Physicochemical Properties of Cross-linked Wheat Starch Affected by L-Ascorbic Acid

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ABSTRACT

There is some evidence on the effects of organic acids on the functional properties of native starches. However, there is little information on such effects on modified starches. The main objective of this study was to determine the effects of L-ascorbic acid (150 mg kg^{-1}, as the permitted level in bakery products) on functional properties of cross-linked wheat starch. Once the starch was treated with L-ascorbic acid, some spots and cracks appeared on the surface of the starch granules, as observed under a scanning electron microscope. Besides, the water solubility increased while water absorption decreased, but intrinsic viscosity of the samples, as determined by a U-tube viscometer, remained unchanged. Also, gelatinization temperature and enthalpy of gelatinization, measured by differential scanning calorimetery, as well as the hardness and elasticity of the starch gels decreased, while adhesiveness and cohesiveness of the gels remained unaffected. In general, it was concluded that the ascorbic acid had some degradation effect on cross-linked wheat starch molecules that could further affect its functional properties.

INTRODUCTION

Starch, as the major polysaccharide in plants, has extensive applications in many food and non-food products. It contributes mostly as a source of energy for human and also affects the physicochemical properties of the foods by acting as thickener, colloidal stabilizer, bulking, water retention, and gelling agents (Dias *et al.,* 1997; Tester *et al.,* 2004). Native starch is a suitable texture stabilizer and regulator in food systems, but restrictions such as low shear and thermal resistance, thermal decomposition, and high affinity towards retrogradation limit its applications in some food products (Copeland *et al.*, 2009; Kaur *et al.,* 2012).

To overcome the shortages of native starch and to generate new functionality, starch modification has been applied for many years. Different modified starches available today are generally produced through physical, chemical, genetical or enzymatic methods (Singh *et al.* 2007; Copeland *et al.* 2009). Chemical modification has a long history and has been used to introduce functional groups into the starch molecules. These changes would consequently alter starch gelatinization, pasting, and retrogradation behavior (Singh *et al.,* 2007; Kaur *et al.,* 2012).

Cross-linking is a common chemical method for starch modification. Using this method, inter- and intra- molecular bonds are introduced at random locations in the starch granules that stabilize and reinforce the granules. Phosphorus oxychloride (POCl$_3$), a mixture of adipic acid and acetic anhydride and vinyl chloride, is the major

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agent used to cross-link food grade starches (Hirsch and Kokini, 2002; Tomasik and Schilling, 2004). The cross-linked starch (CL) shows more resistance against long cooking times, acidity, and harsh shear compared to the native starch (Singh *et al.,* 2007; Kaur *et al.,* 2012).

The functional properties of the cross-linked starch as well as its native counterpart are affected by the components present in a food system such as fat, sugars, salt and acids. Previous studies on the interactions of starch with some of the food components indicated that the functional properties of the starch considerably altered in the presence of these components (Carvalho and Mitchell 2001; Farahnaky *et al.,* 2009; Considine *et al.,* 2011). Most of these studies focused on the effects of food components on native starches, while there is little information on such effects on modified starches.

Organic acids (e.g. ascorbic acid or vitamin C (AA), malic acid and lactic acid) are amongst the common food components. They may be naturally present in many foods or added to regulate pH, produce a sour taste, or to prevent microbial growth. Previous studies showed the effects of AA on some functional properties of native starches. Valles-Pamise *et al.* (1997) reported a decrease in paste viscosity of native cassava as affected by AA. Sriburi *et al.* (1999) reported that AA can depolymerize cassava starch molecules. The effects of AA on the rheological properties of native corn starch pastes were studied by Hirashima *et al.* (2005). Results showed that addition of the AA caused amylose and amylopectin chains to hydrolyze and led to the decrease in the viscosity at low pH values. Recently, the degradation effect of AA on native wheat starch was reported by Majzoobi *et al.* (2012a). Although there is some information on the effects of AA on native starches, no information was found to show such effects on cross-linked wheat starch.

The main objective of this study was to determine the physicochemical properties of cross-linked wheat starch in the presence of AA. Therefore, starch was first modified and then AA was added to the modified starch before and after starch gelatinization and the physicochemical changes were studied using different techniques.

