Faculty Publications in Food Science and Technology

Food Science and Technology Department

2006

Gelatinization and Solubility of Corn Starch during Heating in Excess Water: New Insights

Wajira S. Ratnayake
University of Nebraska-Lincoln, wratnayake2@unl.edu

David S. Jackson
University of Nebraska-Lincoln, djackson1@unl.edu

Gelatinization and Solubility of Corn Starch during Heating in Excess Water: New Insights

Wajira S. Ratnayake and David S. Jackson

Department of Food Science and Technology, University of Nebraska–Lincoln, Lincoln, Nebraska 68583-0919

Corresponding author — D. S. Jackson, tel 402 472-2814, fax 402 472-1693, email david.s.jackson@unl.edu

Abstract
Starch gelatinization is associated with the disruption of granular structure causing starch molecules to disperse in water. This study was designed to examine starch granules as they were heated in water, and their resulting morphological, structural, and solubility traits. The results indicate that starch gelatinization is a more complex process than the previously suggested order-to-disorder transition. The energy absorbed by the granules facilitates the rearrangement or formation of new bonds among molecules prior to the temperatures normally associated with the melting of amylopectin crystallites during gelatinization. It is also evident that amylose plays an important role during the initial stages of corn starch gelatinization.

Keywords: Starch, gelatinization

Introduction

Starch gelatinization is associated with the disruption of granular structure causing starch molecules to dissolve in water and, as such, is one of the starch's most important and unique properties (*1*). Many food products contain partially cooked starch granules that contribute to their functional and structural properties. Therefore, it is important to understand the time and temperature dependence of starch structural changes in water to characterize the gelatinization processes, especially how the granular structure changes and how amylose and amylopectin polymers behave at different water temperatures.

Starch is known as a semicrystalline material (i.e., the granules contain alternating crystalline and amorphous regions (*2−4*)). These crystalline regions are predominantly made up of amylopectin polymers of which the outer branches are hydrogen bonded to each other to form crystallites that unravel during gelatinization (*5−7*). The amorphous regions of granules are mainly composed of amylose and amylopectin branch points (*8*).

Starch granules are insoluble in cold water. When starch is heated in water, granules absorb water and swell. The absorption of water by amorphous regions within the granules destabilizes their crystalline structure, resulting in the loss of birefringence, which is one definition of gelatinization (*9−11*). Upon continuous heating, granules tend to swell to greater extents, and the crystallites melt, resulting in increased molecular motion that eventually leads to complete separation of amylose and amylopectin (*12, 13*). The temperature at which granules lose their birefringence is referred to as the gelatinization temperature; this temperature depends in part on the botanical source of starch (*1*). Tester and Morrison (*7*) reported that starch granular swelling is primarily a property of amylopectin because waxy starch swelled

much more than normal starch did. While there are many other reports available on the role of amylose and amylopectin in gelatinization (*14−16*), there are few reports available on what occurs within the granular structure during progressive heating of a starch/water mixture.

Starch gelatinization and associated properties are determined by various methods, including measuring the birefringence end point, viscosity changes, X-ray diffraction, amylose−iodine binding (blue value), enzymatic digestibility, NMR, light extinction, solubility or sedimentation of swollen granules, and absorption of Congo red (*17*). All these methods measure slightly different physicochemical properties and have unique and inherent advantages and disadvantages. Therefore, it is important to use several different methods to fully characterize starch gelatinization properties.

In food products, starch granules are subjected to different thermal conditions and other unit operations that result in granules with differing stages of partial and full gelatinization; these collectively influence the product's physicochemical properties. The objective of the study was to investigate the progressive morphological changes in starch granules and the structural changes to starch polymers that occur at different temperatures during gelatinization in water.

Materials and Methods

Granular and Molecular Changes of Corn Starch during Heating. Regular corn starch was obtained from Penford Food Ingredients (Englewood, CO). Starch (6 g in 100 mL of distilled water) samples were treated at specific temperatures (ranging from 35 to 90 °C at 5 °C increments) for 30 min in 250 mL conical flasks in a water bath. Samples were filtered through Whatman no. 1 filter papers, and the residue was freeze-dried (Virtis Sentry 8L, The Virtis Co., Gardiner, NY) for 36 h at −55 °C and 50 mTorr vacuum pressure.

