

Two- and Multi-Step Annealing of Cereal Starches in Relation to Gelatinization

YONG-CHENG SHI[†]

Department of Grain Science and Industry, 201 Shellenberger Hall, Kansas State University, Manhattan, Kansas 66506

Two- and multi-step annealing experiments were designed to determine how much gelatinization temperature of waxy rice, waxy barley, and wheat starches could be increased without causing a decrease in gelatinization enthalpy or a decline in X-ray crystallinity. A mixture of starch and excess water was heated in a differential scanning calorimeter (DSC) pan to a specific temperature and maintained there for 0.5–48 h. The experimental approach was first to anneal a starch at a low temperature so that the gelatinization temperature of the starch was increased without causing a decrease in gelatinization enthalpy. The annealing temperature was then raised, but still was kept below the onset gelatinization temperature of the previously annealed starch. When a second- or third-step annealing temperature was high enough, it caused a decrease in crystallinity, even though the holding temperature remained below the onset gelatinization temperature of the previously annealed starch. These results support that gelatinization is a nonequilibrium process and that dissociation of double helices is driven by the swelling of amorphous regions. Small-scale starch slurry annealing was also performed and confirmed the annealing results conducted in DSC pans. A three-phase model of a starch granule, a mobile amorphous phase, a rigid amorphous phase, and a crystalline phase, was used to interpret the annealing results. Annealing seems to be an interplay between a more efficient packing of crystallites in starch granules and swelling of plasticized amorphous regions. There is always a temperature ceiling that can be used to anneal a starch without causing a decrease in crystallinity. That temperature ceiling is starch-specific, dependent on the structure of a starch, and is lower than the original onset gelatinization of a starch.

KEYWORDS: Annealing; gelatinization; starch

INTRODUCTION

Native starches occur in higher plants as granules and are partially crystalline polymers (1, 2). The crystallinity of native starches is about 15–40% as measured by X-ray diffraction (2, 3). In polymer science, “annealing” is used to describe the improvement of crystallization by heating a partially crystalline polymer to a temperature below its melting point (4). The improvement of crystallization includes the growing of crystalline areas, perfection of crystals, and a change to more stable crystal structures. Annealing occurs at a temperature below the melting point (T_m) but above the glass transition (T_g) of a partially crystalline polymer (5), typically at $0.75\text{--}0.88T_m$ (K), for polymers with T_g/T_m ratios of 0.5–0.8. Applying these polymer science concepts to starch, Slade and Levine (6, 7) have emphasized that annealing is a diffusion-controlled, nonequilibrium process. The rate of annealing is governed by William–Landel–Ferry (WLF), rather than Arrhenius, kinetics. The time of annealing is shortest at a temperature just below

T_m and longest at a temperature just above T_g . Both T_g and T_m are dependent on moisture content.

Applying the side-chain liquid-crystalline model to starch, Donald and co-workers (8–11) have proposed that dry starch granules are disordered, having no periodic lamellar structure and little defined crystallinity. Amorphous regions are in a glassy state and are low in mobility. Upon hydration, amorphous lamellar regions are plasticized, and starch changes from a nematic structure to a smectic structure and forms a periodic lamellar structure (8–11). Further heating or prolonged holding at room temperature in the presence of water leads to annealing, or crystal growth and perfection, as evidenced by the increases of gelatinization temperature.

To determine the optimum conditions for annealing, it is important to know the melting temperature of crystallites in starch granules and the glass transition temperature of the amorphous phases in granular starch. The T_m , or gelatinization temperature of starch granules in water, can be measured by differential scanning calorimetry (DSC) (12), but the determination of the T_g of the amorphous phases in granular starch is difficult. Indeed, the location of T_g and its relationship to gelatinization in granular starch have been debated in the

[†] Telephone (785) 532-6771; fax (785) 532-7010; e-mail ycshi@ksu.edu.

literature over the years (13–20). We have recently suggested (21), on the basis of concepts from polymer science (22–26), that there are two types of amorphous phase in granular starch: a mobile amorphous phase and a rigid amorphous phase. The T_g of the rigid amorphous phase is greater than the T_g of the mobile amorphous phase. Using dynamic DSC, a temperature-modulated DSC technique, we have been able to measure a T_g that is overlapped with the gelatinization endothermic peak, and we attribute this T_g to the rigid amorphous phase in granular starch (21). We believe that the T_g of the mobile phase extends over a broad range of temperatures and cannot be easily pinpointed. Using DDSC, however, we have detected a small subzero T_g , which is attributed to part of the mobile amorphous phase in starch granules (21).