MATERIALS AND METHODS

Native wheat starch was purchased from Fars-Glucosin Company (Marvdasht, Iran). Native wheat starch contained 9.24% moisture; 0.66% fat; 0.27% ash; 0.14% protein (all determined according to the Approved Methods of the AACC, 2000) and 26.35% amylose (determined by iodine method according to Williams *et al.,* 1970). AA and other chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). Cross-linked wheat starch was produced in the laboratory using $POCl_3$ as the cross-linking agent at pH 11.3 (Majzoobi *et al.,* 2012b). The number of cross-linking sites of the sample known as the degree of cross-linking was $7.94×10^{-5}$ as determined using the method described by Majzoobi *et al.* (2009).

Sample Preparation

To determine the effect of AA on CL wheat starch before gelatinization, slurries of each starch sample (40%, w/w) were made with distilled water containing AA (150 mg kg^{-1} starch basis). The sample was mixed well with a magnetic stirrer for 30 minutes at 40ºC. The pH value of the samples after addition of AA was 4.04±0.06. When the reaction between AA and CL was over, the NaOH (1 M L^{-1}) was added to adjust the initial pH value from 4.04 to 6.20. The sample was centrifuged at 3,000g for 15 minutes. The supernatant was discarded and the pellet was washed with distilled water three times to remove any salt or remaining acid. The pellet was dried in a vacuum oven at 40˚C to reach moisture content of about 8.5%. The dried starch was hammer-milled and sieved to obtain average particle size of

200 μm. A control was prepared under the same condition without acids.

To study the effects of AA on gelatinized CL starch, slurries of the starch in distilled water (40%, w/w) were prepared and heated in a boiling water bath at 95°C for 30 minutes. To confirm full gelatinization of starch, several samples were taken from the heated starch at the end of heating stage and observed under a light microscope. The absence of intact starch granules in the samples proved full gelatinization of the starch samples. Gelatinized starch was cooled down rapidly in a cold water bath (about 10 min) to reach about 70±2°C and then AA (150 mg kg^{-1} starch basis) was added, mixed by a magnetic stirrer for 30 min, while the temperature was kept constant. Then, the pH of the sample was adjusted to the initial pH value by addition of NaOH (1 M L^{-1}). The sample was washed several times with distilled water and then dried at 40°C to reach moisture content of 9.0%. After drying, the sample was hammer-milled and sieved to obtain average particle size of 200 μm. A control was prepared under the same condition without acid. The produced powder of each sample was used for further experiments.

Determination of the Thermal Properties of the Starches

Diffrential scanning calorimeter (DSC, Mettler Tledo, Switzerland) equipped with a thermal analysis data station was used. Starch (3.5 mg, dry basis) was weighted into a 40 μL aluminium pan and distilled water was added by Hamilton microsyringe to obtain a starch in water suspension contaning 70% water. Samples were sealed hermetically and left at ambient temeparture for 1 hour. The DSC analyser calibrated with indium and an empty aluminium pan was used as a reference. Samples were heated at a rate of 10 °C min^{-1} from 20 to 100°C. From the resulting DSC traces, gelatinization onset temperature (T_o), peak temperature (T_P), conclusion temperature

(T_c) and enthalpy of gelatinization (ΔH) were obtained.

Determination of the Microstructure

The microstructure of the CL starch before gelatinization was studied using a scanning electron microscope (SEM) (Model Leica Cambridge, UK). About 0.1 g of each sample was mounted on an SEM stub with double sided adhesive tape and sputter coated with gold. Scanning electron micrographs were taken using the electron microscope at 20kV.

Determination of the Intrinsic Viscosity

To determine the intrinsic viscosity of the samples before and after gelatinization, solutions of 6 mg mL^{-1} in dimethyl sulfoxide (DMSO) 90% (v/v, in distilled water) were prepared (Majzoobi *et al.,* 2003) and the full solubility of the samples was checked by a light transmission method (Kaur *et al.,* 2004). Then, a U-tube viscometer with the inside diameter of 0.5 mm in a water bath set at (30°C±0.1) was used to measure the relative, reduced, and intrinsic viscosities of the samples using Equations 1-3, respectively (Harding, 1997).

$$\eta_{rel} = \frac{\eta}{\eta_0} = (\frac{t}{t_0}).(\frac{\rho}{\rho_0}) \tag{1}$$

Where, η_{rel} is relative viscosity, t and t_0 are the times required for the sample and solvent (90% DMSO) to pass through the U-tube, respectively, ρ and ρ_0 are the densities of sample solution and solvent, respectively.

