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Crosslinking of starch and its effect on viscosity behaviour

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Abstract: Starch is a widely used component in the food industry. Native starch does not have the desired properties, so it is modified according to the required properties. Out of different methods, crosslinking is one of the widely used ones. There are different crosslinking agents available. The method and mechanism of widely used crosslinking agents like phosphorous oxychloride, adipate and epichlorohydrin are discussed and compared. When starch is crosslinked, its properties vary. The extent of crosslinking can be predicted with the help of properties like viscosity and swelling. They are also compared for crosslinking by different agents.

Keywords: crosslinking mechanism; starch crosslinking; viscosity behaviour.

1 Introduction

Starch is a natural polymer. It is generated by photosynthesis in plants from carbon dioxide and water (Liu et al. 2013). It is a major reserve substance of plant sources. Owing to its renewability (Kadajji and Betageri 2011), low cost and complete biodegradability (Santander-Ortega et al. 2010), starch became a promising candidate in various applications. In view of this, starch has attracted many researchers for many decades (Kumari and Rani 2011, Raafat et al. 2012).

Some limitations like low resistance to moisture, poor processability, poor mechanical properties and dimensional stability for its end products (Moad 2011) etc challenge this promising candidate. Brittleness and water solubility of starch film limits its applications (Woggum et al. 2015). Therefore, native starch is not used directly (Zhang et al. 2015). It is also incompatible with some hydrophobic polymers (Zhang et al. 2015). Starch contains an abundance of hydroxyl groups. The presence of these hydroxyl groups develops a hydrophilic nature in starch, but it becomes a major constraint that seriously limits the development of starch-based materials (Koo et al. 2010).

Many researchers suggested several strategies to overcome these problems. Various modifications like chemical or physical modification of starch granules have been considered, including blending (Chen et al. 2006, Vroman and Tighzert 2009, Fuentes-Zaragoza et al. 2010, Neelam et al. 2012, Sapsford et al. 2013) and chemical modifications.

Some physical methods are gelatinisation, thermal inhibition, osmotic-pressure treatment, glow discharge plasma treatment, ultra-high pressure treatment, freezing, retrogradation, annealing, heat moisture treatment, etc. Physical modification of starch will convert native starch into small-crystalline or cold water-soluble starch. It is due to changing the granular structure of native starch. These sets of techniques are generally given more preference as these do not involve any chemical treatment. Chemical modification produces water-resistant material with various degrees of substitution (Chen et al. 2006, Neelam et al. 2012, Sapsford et al. 2013).

Nowadays, starch is widely used in drug delivery and in food products. It contributes to not only the appearance but also the varying structure and quality of the food item (Fuentes-Zaragoza et al. 2010, Hoover et al. 2010). When such modified starch is used, it affects also the physicochemical properties of foods (Sweedman et al. 2013). Chemical modification affects the *in vitro* digestibility of starch (Horchani et al. 2010, Ashwar et al. 2015).

According to modifications made with starch, its digestibility alters. According to digestibility, there are three classes of starch: resistant starch, delayed digestible starch and fast digestible starch (Walker et al. 2011). The resistant starch takes time to get digested due to its modified structure. According to the modification there are four types of resistant starch: granular starch, physically inaccessible starch, chemically modified starch and high amylose starch (or retrograded starch) (Teacă et al. 2014). When restricted swelling

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and stabilised granular structure is required, crosslinking of starch is an established technique in the food and pharmaceutical industry (Ratnayake and Jackson 2008). The physicochemical and nutritional properties of crosslinked starches have been the focus of many preceding studies (Carmona-Garcia et al. 2009, Koo et al. 2010, Kim et al. 2015).

To modify the structure and properties of biopolymers, chemical modification is one of the most effective methods (Cui 2005). There are many chemical reactions such as crosslinking, esterification, etherification, oxidation and acid hydrolysis that result in modification of starch (Le Corre et al. 2010).