Figure 1. Light microscopic images of corn starch granules in water mixtures heated to specific temperatures (numbers represent the temperature (°C)).
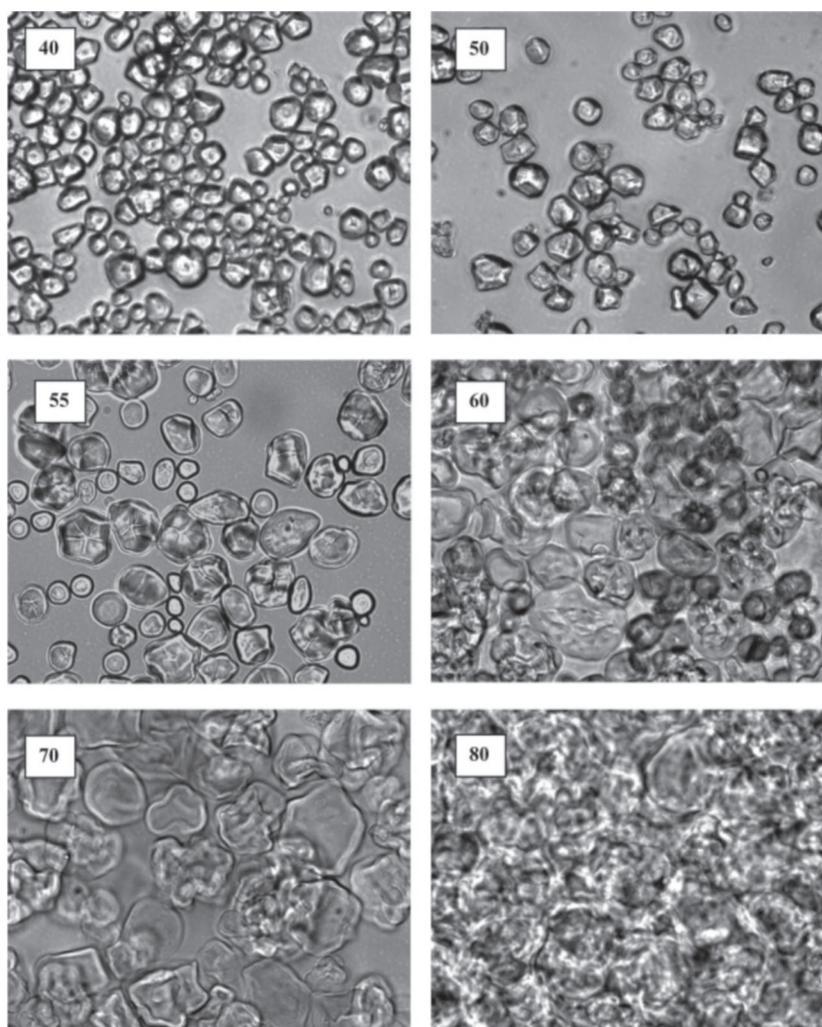
High-Performance Size-Exclusion Chromatography (HPSEC). Samples of 0.5 g dry weight were dispersed in 100 mL of 90% (v/v) reagent grade DMSO (Mallinckrodt Baker Inc., Paris, KY) in water by heating in a boiling water bath for 30 min, with intermittent mixing with a vortex mixer and subsequent aging at 50 °C in an oven for 12 h.

HPSEC was carried out according to Ozcan and Jackson (*18*) with slight modifications as follows: dispersed samples were passed through a 1.2 μm Magna nylon supported membrane (GE Osmonics Labstore, Minnetonka, MN), and 25 μL was injected into a HPSEC system equipped with a Waters 515 HPLC pump, Waters 410 differential refractometer (Waters Co., Milford, MA), and Shodex KS-G, KS 806, 804, 803, and 802 columns (Showa Denko, Tokyo, Japan) connected in a series and maintained at 50 °C. Distilled deionized degassed water was used as mobile phase with a 1 mL/min flow rate. Data were acquired automatically with Astra software Version 4.70.07 (Wyatt Technology Co., Santa Barbara, CA). Relative peak areas of amylopectin and amylose were estimated using Origin peak fitting software (version 6.0, OriginLab Co., Northampton, MA).