Then reduced viscosity (η_{red}) and intrinsic viscosity [η] were determined according to Equations (2) and (3), respectively.

$$\eta_{red} = \frac{\eta_{rel} - 1}{C} \tag{2}$$

Where, *C* is the concentration of the sample.

$$[\eta] = \lim_{c \to 0} \eta_{red} \tag{3}$$

Determination of Water Absorption and Solubility

The method of Leach *et al.* (1959) was used to determine water absorption and solubility of the samples with some modifications. For the ungelatinized sample, suspension containing 3% starch in distilled water was mixed well in a centrifuge tube over a vortex mixer for 5 minutes at room temperature. The tube was incubated in a shaking incubator for 30 minutes at 90°C. Then, it was centrifuged for 30 minutes at 700g. The supernatant was collected and dried at 120°C until a constant weight (about 2 hours) and weighted, while the pellet was weighted straight away. After gelatinization, the suspension prepared after heating was used and the weight of salt produced during neutralization of acid was calculated and subtracted from the weight of soluble materials.

For the gelatinized samples, first, the samples were prepared in the same method as explained in "Sample Preparation" section and the procedure was followed until the pH of the sample was adjusted by NaOH. The sample was centrifuged at 700g for 30 minutes. The weight of the pellet and dried supernatant (dried at 120°C) were obtained. The weight of salt produced during neutralization of acid was calculated and subtracted from the weight of soluble materials.

The water absorption and water solubility of the ungelatinized and gelatinized samples were obtained using Equations (4) and (5), respectively.

Water absorption= Weight of pellet/Weight of dry solid   (4)

Water solubility= (Weight of dissolved solids in the supernatant/Weight of dry solids in the original sample)×100   (5)

Textural Properties of Starch Gels

Starch gels were prepared by dispersion of 15% (w/w) starch in distilled water, heating in a 95°C water bath for 30 minutes and then cooling of the sample to 50°C. To set a gel, the hot paste was transferred into a cylindrical plastic container with diameter and height of 1×1 cm and refrigerated at 4°C for 24 hours. The gels were removed from the container and tested for their textural properties using a texture analyzer (Texture Analyser, TA Plus, Stable Micro System, Godalming, Surrey, England) at 22±1°C. The gels were compressed at a pretest speed of 5 mm s^{-1}, test speed of 2 mm s^{-1}, post test speed of 5.0 mm s^{-1}, time interval of 10 seconds, and strain deformation of 25% using a cylindrical aluminum plunger with diameter of 10 mm. From the resulting force-deformation curves, hardness, adhesiveness, elasticity, and cohesiveness were determined according to Steffe (1996). Since the gels of the gelatinized starch were too weak to be studied by the TPA, the test was performed only for the samples before gelatinization.

Statistical Analysis

The experiments were performed in a completely randomized design and conducted in triplicate. Experimental data were analyzed using Analysis of Variance (ANOVA) and expressed as mean±standard deviation. A Duncan's multiple range test was conducted to assess significant differences among experimental mean values ($P < 0.05$). All statistical computations and analysis were conducted using SAS version 9.1 for Windows.

RESULTS AND DISCUSSION

Granular Morphology

The two types of starch granules including large (A-type) and small (B-type) granules could be obsereved on the micrographs of the CL wheat starch (Figure 1). Some spots could be seen on the surface of the CL starch granules, which were also reported by Majzoobi *et al.* (2009) and Majzoobi *et al.* (2012b). These spots could be related to the effects of chemicals used during modification on the granules.
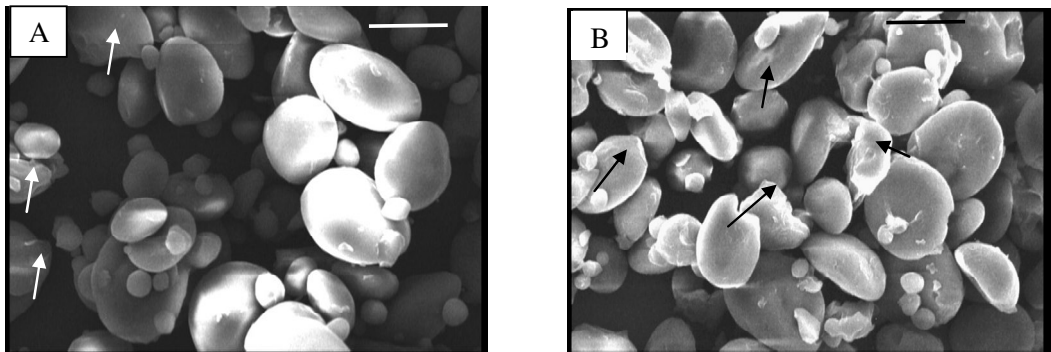
Figure 1. Scanning electron micrographs of the cross-linked wheat starch before (A) and after (B) treatment with L-ascorbic acid. Each bar on the micrographs is 20 µm.

