross-linking, and maximize carboxyl content. They are used to remove toxic, heavy metal ions in water purification (industrial waste water) as biodegradable ion-exchange materials against the petrochemically derived ion-exchange resins.

### 2. Cross-Linking

Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds that act as a bridge between the starch molecules. Important factors in the cross-linking reaction include chemical composition

of reagent, reagent concentration, pH, reaction time and temperature. Because the degree of cross-linking for food starch is very low, the extent of reaction and yield of cross-linked starch are difficult to measure chemically; hence there is a need for physical property measurement. When phosphorus oxy chloride (phosphoryl chloride, POCl<sub>3</sub>, MW153.3) is added to starch slurry under alkaline conditions (pH 8–12), the hydrophilic phosphorus group immediately reacts with the starch hydroxyls, forming a distarch phosphate<sup>65</sup>. Cross-linking alters, not only the physical properties, but also the thermal transition characteristics of starch, although the effect of cross-linking depends on the botanical source of the starch and the cross-linking agent. Decrease in retrogradation rate and increase in gelatinization temperature has been observed with cross-linked starch, and these phenomena are related to the reduced mobility of amorphous chains in the starch granule as a result of intermolecular bridges<sup>105</sup>. However, Jyothi et al.<sup>106</sup> showed that cross-linked starch has more pronounced syneresis than has native starch because of ordered structure in the starch paste, thus resulting in a higher degree of retrogradation.

### 3. Acid Treatment

In acid modification, the hydroxonium ion attacks the glycosidic oxygen atom and hydrolyses the glycosidic linkage. An acid acts on the surface of the starch granule first before it gradually enters the inner region. Acid modification changes the physicochemical properties of starch without destroying its granule structure and the properties of acid-thinned starches differ according to their origin<sup>100</sup>. The gelatinization temperature and the breadth of the gelatinization endotherm have also been shown to increase on acid hydrolysis. The retrogradation rate of acid thinned starch increased as hydrolysis proceeded. The method for the manufacture of acid thinned starch entails treating concentrated starch slurry with mineral acid at temperatures below gelatinization temperature for specific period depending on the desired viscosity or degree of conversion. Effect of different acids (HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub>) under similar conditions of treatment on molecular weight, alkali fluidity number, iodine binding capacity and intrinsic viscosity of various starches has also been studied<sup>107</sup>.

### 4. Oxidation

Oxidized starch is produced by reacting starch with a specified amount of oxidizing reagent under controlled temperature and pH<sup>108</sup>. Oxidation causes depolymerisation, which results in a lower dispersion viscosity and introduces carbonyl and carboxyl groups, which retard Recrystallization<sup>109</sup>.

### 5. Dual Modification

These include methods that involve the chemical reaction in the presence of a specific physical environment or an enzymatic treatment that make serve to enhance the rate of derivatization or can enhance the degree of substitution in some instances. Microwave assisted esterification to produce starch maleate using the dry method had a reaction efficiency of up to 98% and a reaction time of less than 5 min. This is thought to be an efficient method in esterifying starch<sup>110</sup>. The high efficiency in producing succinylated cassava starch with microwave assistance was also observed by Jyothi et al, (2005)<sup>111</sup>. This is a good method to decrease the use of chemicals to enhance production. Microwave and ultrasound irradiation was used for the Esterification of carboxymethyl cold-water-soluble potato starch with octenyl succinic anhydride. They were positively able to shorten the esterification time from a few hours to a few minutes. The derivatives displayed excellent emulsifying and surfactant performance properties<sup>112</sup>. A combined method of modification using crosslinking and phosphorylation on rice starch provided modified rice starch with good freeze-thaw stability. Modified corn and mung bean starch was prepared by treating native starch with a mixture of fungal  $\alpha$ -amylase and glucoamylase followed by hydroxypropylation with propylene oxide. This partial enzymatic hydrolysis produces modified starch that possesses significantly different functional properties to that of hydroxypropyl starch prepared under normal conditions<sup>113</sup>.

Rajan et al., 2007 have also demonstrated that the use of fungal lipase to enhance the Esterification of cassava starch using recovered coconut oil gives thermoplastic starch which has got wide use in plastic industry, pharmaceutical industries, and in biomedical applications such as materials for bone fixation and replacements, carriers for controlled release of drugs and other bioactive agents. Unlike chemical esterification, enzymatic esterification is ecofriendly and avoids the use of nasty solvents.