Crosslinking is the most widely used method for modification of native starch. Swelling of the starch granule under cooking conditions will be restricted due to crosslinking. Crosslinking may also prevent gelatinisation of starch. Even a very low level of reagent can crosslink starch (Cui 2005). Crosslinking of starch depends on factors like source of starch, crosslinking reagent composition and concentration, the extent of substitution, reaction time, temperature, pH, etc (Koo et al. 2010, Ashogbon and Akintayo 2014, Blennow 2015).

There are many known crosslinking reagents. Out of these, phosphoryl chloride, adipic acetic mixed anhydride, sodium trimetaphosphate (STMP) and mixtures of STMP and sodium tripolyphosphates (STPP) are crosslinking agents for food grade starch that are permitted by the FDA. Earlier, epichlorohydrin was also used. But chlorohydrins are a carcinogen, so it is now out of use in the U.S. by manufacturers of crosslinked starch (Singh et al. 2012, De Oliveira et al. 2014).

2 Mechanism of crosslinking with different reagents

There are two types of hydroxyl groups attached to starch: (6-OH) is primary and (2-OH and 3-OH) are secondary. The reaction of these hydroxyl groups with multifunctional reagents results in crosslinked starches. In a granule of starch, many chains are found in closer proximity, and such reactions not only take place between single chains, but it links side by side chains as well. These crosslinking agents form either ether or ester inter-molecular linkages between hydroxyl groups on starch molecules (Koo et al. 2010, Whistler 2012). A small amount of multifunctional reagent is enough to interconnect starch molecules by crosslinking reactions (Singh et al. 2012).

2.1 Crosslinking with phosphorous oxychloride

Phosphorous oxychloride gives a rapid reaction with starch to produce a distarch phosphate. The efficiency of crosslinking is pH dependent. Above pH 11, the most efficient crosslinking reaction can be carried out. The presence of sodium sulphate [2% w/w dry base (db) starch] enhances the rate and efficiency further (Singh et al. 2010, Gao et al. 2014).

Phosphoryl chloride contains three chlorides. Upon addition of phosphoryl chloride to starch solution, the first chloride ion [hl (half life)=0.01 s] will form a phosphorous dichloride after reaction with water immediately at 25°C (Delley et al. 2012) to form a phosphorous dichloride, which is likely the crosslinking agent. Simultaneously, the water molecule reacts with the remaining two chlorides (hl=~4 min) of phosphorous dichloride at 25°C. Phosphoryl chloride does not get enough time to diffuse into the starch granules. Practically, the addition of phosphoryl chloride to a starch solution should be as fast as possible in order to generate phosphorous dichloride *in situ*, which then diffuses into the granule to effect crosslinking (Cui 2005, Singh et al. 2012).

2.1.1 Crosslinking of corn starches with $POCl_3$ under ultra-high pressure

Kim et al. (2012) suggested a method to crosslink starches with $POCl_3$ (Figure 1). As per this method, dissolve anhydrous sodium sulphate (10.0%, w/w) in 80 ml of double-distilled water. Suspend starch (20 g, w/w, db) in solution under rapid stirring. Add 0.1% w/w $POCl_3$ in it. Adjust pH around 11.5 with 1 M NaOH. Carry out reaction for 120 min at 45°C. After the reaction, neutralise mass to pH 5.5 with

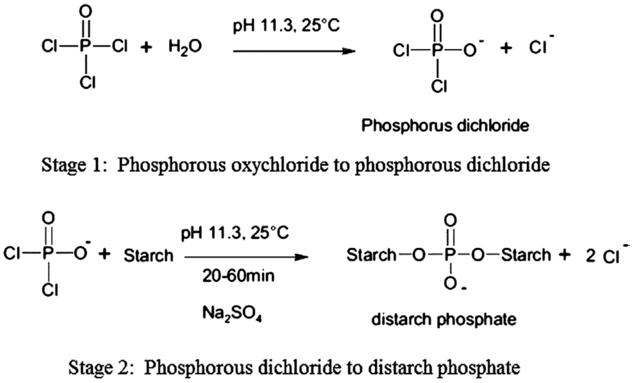

Figure 1: Mechanism of crosslinking of corn starches with phosphorous oxychloride ($POCl_3$).