There are two methods to anneal starch: “warm-water treatment” (WWT) and “heat–moisture treatment” (HMT) (27). Warm-water treatment is generally done in starch slurries with excess water (>60%) or at intermediate water content (40–55%), whereas heat–moisture treatment is done under limited-water conditions (<40% w/w) and often no “free” visible water is observed in the mixture of starch and water. Blanshard (28) defines annealing of starch as “a process whereby a material is held at a temperature somewhat lower than its melting temperature which permits modest molecular reorganization to occur and a more organized structure of lower free energy to form” and considers that heat–moisture treatment is “an allied approach.” The definition of annealing of starch and most early studies have been recently discussed in a number of reviews (29–31). In contrast to the classification of annealing of starch suggested by Kuge and Kitamura (27), in these reviews (29–31), annealing of starch is considered to be a hydrothermal modification under excess water conditions and does not include HMT. Heat–moisture treatment is often distinguished from warm-water treatment because high temperatures can be applied under limited-water conditions. Even in the case of annealing of starch slurries, however, high temperatures may be used when a “swelling-inhibiting agent”, such as a salt, is used, and a significant annealing effect can be achieved (32). To be consistent with polymer science, we should define annealing of starch as a physical treatment that involves incubation of starch granules at temperatures above the T_g and below T_m (the gelatinization temperature). That definition includes both WWT and HMT.

In general, warm-water treatment of starch increases the gelatinization temperature and narrows the gelatinization temperature range, as observed by many researchers (29–31, 33, 34). It has been suggested that WWT increases homogeneity of both individual and populations of starches granules through the process of annealing of starch crystallites (27) or decreases the size distribution of crystallites (35, 36). Crystalline type is not altered by WWT, as determined by wide-angle X-ray diffraction (29, 37), and solid-state NMR results suggest that the double-helix content is unchanged by WWT (30, 38, 39). However, small-angle X-ray scattering (SAXS) shows a more intense 9 nm scattering maximum for potato starch after WWT, compared with that for native potato starch, suggesting a more efficient packing of crystallites in dense lamellae (40, 41). In contrast to WWT, HMT of starches broadens the gelatinization temperature range (41–45), indicating that HMT increases heterogeneity of granular structure (27). Heat–moisture treatment gradually changes the B-type of X-ray pattern of native potato starch to the A-type crystalline pattern when treatment temperature increases (29, 41). Moreover, heat–moisture-treated samples give a diffused SAXS background, suggesting that the

stacked lamellae are disrupted by the HMT process (41). After partial acid hydrolysis of high amylose maize starch, both WWT and HMT increase the yield of boiling-stable granular resistant starch, with greatest yield observed for HMT (46).

Although the gelatinization temperature of a starch usually increases after annealing, gelatinization enthalpies have been reported that increased or were unchanged (30). Two- or multi-step processes have been applied to anneal starches (30, 39, 40, 43), but how high can gelatinization temperature be increased by annealing? Is there a limit? These questions have not been clearly addressed. In this study, two- and multi-step annealing experiments have been designed to determine how much gelatinization temperature can be increased in excess-water conditions without a significant reduction in gelatinization enthalpy or a decline in X-ray crystallinity. The experimental approach is first to anneal a starch at a low temperature so that the gelatinization temperature of the starch is increased without causing a decrease in gelatinization enthalpy. The annealing temperature is then raised, but still is kept below the onset gelatinization temperature of the previously annealed starch. The results could help us further understand annealing mechanisms and the internal structure of starch granules.

MATERIALS AND METHODS

Materials. Cultivars ‘RD4’ and ‘IR29’ of milled waxy rice were obtained from the Pothum Tharis Rice Research Center (Thanyaburi, Thailand) and the International Rice Research Institute (Philippines), respectively. Hull-less waxy barley (WB), cultivar ‘Wanuber’, was obtained from the Montana Agricultural Experimental Station (Bozeman, MT). Starch was isolated from waxy rice (47) and barley (48) as previously reported (49). Wheat starch was provided by MGP Ingredients, Inc. (Atchison, KS).

Differential Scanning Calorimetry (DSC). Unless noted otherwise, DSC studies were performed with a Perkin-Elmer DSC-2 (Norwalk, CT) as previously described (49). The instrument was calibrated with indium.

One-step annealing of starch was carried out by heating (10 °C/min) a mixture of starch and water (1:3 w/w) in a DSC pan from 27 °C (300 K) to a specific temperature and holding it there for a certain time. The DSC pan was then rapidly cooled (320 °C/min) to 7 °C (280 K) and scanned from 7 °C (280 K) to 127 °C (400 K). The second-step annealing of starch was carried out by raising the temperature to another specific temperature after the first annealing and holding it there for a certain time. The DSC pan was then rapidly cooled (320 °C/min) to 7 °C and scanned from 7 to 127 °C.

Small-Scale Annealing. Starch (10.0 g, dry weight) was slurried in water (35% total solids) in a round-bottom flask. The slurry was mixed and heated (annealed) in a water bath to a specific temperature of the starch slurry and held for a certain time. After the first annealing, the temperature was increased to another specific temperature, and the starch slurry was held for a second annealing. To determine if there was any soluble material before and after annealing, starch slurry (2 mL) was centrifuged at 13000 rpm for 3 min (Hermle 2180M, National Labnet Co., Woodbridge, NJ). The concentration of the soluble material in the supernatant was measured by using a portable refractometer (Fisher Scientific, catalog no. 13-946-20). After the annealing process, the starch slurry was cooled to room temperature and added to absolute ethanol (100 mL) with agitation. The mixture was centrifuged and washed with acetone once, and starch was air-dried and allowed to gain moisture to about 11%. A part of the starch slurries, before and after annealing, was also directly taken, weighed in DSC pans, and analyzed by DSC.