Total Soluble Starch. Total soluble starch was estimated by the phenol-sulfuric acid method (*19*) using portions of the same samples prepared for HPSEC analysis after filtration (as described previously). Absorbance was measured at 490 nm using a Unico 1100RS spectrophotometer (United Products and Instruments, Dayton, NJ) against a reagent blank. Total soluble starch was estimated using a standard curve prepared using a glucose standard series. All chemicals were of ACS certified grade.

Light Microscopy. Diluted starch samples (~0.1 g in 25 mL of water) were heated in a glass Petri dish on a hot plate for 5−10 min (depending on the desired end temperature). After reaching the experimentally specified temperature (between 30 and 85 °C at 5 °C

intervals, i.e., 35, 40, and 45 °C, etc.) the Petri dish was promptly viewed using a light microscope (Nikon Eclipse TE300, Nikon USA, Melville, NY) and observed at a 1 × 400 magnification. Sample temperatures, before and during microscopic observation, were monitored using an infrared thermometer. Images were acquired and recorded using Magnafire software (version 2.1, Optronics, Goleta, CA).

Scanning Electron Microscopy (SEM). Starch samples were mounted on metal stubs and coated with gold−palladium (~20 nm thickness) using a Hummer sputter coating system (Anatech Ltd., Union City, CA). Samples were then observed using a Hitachi S-3000N scanning electron microscope (Hitachi Science Systems, Tokyo) at an acceleration potential of 15 kV. Pictures were captured by automatic image capturing software (Hitachi High-Technologies, Pleasanton, CA). Magnifications are as indicated at the bottom of each figure.

Differential Scanning Calorimetry (DSC). Starch samples (~10 mg, db) with ~55 μL of distilled water (i.e., starch in excess water) were hermetically sealed in DSC pans (Perkin-Elmer Pan Sell Kit 0319-1525/1526/1535) and kept at room temperature for 3−4 h. Next, samples were scanned against a blank (empty pan) using Perkin-Elmer Pyris 1 differential scanning calorimeter (Perkin-Elmer Co., Norwalk, CT) from 25 to 90 °C at a 10 °C/min scanning rate. Pyris−Version 3.52 (Perkin-Elmer Co., Norwalk, CT) software was used to collect data and analyze onset (T_o), peak (T_p), and end (T_c) temperatures and the transition enthalpy (ΔH).

Statistical Analysis. Tukey's HSD test (SigmaStat version 2.0 software, Jandel Scientific/SPSS Inc., Chicago, IL) was used to compare mean differences of dispersed amylose and amylopectin proportions. All values used for analyses were averages of at least three independent replicates.
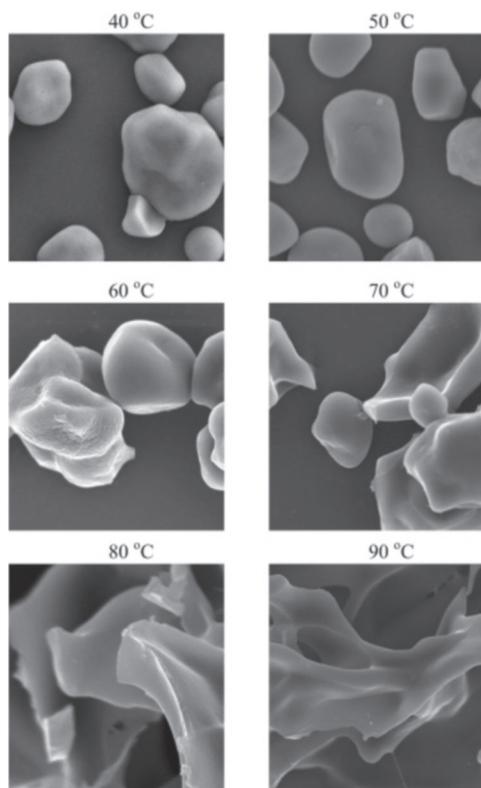
Figure 2. SEM images of temperature treated and freeze-dried corn starch samples (2500×).