Comparison of Figures 1-A and -B shows that the number of spots and cracks on the AA treated granules were slightly more than AA untreated CL starch. These spots were more obvious on A-type granules. On the surface of the granules, there were some proteins called "starch granules-associated proteins". The properties of starch granules-associated proteins have been well demonstrated by Baldwin (2001). It is possible that the AA affected these proteins and altered the appearance of the granules. Moreover, it is possible that the reduction in the pH of the media in the presence of AA caused some changes to the surface of the granules. The morphological changes may affect physicochemical properties of the starch.

Water Solubility and Absorption

Table 1 shows that in the presence of AA, the water solubility of the CL starch increased significantly from 4.00 to 5.16%.

A remarkable increase in the water solubility of the CL starch was observed after gelatinization (7.00% for the gelatinized cross-linked starch) that increased further by addition of ascorbic acid (10.06% for the gelatinized cross-linked starch). Determination of the water absorption (Table 1) showed that the CL starch had the highest water absorption (4.50%) that reduced significantly to 2.72% after addition of AA. Gelatinized CL starch had lower water absorption compared to the ungelatinized sample (2.50 *vs.* 4.5%). Addition of AA further decreased the water absorption to 1.68%. The experimental condition used to determine starch solubilization in this study measures the amount of starch molecules that remain in the supernatant after centrifugation of the heated starch suspension. Generally, smaller molecules remain in the supernatant. Therefore, it may be concluded that addition of AA resulted in lower molecular weight starch molecules of higher water solubility. For the gelatinized samples, it is possible

Table 1. Water solubility and absorption of the cross-linked starch in the presence of ascorbic acid before and after gelatinization.[a]

Samples	Water solubility (%)	Water absorption (%)
Cross-linked starch	4.00 ± 0.55[d]	4.50 ± 0.70[a]
Cross-linked starch + ascorbic acid	5.16 ± 0.35[c]	2.72 ± 0.11[b]
Gel[1]. Cross-linked starch	7.00 ± 1.10[b]	2.50 ± 0.74[b]
Gel. Cross-linked + ascorbic acid	10.06 ± 0.58[a]	1.68 ± 0.30[c]

[a] Different letters in each column show significant statistical difference between the values (P< 0.05).

that AA had some degradation effects on starch molecules resulting in higher starch solubility. Degradation of native wheat starch and some other polysaccharides (e.g. β-glucan, carboxymethylcellulose, xanthan gum, pectin, gellan, welan, scleroglucan, alginate, hydroxyethylcellulose and κ-carrageenan) by AA has been reported by Kivelä *et al.* (2009), Paquet *et al.* (2010) and Majzoobi *et al.* (2012a). The mechanism of polysaccharide depolymerization in the presence of AA may be related to the formation of free radicals rising from AA or its oxidation products. For the ungelatinized samples, the formation of some pores and spots on the surface of the granules (see Figure 1) may allow more starch molecules to exit from the granules and AA to enter the granules causing starch degradation, resulting in higher water solubility. The formation of the pores and spots on the surface of the granules as well as starch degradation may reduce the water absorption. Moreover, AA can enter the granules and cause some starch degradation. According to the results, gelatinized starches were more susceptible to the AA compared to the ungelatinized sample, since higher water solubility and lower water absorption were obtained for these samples.