X-ray Diffraction. X-ray diffraction patterns of native and annealed starches were recorded on a Philips X-ray diffractometer, using Cu K α radiation at 35 kV and 20 mA, a theta compensating slit, and a diffracted beam monochromator (49). Relative crystallinity was estimated from the ratio of the area of peaks to the total area of a diffractogram (50).

Table 1. Effects of Annealing Time on Gelatinization of Waxy Rice (RD4) Starch^a

annealing time (h)	gelatinization properties ^b			
	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
none	76.2	81.2	87.1	19.2
0.5	81.4	84.6	88.4	18.0
1.0	82.7	85.9	89.8	18.8
2.0	83.3	86.5	90.0	18.8
24.0	85.7	88.0	93.1	19.6
48.0	86.9	90.0	93.7	19.6
SD ^c	0.3	0.3	0.4	0.3

^a Annealing was conducted in three parts of water at 70 °C, which was 6 °C below the onset gelatinization temperature of waxy rice starch before annealing. ^b T_o, T_p, and T_c were onset, peak, and end gelatinization temperatures, respectively. ΔH was gelatinization enthalpy. ^c SD = pooled standard deviation from duplicate measurements of DSC parameters.

Table 2. Effects of Annealing Time on Gelatinization of Wheat Starch^a

annealing time (h)	gelatinization properties ^b			
	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
none	58.4	63.2	69.0	10.0
0.5	64.0	67.0	70.6	8.8
4.0	67.0	69.4	73.2	8.8
SD ^c	0.4	0.3	0.4	0.4

^a Annealing was conducted in three parts of water at 52 °C, which was 6 °C below the onset gelatinization of wheat starch before annealing. ^b T_o, T_p, and T_c were onset, peak, and end gelatinization temperatures, respectively. ΔH was gelatinization enthalpy. ^c SD = pooled standard deviation from duplicate measurements of DSC parameters.

RESULTS

Effects of Annealing Time and Temperature. Table 1 shows the effects of annealing time on gelatinization of waxy rice (RD4) starch. The starch was annealed at 70 °C, which was 6 °C below the onset gelatinization temperature (T_o) of the waxy starch before annealing. The T_o of annealed waxy rice (RD4) starches increased with annealing time, whereas there was little change in enthalpy values (ΔH) (Table 1). Moreover, the increase in T_o was 5 °C in the first 30 min, and this was fast. For the commercial wheat starch, T_o increased 6 °C in the first 30 min (Table 2), similar to what was observed for waxy rice (RD4) starch. The rate of increase slowed with time after that. When the waxy rice (RD4) starch was annealed for 48 h instead of 24 h, T_o increased only 1 °C (Table 1). As expected, the gelatinization range was narrowed after annealing, as evidenced by the decrease in the difference between the end gelatinization temperature (T_c) and T_o (Tables 1 and 2).

Annealing temperature exerted a significant impact on gelatinization enthalpy (ΔH) values after annealing (Table 3). As reviewed by Jacobs and Delcour (30), many researchers have reported an increase or no change in ΔH value after annealing. In this study, the ΔH value increased, decreased, or remained unchanged after annealing depending on the annealing temperature. For waxy rice (RD4) starch, annealing 2 h at 65 °C, 11 °C below the onset gelatinization temperature, increased ΔH slightly from 19.2 to 21.3 J/g. The enthalpy value (ΔH) remained unchanged when the sample was held for 2 h at 70 °C, 6 °C below the onset gelatinization temperature of the starch before annealing. Annealing the waxy rice (RD4) and waxy barley (WB) starches at a temperature close to T_o decreased ΔH (Table 3).

What annealing temperature should be used to avoid the decrease in ΔH? The answer depends on the type of starch

Table 3. Effects of Annealing Temperature on Gelatinization of Waxy Rice (RD4) and Waxy Barley (WB) Starches^a

sample	annealing temp (°C)	ΔT ^b (°C)	T _o ^c (°C)	ΔH ^d (J/g)	
RD4	none		76.2	19.2	
	77		88.0	2.5	
	76	0	87.7	6.3	
	75	1	86.9	9.6	
	74	2	86.2	13.0	
	73	3	85.1	14.2	
	72	4	84.4	15.5	
	71	5	84.1	18.4	
	70	6	83.3	18.8	
	65	11	81.3	21.3	
	SD ^e			0.3	0.4
	WB	none		58.1	13.8
		58	0	71.4	1.7
53		5	67.0	8.7	
52		6	66.1	11.3	
51		7	65.9	12.5	
50		8	64.9	13.0	
49		9	64.7	14.2	
SD				0.4	0.3

^a Annealing time was 2 h. ^b ΔT = onset gelatinization temperature of starch before annealing – annealing temperature. ^c T_o was onset gelatinization temperature. ^d ΔH was gelatinization enthalpy. ^e SD = pooled standard deviation from duplicate measurements of DSC parameters.