Results and Discussion

During heating in water, starch granules remained intact up to 50 °C (Figure 1); this observation is in agreement with Jing-ming and Sen-lin (*20*). It appears that the outermost layers (or skin) of the granules tend to maintain granular integrity during heating to 60 °C, before they disintegrate as the internal structure and free movement of starch polymers destabilize the granule's internal structure. Jing-ming and Sen-lin (*20*) reported that granules lost their integrity and formed a molecular network at 64 °C. In con-

trast, our results showed that complete granular disruption and the formation of a gelatinized solution did not occur below 70 °C. The differences might be due to concentration differences (1 g of starch in 10 mL of water vs 0.1 g in 25 mL of water in this study) (*8, 17*) and plant source (i.e., variety and cultivar (*21*)). As observed by light microscopy, the large starch granules swelled first and began to break apart at around 55−60 °C; small granules did not start to disintegrate until 65 °C (data not shown). SEM pictures of dried samples (Figure 2) confirmed the light microscopy observations. Almost all the granules were irreversibly disrupted at or before 80 °C.

DSC results (onset, peak, end temperatures, and enthalpies) were determined averaging three independent replicates. DSC enthalpies of heat-treated samples steadily increased ($p < 0.05$), from ~14 to ~16 J/g until 50 °C and then disappearing gradually between 55 and 75 °C. No enthalpies were observed at the 70−85 °C range, indicating the absence of crystalline domains. The increase in DSC enthalpies from 35 to 50 °C (Figure 3), without viewable light or SEM microscopic changes in granular morphological characteristics (Figures 1 and 2), indicates molecular level reorganizations or interactions taking place within the granules at low temperatures before gelatinization. This increase in melting enthalpy from 35 to 50 °C (from ~14 to ~16 J/g) was essentially linear ($y = 0.16x + 8.65$; where y = enthalpy (J/g), x = treatment temperature (°C), $R^2 = 0.93$, and $p = 0.03$). These molecular interactions also cause the DSC peak temperatures to decrease (from 72.7 to 70.6 °C between 35 and 55 °C, significant at $p < 0.05$) and then increase (from 72.7 to 80.3 °C between 55 and 65 °C, significant at $p < 0.05$) (Figure 3). These DSC trends, taking place in corn starch just before gelatinization, are different from what is characterized as annealing. Annealing is a process by which a material is held at a temperature somewhat lower than its melting temperature, permitting modest molecular reorganization and a more organized structure of a lower free energy to form (*22*). Annealing is characterized by narrowed gelatinization temperature ranges and increased gelatinization peak temperatures and enthalpies of annealed starches as compared to their native counterparts (*1, 23, 24*). The only similarity between annealing and observed DSC trends here is the gradual
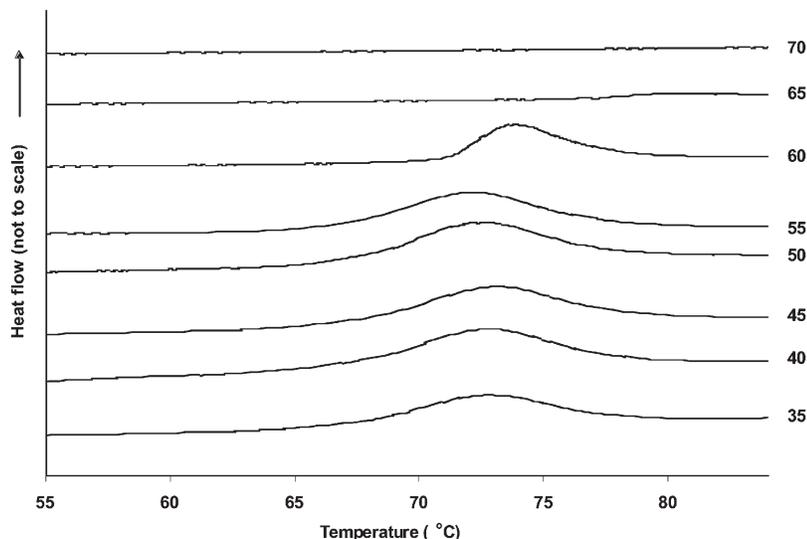
Figure 3. Representative DSC endotherms of temperature treated (treatment temperatures (°C) are indicated next to each profile) corn starch samples. No detectable enthalpies were noted in treatments >65 °C.