1 M HCl. Separate and dry the polymer. Kim et al. (2012) also suggested a method to crosslink starches with $POCl_3$ under ultra-high pressure. It completes the crosslinking reaction within 15 min at 400 MPa.

2.2 Crosslinking with sodium trimetaphosphate

STMP is a nonhazardous solid crosslinking agent for starch (Cui 2005). It gives slow reaction for crosslinking of starch. It produces distarch phosphate as a result of reaction (Figure 2). The presence of sodium sulphate or rise in pH accelerates the rate (Singh et al. 2012).

2.2.1 Preparation of crosslinked corn starch

Shi et al. (2013) suggested a method to crosslink starch with a mixture of STMP/STPP (99/1% w/w). Mix corn starch with a mixture of STMP/STPP (99/1% w/w) and dissolve in water. Adjust the pH to 11.0 with 0.1 N NaOH. Maintain 45°C temperature for 3 h in a shaking water bath. Neutralise the suspension to pH 6.0 with 0.1 N HCl. After washing, dry at 40°C for 24 h in an oven (Zhang et al. 2014).

2.3 Crosslinking with adipate

Adipate gives a rapid crosslinking reaction with starch at pH 8 (Figure 3). The starch solution pH is maintained by addition of mixed acetic/adipic acid anhydride. Often, along with crosslinking of adipate, acetylation of hydroxyls also takes place. These two reactions produce a doubly modified starch (Singh et al. 2012). This reagent is required in small quantity, so it is very difficult to measure the extent of crosslinking. To carry out crosslinking with phosphorylating reagents, ^{31}P-NMR could be used to measure the extent of crosslinking (Cui 2005, Hanashiro 2015).

2.4 Crosslinking with epichlorohydrin

The mechanism of the epichlorohydrin (1-chloro-2, 3-epoxypropane; MW 92.5) reaction with starch occurs over a series of steps (Zhang et al. 2014). Epichlorohydrin gives multifunctional reaction with starch. As the reaction proceeds, a single crosslink forms consuming either one or two molecules of epichlorohydrin. Diester and diglycerol molecules form in crosslinked starch molecules.

To crosslink starch with epichlorohydrin, take a starch solution and add epichlorohydrin in lower concentration. Stir the reaction mass, and maintain the temperature at 40°C for 17 h. To end the reaction, add 3% solution of HCl and bring pH to 5.25. Filter, wash and dry the mass (Zhang et al. 2014).

3 Functional properties

The extent of crosslinking depends not only on parameters like the crosslinking agent and its concentration, temperature, reaction time and pH, but also on the treatment conditions of raw starch and on how the starch is prepared in the final application. When the degree of crosslinking is very low, the extent and yield of crosslinking of starch

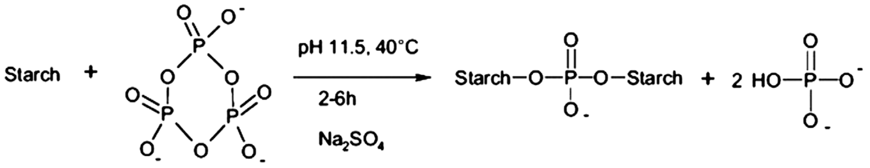

Figure 2: Crosslinking of corn starch with sodium trimetaphosphate.