Table 4. Effects of Annealing Temperature on Gelatinization of Wheat Starch^a

annealing temp (°C)	ΔT ^b (°C)	T _o ^c (°C)	ΔH ^d (J/g)
none		58.4	10.0
56	2	66.9	3.8
55	3	66.0	5.0
54	4	65.5	6.3
53	5	65.0	7.9
52	6	64.0	8.8
51	7	63.5	9.2
SD ^e		0.3	0.3

^a Annealing time was 30 min. ^b ΔT = onset gelatinization temperature of wheat starch before annealing – annealing temperature. ^c T_o was onset gelatinization temperature. ^d ΔH was gelatinization enthalpy. ^e SD = pooled standard deviation from duplicate measurements of DSC parameters.

(Tables 3 and 4). For waxy rice (RD4) starch, which has a high gelatinization temperature, holding the starch 6 °C or more below the onset gelatinization of the starch did not lower the ΔH. For waxy barley, however, which has a lower gelatinization temperature than waxy rice (RD4) starch, the annealing temperature had to be at least 9 °C below the T_o of waxy barley to prevent ΔH from decreasing. For wheat starch, which has the same T_o as waxy barley but contains amylose, the annealing temperature had to be 7 °C below its T_o to prevent ΔH from decreasing (Table 4). A decrease in ΔH indicates a partial melting of crystallites and swelling of starch granules. These effects of annealing temperature on different starches are important to the industrial isolation of cereal starches. A low steeping temperature must be used when a grain containing starch with a low gelatinization temperature is wet-milled. Otherwise, swelling of starch granules occurs, dewatering becomes difficult, and recovery yield of starch is reduced.

Two-Step Annealing. Results of the single-temperature annealing (Tables 1 and 2) show that the increase in T_o is limited, even if a starch is annealed for a long time. Because T_o increases after annealing, could we raise the annealing

Table 5. Comparison of Two- and One-Step Annealings of Waxy Rice (RD4) Starch

annealing conditions		gelatinization properties ^a			
temp (°C)	time (h)	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
none		76.2	81.2	87.1	19.2
two-step					
70	2	83.3	86.5	90.0	18.8
77	0.2	84.1	87.0	90.0	12.5
	0.5	85.9	88.6	90.6	10.9
	2.0	87.4	89.4	92.0	9.6
	4.0	88.4	90.3	93.0	9.2
	16.0	90.0	91.8	94.6	9.2
SD ^b		0.3	0.3	0.4	0.4
one-step					
77	0.2	84.7	87.1	89.9	2.5
	0.5	86.4	88.5	91.4	2.5
	2.0	88.0	89.6	91.8	2.5
SD		0.4	0.3	0.4	0.3

^a T_o, T_p, and T_c were onset, peak, and end gelatinization temperatures, respectively. ΔH was gelatinization enthalpy. ^b SD = pooled standard deviation from duplicate measurements of DSC parameters.

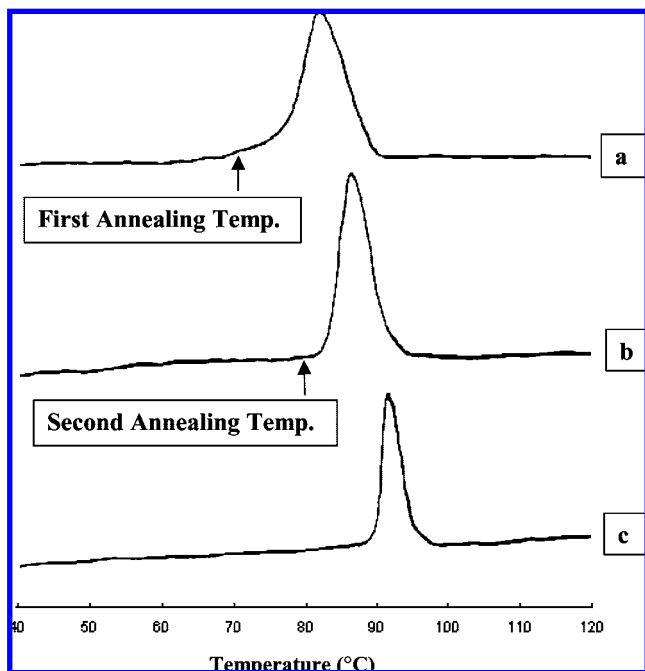


Figure 1. Two-step annealing for waxy rice (RD4) starch: (a) DSC curve of native waxy rice (RD4) starch before annealing [on the basis of this DSC curve, the first annealing temperature (70 °C), 6 °C below the T_o of the starch, was chosen]; (b) DSC curve of the first annealed waxy rice (RD4) starch [the first annealing was carried out at 70 °C for 2 h; on the basis of this DSC curve, the second annealing temperature (77 °C), 6 °C below the T_o of the first annealed starch, was chosen]; (c) DSC of the second annealed waxy rice (RD4) starch (the second annealing was carried out at 77 °C for 4 h).