Table 1. Relative Proportions of Amylose and Amylopectin in Heat-Treated Corn Starch/Water (6% w/v) Samples

temperature treatment (°C)	amylose (%)[a]
35	29.12 ± 1.58[c]
40	26.10 ± 0.87[b]
45	26.79 ± 0.92[b,c]
50	25.89 ± 0.57[b]
55	25.35 ± 0.83[b]
60	22.89 ± 0.21[a]
65	21.86 ± 0.52[a]
70	21.43 ± 0.73[a]
75	27.82 ± 0.86[b]
80	28.80 ± 0.47[c]
85	34.24 ± 0.56[d]

a. Means having the same letter are not significantly different by Tukey's HSD test ($p < 0.001$).

increase in melting enthalpy. It is likely that the energy provided before the initiation of gelatinization causes the formation of new, but less stable, molecular interactions within granules that result in progressively lower DSC peak temperatures between 45 and 55 °C treatments.

The changes that take place during gelatinization have been attributed to water availability (*9*), crystal stability within granules (*25*), differences between amorphous and crystalline regions (*26*), and glass transition related progressive melting (*27*). However, aspects of these explanations are contradictory, and it is believed that enthalpic transitions during gelatinization represent numerous changes in starch granules (*6*). Our results indicated that although the granular morphology is not affected by heating until 55 °C, the internal structures of the granules undergo considerable changes as indicated by DSC enthalpic changes (Figure 3) and HPSEC results. Relative proportions of amylose dispersed in each sample are given in Table 1. The relative amounts of amylose dispersed before complete granular disruption were higher ($p < 0.001$) at low temperatures (35−45 °C), indicating a greater role of amylose in the gelatinization process during its initial stages. The high amounts ($p < 0.001$) of amylose dispersed after the complete granular disruption, at high temperatures, might be due to the increased physical dispersion of starch polymers. The retention times of each HPSEC peak (representing amylopectin and amylose) did not change ($p > 0.05$) among the treatments, demonstrating the absence of depolymerization. Our results indicate that the starch gelatinization process involves different and more complex structural changes than simple order-to-disorder phase transition of granules.

Conclusions. The results indicate that starch gelatinization is a more complex process than the previously suggested order-to-disorder transition. Energy absorbed by granules not only unfolds amylopectin double helices during gelatinization but also facilitates rearrangement or formation of new bonds among molecules at lower temperatures before gelatinization takes place. An array of new amylopectin crystallites having different stabilities is formed during this structural reordering process. Amylose likely plays a major role in the early stages of the gelatinization process.

References

(1) Lineback, D. R. Current concepts of starch structure and its impact on properties. *J. Jpn. Soc. Starch Sci.* 1986, *33*, 80−88.

(2) Waigh, T. A.; Hopkinson, I.; Donald, A. M. Analysis of the native structure of starch granules with X-ray microfocus diffraction. *Macromolecules* 1997, *30*, 3813−3820.

(3) Wang, T. L.; Bogracheva, T. Y.; Hedley, C. L. Starch: As simple as A, B, C? *J. Exp. Bot.* 1998, *49*, 481−502.

(4) Zobel, H. F. Molecules to granules: A comprehensive starch review. *Starch/Staerke* 1988, *40*, 1−7.