Intrinsic Viscosity

Low intrinsic viscosity (Figure 2) was observed for the gelatinized starch compared to the ungelatinized sample that may be related to the changes in the molecular conformation after starch gelatinization. Nevertheless, no significant changes in the intrinsic viscosity were observed after addition of AA to the CL before and after gelatinization. Majzoobi *et al.* (2012a) used similar technique and found that the intrinsic viscosity of the native wheat starch decreased in the presence of AA. In this study, such changes were not observed for the CL starch. However, the possibility of molecular degradation of the CL starch in the presence of AA was concluded from the water solubility and absorption results (Table 1). This contradiction may be related to the different factors affecting intrinsic viscosity results and possibly the sensitivity of this method. Harding (1997) well demonstrated that the intrinsic viscosity is a measure of the occupied hydrodynamic volume of a macromolecule in solution and, therefore, a reflection of its size and shape. Therefore, it may be concluded that the possible changes in the size and shape of the samples mask the changes due to the molecular weight reduction. It is also
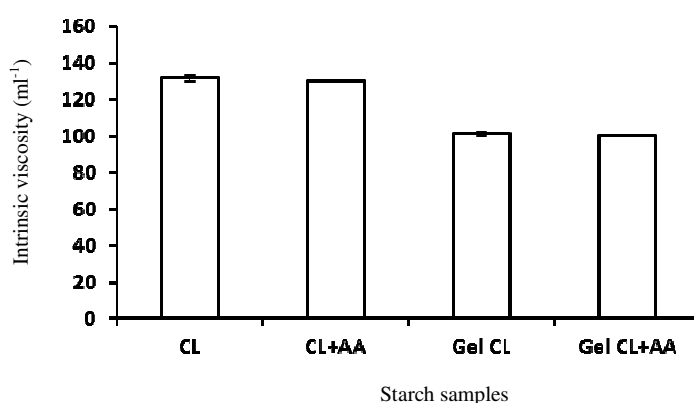
Figure 2. Effect of L-ascorbic acid on the intrinsic viscosity of the cross-linked wheat starch. CL: cross-linked wheat starch before gelatinization; CL+AA: Cross-linked wheat starch treated with ascorbic acid before gelatinization; Gel CL: Gelatinized cross-linked wheat starch, Gel CL+AA: gelatinized wheat starch treated with ascorbic acid. Different letters on the bars indicates statistical difference (P< 0.05).
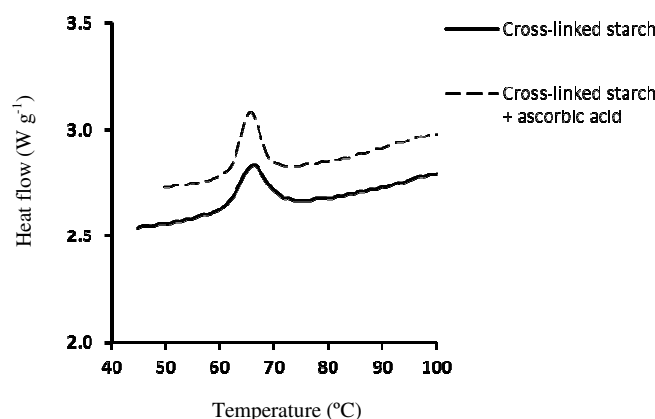
Figure 3. DSC curves of the cross-linked wheat starch before and after treatment with L-ascorbic acid.

possible that the degradation of starch could not take place for all starch molecules during the experiment, resulting in no distinct change in intrinsic viscosity.

Thermal Properties of Starch

The DSC traces and the related data are given in Figure 3 and Table 2, respectively. The results showed that AA significantly reduced the gelatinization transition temperature (T_o, T_p, T_c) and enthalpy of gelatinization (ΔH) of the CL starch. For the gelatinized samples no gelatinization peak could be obtained from the DSC and, hence, the data are not presented. Addition of AA significantly reduced T_p, T_c and ΔH of the CL starch, but had no significant effect on the T_o of the samples. Addition of AA also reduced the range of T_c-T_o of the sample. These changes indicated that lower temperature was required for gelatinization of the CL starch in the presence of AA. Pores and spots that formed on the surface of the starch granules, as

observed using SEM (Figure 1), may facilitate water penetration into the starch granules at lower temperatures. ΔH is the net sum of all endothermic processes that take place during heating and provides an overall assessment of starch crystallinity (quantity and quality). It is a sign of the loss of molecular order within the granules and corresponds to the number of double helices that disentangle and melt during gelatinization (Cooke and Gidly 1992; Ratnayake *et al.,* 2009). The lower ΔH of the acid treated starch suggested a lower percentage of organized arrangements or lower stability of the crystals.