temperature to further increase T_o without causing a decrease in ΔH or swelling of starch granules? **Table 5** shows the effects of two-step annealing on gelatinization of waxy rice (RD4) starch. The design of the two-step annealing experiment is depicted in **Figure 1**. During the first annealing, the starch and water mixture was held at 70 °C, 6 °C below the onset gelatinization of the waxy rice (RD4) starch. The T_o increased

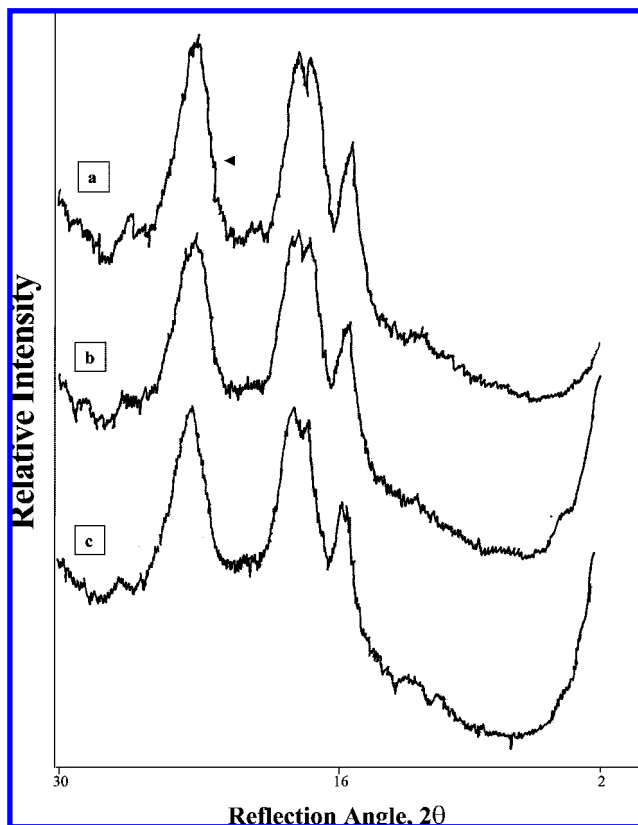


Figure 2. X-ray diffractograms of waxy rice (RD4) starch, before and after annealing: (a) waxy rice (RD4) starch before annealing; (b) waxy rice (RD4) starch annealed at 70 °C for 30 min and then at 77 °C for 30 min; (c) waxy rice (RD4) starch annealed at 70 °C for 30 min and then at 77 °C for 16 h.

from 76.2 to 83.3 °C after 2 h, whereas ΔH was unchanged. The annealing temperature was then increased to 77 °C, 6 °C below the onset gelatinization of the annealed waxy rice starch. The T_o continued to increase with time, as expected (**Table 5**), but the ΔH value decreased, even though the second annealing temperature was 6 °C below the onset gelatinization temperature of the first-step annealed waxy rice (RD4) starch. The ΔH value decreased from 18.8 to 9.2 J/g in 4 h, but remained unchanged after that. If we directly held the waxy rice starch and water mixture at 77 °C without the first annealing step at 70 °C, the ΔH value decreased from 19.2 to 2.5 J/g in 10 min (**Table 5**). These results show that the crystallites were better packed and preserved when the starch was preannealed. X-ray diffractograms confirmed that the crystallinity of waxy rice (RD4) starch decreased from 54 to 45% after it was annealed in two steps at 70 °C for 30 min and then at 77 °C for 30 min (**Figure 2**). Holding at 77 °C for 16 h during the second annealing step did not cause further reduction in X-ray crystallinity (**Figure 2**).

A similar trend was observed when a two-step annealing was applied to wheat starch (**Table 6**). **Figure 3** shows the X-ray diffractograms of wheat starch before and after two-step annealing. The estimated X-ray crystallinity of the wheat starch was 37%. After the wheat starch was annealed at 51 °C for 30 min and then at 56 °C for 30 min, its crystallinity decreased to 27%. Holding at 56 °C for 16 h during the second annealing step further reduced the crystallinity of the wheat starch to 22%. The wheat starch had a lower degree of crystallinity compared to the waxy rice (RD4) starch. The two-step annealing caused a significant reduction in crystallinity, even though the second-step annealing was held below the onset gelatinization temperature of the first-step annealed wheat starch.