(5) Biliaderis, C. G. Structures and phase transitions of starch polymers. In *Polysaccharide association structures in food*; Walter, R. H. Ed.; Marcel Dekker Inc.: New York, 1998; pp 57−168.

(6) Sahai, D.; Jackson, D. S. Enthalpic transitions of native starch granules. *Cereal Chem.* 1999, *76*, 444−448.

(7) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches I. Effects of amylopectin, amylose and lipids. *Cereal Chem.* 1990, *67*, 551−559.

(8) Donald, A. M. Plasticization and self-assembly in the starch granule. *Cereal Chem.* 2001, *78*, 307−314.

(9) Donovan, J. W. Phase transitions of starch water system. *Biopolymers* 1979, *18*, 263−275.

(10) Biliaderis, C. G. Thermal analysis of food carbohydrates. In *Thermal analysis of foods*; Harwalkar, V. R., Ma, C.-Y., Eds.; Elsevier Applied Sciences: London, 1990; pp 169−220.

(11) Parker, R.; Ring, S. G. Aspects of the physical chemistry of starch. *J. Cereal Sci.* 2001, *34*, 1−17.

(12) Keetals, C. J. A. M.; van Vliet, T.; Walstra, P. Gelation and retrogradation of concentrated starch systems: 3. Effect of concentration and heating temperature. *Food Hydrocolloids* 1996, *10*, 363−368.

(13) Levin, H.; Slade, L. Influence of glassy and rubbery state of thermal, mechanical, and structural properties of doughs and baked products. In *Dough rheology and baked product texture, theory and practice*; Faridi, H., Faubion, H., Eds.; Van Nostrand Reinhold AVI: New York, 1990; pp 157−330.

(14) Sasaki, T.; Matsuki, J. Effect of wheat starch structure on swelling power. *Cereal Chem.* 1998, *75*, 525−529.

(15) Jenkins, P. J.; Donald, A. M. Gelatinization of starch: A combined SAXS/WASX/DSC and SANS study. *Carbohydr. Res.* 1998, *308*, 133−147.

(16) Fredriksson, H.; Silverio, J.; Andersson, R.; Eliasson, A. C. Aman, P. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydr. Polym.* 1998, *35*, 119−134.

(17) Lund, D. Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. *Crit. Rev. Food Sci. Nutr.* 1984, *20*, 249–273.

(18) Ozcan, S.; Jackson, D. S. The impact of thermal events on amylase–fatty acid complexes. *Starch/Staerke* 2002, *54*, 593–602.

(19) Dubios, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956, *28*, 350–356.

(20) Jing-ming, L.; Sen-lin, Z. Scanning electron microscope study on gelatinization of starch granules in excess water. *Starch/Staerke* 1990, *42*, 96–98.

(21) Yamin, F. F.; Svendsen, L.; White, P. J. Thermal properties of corn starch extraction intermediates by differential scanning calorimetry. *Cereal Chem.* 1997, *74*, 407–411.

(22) Blanshard, J. M. V. Starch granule structure and function: A physicochemical approach. In *Starch: Properties and potential*; Gaillard, T., Ed.; John Wiley and Son: Chichester, UK, 1987; pp 16–54.

(23) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches. II. Waxy starches. *Cereal Chem.* 1990, *67*, 558–563.

(24) Gomes, A. M. M.; da Silva, C. E. M.; Ricardo, N. M. P. S.; Sasaki, J. M.; Germani, R. Impact of annealing in the physicochemical properties of unfermented cassava starch. *Starch/Staerke* 2004, *56*, 419–423.

(25) Evans, I. D.; Haisman, D. R. The effect of solutes on the gelatinization temperature range of potato starch. *Starch/Staerke* 1982, *34*, 224–231.

(26) Nakazawa, F.; Noguchi, S.; Takahashi, J. Thermal equilibrium state of starch–water mixture studied by differential scanning calorimetry. *Agric. Biol. Chem.* 1984, *48*, 2647–2653.

(27) Biliaderis, C.; Page, C. M.; Maurice, T. J.; Juliano, B. O. Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch. *J. Agric. Food Chem.* 1986, *34*, 6–14.