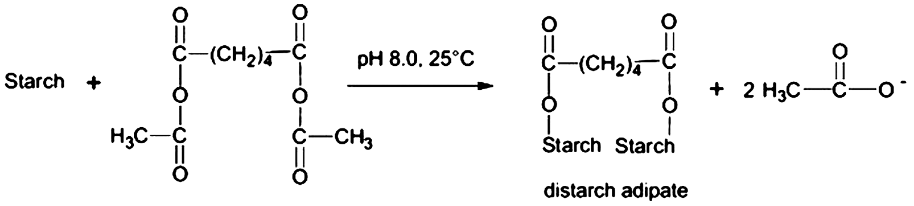

Figure 3: Crosslinking of corn starch with adipate (mixed acetic/adipic acid anhydride).

become difficult to measure chemically; hence, there is a need for physical property measurement (Zhang et al. 2014). There are some physical indications like change in swelling, pasting curves, optical clarity and fluidity of starch granules. One can use these properties to measure the extent of crosslinking (Singh et al. 2012).

Swelling and viscosity of the crosslinked starch are the most useful properties to measure the extent of the crosslinking (Zhang et al. 2014). When the viscosity behaviour of crosslinked starch is studied on rapid viscosity analyser (RVA), it shows that the peak viscosity of crosslinked starch is higher than that of native starch (Chantaro et al. 2013). The strength of the additional covalent bonds of the crosslinked starch enables the granules to withstand the 95°C temperature and not dissolve at >30 min (Zhang et al. 2014).

The viscosity breakdown of crosslinked starch is reduced compared with that of native starch. The crosslinked molecules are bonded with each other, increase mechanical strength of the molecule, keep the swollen granules intact and, hence, prevent loss of viscosity and provide resistance to mechanical shear (Cui 2005). Starch with a higher level of crosslinking will show a lower peak viscosity compared to starch with low level of crosslinking. This may be due to the higher density of crosslinks; it is also consistent with the swelling behaviour, which decreases with increase in crosslinking agent concentration (Figure 1). The level of crosslinking varies directly with the concentration of the crosslinking agent, time and temperature. The greater the degree of crosslinking, the smaller the granule volume. As a result, at the same concentration, the highly crosslinked starch shows lower peak viscosity than the lower crosslinked starch.

Increasing the level of crosslinking eventually will reduce granule swelling. At very high crosslinking levels, the crosslinks completely prevent the granule from swelling. Such starch cannot even be gelatinised (Cui 2005, Chantaro et al. 2013).

Figure 4 shows the amylograms (viscosity behaviour) of crosslinked and native corn starch. When phosphoryl chloride is used to crosslink starch, the highest peak viscosity is achieved with the lowest concentration (e.g. 0.013%, w/w). When the quantity of phosphoryl chloride increases (from 0.013 to 0.038%), a steady decline in both peak viscosity and cool paste viscosity is found (Cui 2005).

At higher swelling, starch molecules become quite fragile. Upon prolonged heating or agitation they tend to be fragmented. Due to such fragmentation, its viscosity falls rapidly. In RVA study this behaviour can be identified. Most of the starches are very sensitive to acid, which also results in a rapid breakdown in viscosity. Crosslinking helps the starch granule to hold its structure against this breakdown, so crosslinked starch does not show rupture and loss of viscosity under acidic conditions (Kahraman et al. 2015). The RVA viscosity curves for crosslinked and native waxy corn starch cooked at pH 5 and pH 7 are found to be the same.

RVA study of raw waxy corn starch shows a sharp reduction in viscosity under acid conditions, whereas crosslinked starch retains higher working viscosity and shows less viscosity breakdown (Cui 2005). At a high level of crosslinking, native waxy corn starch gives