Table 6. Effects of Two-Step Annealing on Wheat Starch

annealing conditions		gelatinization properties ^a			
temp (°C)	time (min)	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
none		58.4	63.2	69.0	10.0
two-step					
51	30	63.5	66.0	69.2	9.2
56	30	65.9	68.0	72.5	6.7
one-step					
56	30	66.9	70.0	75.8	3.8
SD ^b		0.3	0.3	0.4	0.3

^a T_o, T_p, and T_c were onset, peak, and end gelatinization temperatures, respectively. ΔH was gelatinization enthalpy. ^b SD = pooled standard deviation from duplicate measurements of DSC parameters.

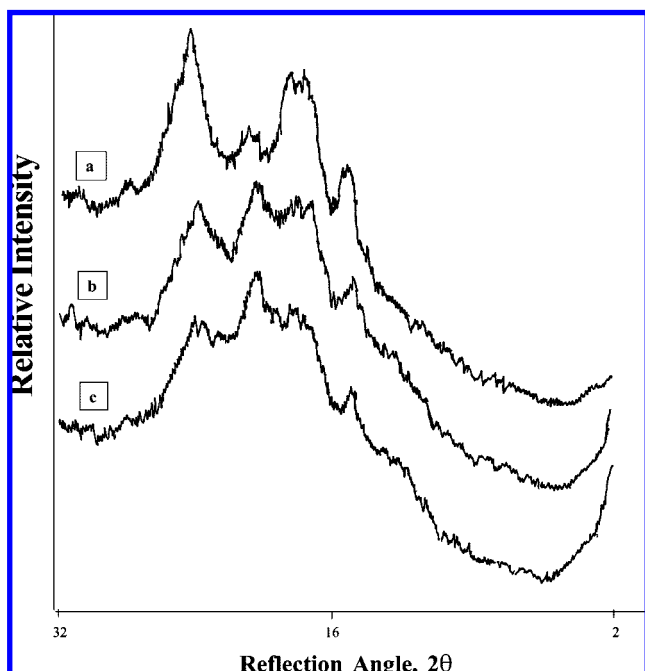


Figure 3. X-ray diffractograms of wheat starch, before and after annealing: (a) wheat starch before annealing; (b) wheat starch annealed at 51 °C for 30 min and then at 56 °C for 30 min; (c) wheat starch annealed at 51 °C for 30 min and then at 56 °C for 16 h.

The two-step annealing results in this study are different from the work by Jacobs, Delcour, and co-workers, who have reported no decrease in ΔH after the two-step annealing of wheat starch (30, 39, 40). In their work, wheat starch had an onset gelatinization temperature of 54.4 °C. The wheat starch suspension (1:2 w/w) was first annealed at 48 °C for 24 h, which increased T_o to 61.6 °C, and then was annealed at 53 °C for 24 h. The suspension was filtered on a Büchner funnel after two-step annealing, and the wheat starch was air-dried and analyzed by DSC. In another study by Nakazawa and Wang (43), annealed starches also were filtered and air-dried at room temperature. After multistep annealing, a significant decrease in ΔH values was reported for potato starch and HYLON VII starch, whereas ΔH values increased for other starches such as common corn and waxy corn starches. In this study, annealing was carried out directly in DSC pans as well as in small-scale starch suspension. When annealing was conducted in DSC pans, gelatinization results were obtained without the isolation of starch (Table 6). Soluble materials after annealing, if any, would be retained in the DSC pans. In the small-scale slurry annealing experiments, the starch samples were recovered with

Table 7. Effects of Multistep Annealing of Waxy Rice (RD4) Starch

annealing conditions	T _o ^a (°C)	ΔH ^b (J/g)
none	76.2	19.2
one-step 65 °C, 30 min	79.6	20.1
two-step 65 °C, 30 min 70 °C, 30 min	82.1	18.4
three-step 65 °C, 30 min 70 °C, 30 min 75 °C, 30 min	84.8	15.9
four-step 65 °C, 30 min 70 °C, 30 min 75 °C, 30 min 80 °C, 30 min	87.3	2.1
SD ^c	0.4	0.4

^a T_o was onset gelatinization temperature. ^b ΔH was gelatinization enthalpy. ^c SD = pooled standard deviation from duplicate measurements of DSC parameters.

Table 8. Effects of Multistep Annealing of Waxy Rice (IR29) Starch

annealing conditions	T _o ^a (°C)	ΔH ^b (J/g)
no annealing	67.3	15.0
one-step 58 °C, 30 min	71.8	15.5
two-step 58 °C, 30 min 63 °C, 30 min	74.4	15.9
three-step 58 °C, 30 min 63 °C, 30 min 68 °C, 30 min	77.6	8.4
SD ^c	0.3	0.4

^a T_o was onset gelatinization temperature. ^b ΔH was gelatinization enthalpy. ^c SD = pooled standard deviation from duplicate measurements of DSC parameters.

alcohol precipitation to ensure a total recovery of starch and used for the X-ray diffraction work presented in this study (Figure 3). In addition, the amount of soluble material during annealing was monitored in the small-scale annealing experiments. No soluble material was detected in the supernatant of wheat starch slurry before annealing or wheat starch slurry annealed at 51 °C. However, soluble materials (0.71%) were detected in the supernatant of wheat starch slurry after the second annealing at 56 °C in this study. Moreover, the wheat starch slurry had increased thickness after two-step annealing. These results suggest that partial swelling of the wheat starch granules had occurred during the second annealing step. To further confirm this, a part of the wheat starch slurries (35% solids) before and after annealing was directly taken, weighed in DSC pans, and analyzed by DSC. In agreement with the X-ray results (Figure 3), the ΔH value decreased more than 25% after two-step annealing.