### ENZYMATIC MODIFICATIONS

This involves the exposure of starch suspensions to a no of enzymes primarily including hydrolyzing enzymes that tend to produce highly functional derivatives. Origin of this technique can be dated back to the times when glucose syrup or high fructose corn syrup was produced. The enzymes amylomaltases( $\alpha$ -1,4- $\alpha$ -1,4 glucosyl transferases) found in eukarya, bacteria and archea representatives, breaks an  $\alpha$ -1,4 bond between two glucose units to subsequently make a novel  $\alpha$ -1,4 bond producing a modified starch that can be used in foodstuffs, cosmetics, pharmaceuticals, detergents, adhesives and drilling fluids. It is also a good

source of plant-derived substitute for gelatin except that it forms a turbid gel whereas gelatin gels are transparent<sup>114</sup>. In the study by Hansen, Blennow, Pedersen, Nørgaard, and Engelsen (2008)<sup>115</sup> on gel texture formed in the modification of potato, high-amylose potato, maize and pea starch with amyloamylase (AM) isolated from the hyperthermophilic bacterium *Thermus thermophilus*, there was an improvement in gel texture compared to the parent starch. All the modified starches showed broadened amylopectin chain length profiles. Cyclomaltodextrinase (CDase; EC 3.2.1.54), isolated from alkalophilic *Bacillus* sp. I-5 (CDase I-5) was used to modify rice starch to produce low-amylose starch products. The amylose content was found to have decreased significantly from 28.5 to 9% while there was no significant change in the side chain length distribution of the amylopectin. Storage of the modified rice starch at 4 °C for 7 days, showed that the retrogradation rate had significantly retarded compared to the control sample<sup>116</sup>.

Treatment of maize starch with  $\beta$ -amylase,  $\beta$ -amylase and transglucosidase, maltogenic  $\alpha$ -amylase, and maltogenic  $\alpha$ -amylase and transglucosidase, resulted into significant reduction in digestion rate by 14.5%, 29.0%, 19.8%, and 31.0%, respectively producing resistant starch with reduced glycemic index that can be used in diabetes, prediabetes, cardiovascular disease and obesity. An increase in the starch branch density and crystalline structure in the modified starches was thought to contribute to the slow digestion<sup>117</sup>.

Kim & Robyt, 2000 worked with Cyclomaltodextrin Glucanoyltransferase (CGTase, EC 2.4.1.19) in the presence of isoamylase to produce cyclodextrins (CDs) with a maximum yield of 3.4 and 100% retention inside waxy maize starch granules. Cyclomaltodextrins are also formed in situ, with the retention of CDs in the granule and this leads to the production of a new material that has properties of starch granules and Cyclomaltodextrins. Formation of complexes of organic molecules with Cyclomaltodextrins provides stabilization of light, heat and oxygen-sensitive materials in the starch granules and also a mechanism for their slow release besides providing special tastes, odors and flavors to the starch granules.

### Genetic Modification

These set of techniques involve transgenic technology that targets the enzymes involved in starch biosynthesis thus avails the advantage over environmentally hazardous post harvest chemical or enzymatic modifications. Genetic modification can be carried out by the traditional plant-breeding techniques or through biotechnology.

#### 1. Amylose-Free Starch

Amylose-free waxy starch was produced by the simultaneous antisense downregulation of three starch synthase genes (Granular Bound Starch Synthetase, Starch Synthetase II and Starch Synthetase III), that resulted in waxy starch with small amylopectin chains. Generally mutant maize is grown on commercial scale, through modified corn, wheat, sorghum, amaranth are also available. Waxy starch thus produced is generally used in food industry as it gelatinizes easily, yielding clear pastes that will not gel.

#### 2. High-Amylose Starch

High-amylose starch in cereals is produced by a mutation in the gene that encodes starch-branching enzyme (SBE) IIb, which is also known as 'amylose extender (ae)'. In potato, discovery of the corresponding gene and the downregulation of its expression in tubers using antisense techniques enabled the production of starches that have slightly increased amylose levels<sup>89</sup>. However, both SBEI and SBEII had to be inhibited to create starches with amylose levels of more than 60%<sup>90</sup>. Recently, a more efficient method of inhibiting gene function using single domain antibodies against SBEII was used to produce starches that had even higher amylose levels<sup>91</sup>. The derivative thus produced serves as an important precursor for the development of coating polymers that are resistant to enzymatic degradation and can be used in colon-targeted drug delivery systems. High-amylose starches can also be processed into 'resistant starch', which has nutritional benefits<sup>118</sup>.

**3. Altered amylopectin structure Amylopectin synthesis** is governed by a no of enzymes including starch Synthetase, branching enzymes and debranching enzymes each of which also has an isoforms. Therefore the downregulation of any one enzyme fails to produce an entirely new amylopectin features. However the inhibition of SS II and SS III isoforms in a few rice species demonstrates functional derivatives with low gelatinization temperature (below 50 °C).<sup>119</sup>

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