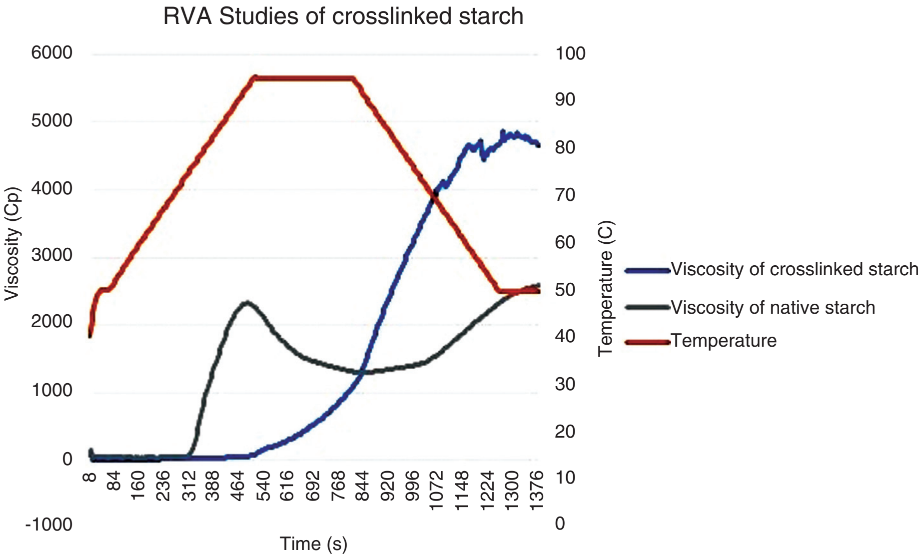

Figure 4: Effect of mild crosslinking on viscosity of corn starch and native corn starch.

cohesive, rubbery and stringy texture upon cooking, whereas crosslinked starch gives paste-like texture with good rheological properties, which makes it suitable as a thickener in food applications (Kahraman et al. 2015).

3.1 Determination of the degree of crosslinking

Kaur, Singh and Singh suggested a viscosity-based method to measure the degree of crosslinking of modified starches (Kaur et al. 2004). The viscosity of the starch solution (25% w/w/) is to be measured with temperature in RVA or controlled-stress rheometer equipped with a starch pasting cell. On the basis of peak viscosity, the degree of crosslinking can be calculated by using the following equation:

$$\text{Degree of cross linking} = (\text{A-B}) / \text{A} \times 100$$

where A is the peak viscosity of the control sample (without crosslinking agent) and B is the peak viscosity of the crosslinked starch.

For the experiments carried out to study the viscosity behaviour of starch and crosslinked starch, RVA was used. There are programmed heating and cooling cycles in standard RVA instruments. Starch solution in measured concentration is placed in the RVA, and the temperature of the starch solution is raised from 50 to 95°C at a constant rate of 11°C/min and held at 95°C for 2 min. Then the paste is cooled down to 50°C at 11°C/min and finally kept at 50°C for 2 min. During this total cycle, viscosity is measured continuously. The peak viscosity of the sample will be shown on the amylograph produced by the RVA.

4 Applications

When starch is crosslinked, its original characteristics will be altered. It shows properties like slow gelatinisation rate, stable viscosity in acidic condition, low initial viscosity, high shear during the homogenisation process, rapid rise in temperature and high heat transfer (Cui 2005). For appropriate viscosity, gelatinisation and textural properties for food applications, crosslinking is often used in combination with other methods like esterification, etherification, hydrolysis, oxidation, etc. This alteration makes crosslinked starch more suitable for applications like salad dressings, canned foods, etc. In applications like deep-fried foods, pudding, fruit filling, gravies, soups, sauces, baby foods, etc., crosslinked starches are widely applied. The use of drum-dried crosslinked starches to develop pulpy texture in food systems is common. For example, waxy corn has low amylose content, and so it is used to improve crumb softness and cake volume (Whistler 2012).

5 Conclusion

The degree of swelling, peak viscosities and intergranular interaction of crosslinking starch varies inversely with the concentration of the crosslinking agent.

Among all these agents studied here, phosphorous oxychloride is the fastest one. At equal swell ratios, it imparts higher viscosity because the concentration of crosslinks at the surface makes the granules more rigid or because there is increased friction between granules (Shi et al. 2013).

From this study, we can conclude that there is a strong relation between factors like type and concentration of crosslinking agent, reaction time, pH, etc. They all affect the degree of crosslinking, which can be identified from viscosity and the degree of swelling.

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