Multistep Annealing. To further determine how high gelatinization temperature can be achieved by annealing and to understand the thermal behavior of starch granules, multistep annealing experiments were designed (Tables 7 and 8). For

waxy rice starch (RD4), the onset gelatinization temperature was 76.2 °C before annealing. The first annealing began at 65 °C, 11 °C below the onset gelatinization temperature of the waxy rice (RD4) starch. The ΔH value slightly increased after the first annealing. When the annealing temperature was increased to 70 °C during the second annealing step, ΔH remained little changed and T_0 increased to 82.1 °C. When the annealing temperature was increased to 75 °C during the third annealing step, T_0 continued to increase, but ΔH decreased from 18.4 to 15.9 J/g, even though the third annealing temperature was 7 °C below the T_0 of the second-step annealed starch. Moreover, the fourth annealing step led to a dramatic decline in ΔH , from 15.9 to 2.1 J/g, when the annealing was conducted at 80 °C for 30 min. The fourth-step annealing temperature, 80 °C, was almost 5 °C below the onset gelatinization temperature of the waxy rice (RD4) starch after the three-step annealing.

Multistep annealing was also done on another waxy rice starch (IR 29), which had an onset gelatinization temperature of 67.3 °C. The first-step annealing (58 °C) and second-step annealing (63 °C) did not cause any decline in ΔH (Table 8). When the waxy rice (IR 29) starch was annealed at 68 °C for 30 min during the third annealing, ΔH decreased from 15.9 to 8.4 J/g. Once again, the ΔH decreased significantly, even though the third annealing temperature, 68 °C, was 6 °C below the onset gelatinization temperature of the waxy rice (IR 29) starch after the second annealing. It should be noted that even though the third annealing temperature (68 °C) was below the T_0 of the waxy rice (IR 29) starch after the second annealing, it was above the original T_0 of the waxy rice (IR 29) starch before annealing, which is 67.3 °C.

It seems that, no matter how a starch is preannealed, the ΔH value drops and crystallinity decreases if the annealing temperature is close to the original T_0 of that starch. Two- or multi-step annealing could achieve an increase in T_0 in a short time, but there is always a temperature ceiling beyond which annealing causes a decrease in crystallinity. That temperature ceiling seems to be starch-specific and is below the original onset gelatinization of a starch.

DISCUSSION

Annealing and gelatinization of starch have been subjects of study for a long time, yet the underlying molecular mechanisms of annealing and gelatinization are not totally clear.

Why Is Annealing Possible at Low Temperature? It has been suggested (6, 7) that annealing cannot occur unless the annealing temperature is above the T_g of the amorphous phase in granular starch. The problem is that T_g is hard to detect and quantify in granular starch. Because of the difficulty associated with measuring T_g , there is confusion in the literature. For instance, in a review on annealing of starch, Tester and Debon (31) cited that some authors (17, 50) claimed that starches could be annealed below T_g , but that claim is not possible if holding at a temperature above T_g is a prerequisite of the annealing process. As observed in this work (Tables 1–4), other studies (17, 38) also have shown that annealing occurs substantially below the onset of gelatinization. This would suggest that T_g must be substantially below T_0 if T_g must be exceeded as a condition for annealing to occur (17, 38). In fact, annealing of starch has been reported (6, 38, 52) to occur at temperatures as low as 25 °C. Jacobs and Delcour (30) stated that the phenomenon of annealing at 25 °C seems to conflict with the proposed location of T_g just preceding the DSC gelatinization peak (6, 7, 13, 15), and they suggested that the operative T_g may change in the presence of water due to the effect of dynamic plasticization. In contrast, Noel and Ring offered (20) a different

view on the location of T_g in granular starch. They suggested that amorphous regions of starch granules were plasticized at the onset of gelatinization and that the T_g of amorphous regions in granular starch occurred below the onset temperature (T_0) of gelatinization. It seems that no consensus has been reached to explain the low-temperature annealing phenomenon largely because of disagreement on the location of T_g in granular starch.