Physical Properties of Starch Gels

Determination of the textural properties of the samples (Table 3) showed that the CL starch gel became remarkably softer in the presence of AA. The elasticity of the gel also decreased slightly. Adhesiveness and cohesiveness of the gels remained unchanged. The gel firmness is mainly caused by

Table 2. Thermal properties of the cross-linked starch with and without ascorbic acid.[a]

Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)	ΔH (J/g)
Cross-linked starch	60.99±0.07[a]	65.43±0.28[a]	69.88±0.21[a]	8.89±0.14[a]	9.28±0.06[a]
Cross-linked starch+ascorbic acid	60.27±0.22[a]	64.35±0.22[b]	67.74±0.16[b]	7.47±0.20[b]	8.37±0.45[b]

[a] Different letters in each column show significant statistical difference between the values (P< 0.05).

Table 3. Textural properties of the cross-linked starch gels with and without ascorbic acid. [a]

Sample	Hardness (N)	Adhesiveness (N)	Elasticity	Cohesiveness
Cross-linked starch	0.54 ± 0.14^a	0.05 ± 0.00^a	0.99 ± 0.01^a	0.69 ± 0.03^a
Cross-linked starch+ ascorbic acid	0.20 ± 0.03^b	0.04 ± 0.02^a	0.92 ± 0.03^b	0.67 ± 0.07^a

[a] Different letters in each column show significant statistical difference between the values ($P< 0.05$).

retrogradation of starch gel, which is associated with the re-crystallization of amylopectin, leading to harder gels (Miles *et al.,* 1985). Mua and Jackson (1997) reported that the starches that exhibit harder gels tend to have longer amylopectin chains. Therefore, AA may reduce the chain length of the starch molecules resulting in softer gel textures.

CONCLUSIONS

It is generally accepted that cross-linking increases the stability of the starch against acids. However, some physicochemical changes may occur in the presence of different acids that should be considered for the appropriated application of the CL starches. The results of this study showed that AA can affect the functional properties of the CL wheat starch before and after gelatinization. The increase in water solubility, decrease in water absorption, changes in the morphological structure of the granules, decrease in the gelatinization temperature and enthalpy of gelatinization, and decrease in the starch gel hardness may all confirm the molecular degradation of the CL starch in the presence of AA. Most of the results were obtained for the ungelatinized starch, however, further work is required to confirm such effects on the gelatinized starch.

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م. مجذوبی، پ. بی‌پروا، ا. فرحناکی، و ف. بدیعی

چکیده

شواهدی وجود دارد که نشان می دهد اسیدهای آلی بر خصوصیات عملکردی نشاسته های طبیعی موثر می باشند. این در حالی است که اطلاعات بسیار کمی از چنین تاثیراتی بر نشاسته های اصلاح شده در اختیار می باشد. هدف اصلی از این تحقیق تعیین تاثیر ال-اسکوربیک اسید (۱۵ میلیگرم بر کیلوگرم به عنوان سطح مجاز برای استفاده از اسکوربیک اسید در محصولات نانوایی) بر خواص عملکردی نشاسته با اتصالات عرضی گندم بود. تغییرات ایجاد شده بر روی نشاسته تحت تاثیر ال-اسکوربیک اسید شامل: ظهور تعدادی لک و شیار بر سطح گرانولهای نشاسته که توسط میکروسکپ الکترونی روبشی قابل مشاهده بود، افزایش حلالیت در آب و کاهش جذب آب نشاسته ، عدم تغییر ویسکوزیته ذاتی که توسط ویسکومتر یو-شکل بررسی شد، کاهش دمای ژلاتینه شدن و آنتالپی ژلاتینه شدن که توسط دستگاه اسکن حرارتی افتراقی مطالعه شد، کاهش سفتی و الاستیسیته ژلها و عدم تغییر پیوستگی وچسبندگی آنها. در مجموع، نتیجه گیری شد که اسکوربیک اسید دارای اثرات تجزیه کنندگی بر مولکولهای نشاسته با اتصالات عرضی گندم است که این خود می تواند بر سایر خصوصیات نشاسته موثر باشد.