If one considers a three-phase model (12, 21) of a starch granule, a mobile amorphous phase, a rigid amorphous phase, and a crystalline phase, and distinguishes the two types of amorphous phase in a starch granule, the annealing results in this study and the low-temperature annealing phenomenon could be explained. We have proposed (21) that the rigid amorphous phase in granular starch corresponds to the interlamellar noncrystalline regions close to crystallites, and its T_g is overlapped with the gelatinization of starch. On the other hand, the mobile amorphous fraction was assigned to mainly amorphous growth rings (bulk amorphous regions), and its T_g is below T_0 of gelatinization and probably extends over a broad range of temperatures. Using dynamic DSC, we have observed two glass transitions in granular starch in water: a T_g that overlaps with gelatinization and a small subzero T_g which is attributed to part of the mobile amorphous phase in granular starch (21). If we consider the heterogeneous nature of starch granules, and there is more than one T_g value in granular starch, annealing at room temperature is possible because it is higher than the T_g of part of the amorphous phase. Room temperature is high enough to plasticize part of the amorphous phase and increase the mobility and entropy of the part of the chains within amorphous lamellar regions of the semicrystalline growth ring (53). At room temperature, however, the rate of annealing is slow, and the rate of annealing is fast when an annealing temperature is high and is close to T_0 , as observed in this study and suggested in previous work (6, 38, 54, 55).

In addition to the dynamic DSC evidence (21), a recent atomic force microscopy (AFM) study by Ridout et al. (56) seems to support the three-phase model of a starch granule. Using AFM, these authors have noted bright and dark bands in pea starches and suggested that both bright and dark bands contain blocklets and both are partially crystalline. In the force modulation images, bright bands reflect regions containing hard and unswollen patches, whereas dark bands reflect swollen regions. One may postulate that the amorphous fraction in the swollen regions they have observed corresponds to the mobile amorphous phase in granular starch, whereas the amorphous fraction in hard, unswollen patches corresponds to the rigid amorphous phase. It is also interesting to note that Chinachoti et al. have suggested (57) different types of amorphous regions in cassava starch granules based on NMR results. A 10 μ s T_2 (transverse relaxation time) peak and a small 80 μ s T_2 peak have been suggested to represent the rigid amylopectin, and a 800 μ s T_2 peak represents the mobile components. Gelatinization leads to an increase in the mobile components and a decrease in the rigid population.

How Is the Amorphous Phase in Granular Starch Affected by Annealing? Annealing is often described as a crystal growth and perfection process (4, 6, 27–31), but what happens to the amorphous regions in starch granules during annealing is not well documented. If one considers that there are two types of amorphous phase in a starch granule, how do the mobile amorphous phase and the rigid amorphous phase change during annealing? Do amorphous regions become more rigid or softer after annealing? Does the T_g of the amorphous phases increase or decrease during annealing? Without distinguishing the two types of the amorphous phases in a starch granule, Tester and Debon (31) have suggested that the amorphous phase after

annealing “probably becomes more ‘glassy’ (more rigid and less mobile) whereupon T_g is elevated.” Using DDSC, we have found (21) that the rigid amorphous phase T_g of waxy maize starch does increase after annealing. However, the ΔC_p of the rigid amorphous phase decreases after annealing, suggesting that the amount of the rigid amorphous fraction decreases and part of the rigid amorphous phase is plasticized. This leads us to believe that the mobile amorphous phase is plasticized during annealing because the T_g of the mobile amorphous phase is lower than the T_g of the rigid amorphous phase, on the basis of concepts from polymer science (22–26). In other words, T_g of the mobile amorphous fraction *decreases* after annealing. This is consistent with the finding (11, 58) that water enters amorphous background regions (mobile amorphous phase) first, and this is where all the initial swelling is concentrated. As a result of annealing, the mobile amorphous phase becomes “softer” and its T_g decreases, whereas the T_g of the rigid amorphous phase, as well as T_m , increases (21).

Why Is Starch Crystallinity Decreased Even Though Annealing Temperature Is below the T_o of a Starch? Annealing can increase T_o of a starch without decreasing ΔH or X-ray crystallinity if the annealing temperature is carefully chosen and low enough, compared with T_o (Tables 3 and 4). However, starch crystallinity decreases when the annealing temperature is close to the onset gelatinization temperature. Two- or multi-step annealing is an effective way to achieve annealing in a short time and make crystallites well packed and more resistant to destruction by thermal energy (Tables 5 and 6). Nevertheless, if the second or third annealing is done at a temperature below the T_o of the annealed starch, yet close to or above the original T_o of the starch, starch crystallinity still decreases (Tables 5 and 6). These results further demonstrate and support that amorphous regions play a critical role in the process of gelatinization (6, 7, 10, 13, 53, 59–61) because the crystalline phase and amorphous phase are interconnected in starch granules (1, 8, 9). It has been proposed (6, 7) that gelatinization is a nonequilibrium melting process and actually a continuum of a relaxation process that occurs during heating of starch in water. During annealing, amorphous growth rings adsorb water, accompanied by swelling within this region (11, 58). The plasticization of the amorphous background and part of the lamellar regions provides mobility and allows alignment of double helices and a more efficient packing of crystallites in dense lamellae, but swelling in these regions also creates a stress on crystallites (59). It seems that two events take place at the same time inside starch granules during annealing. On the one hand, annealing results in a more efficient packing of crystallites and crystal perfection. On the other hand, the combination of thermal energy and water causes the swelling of plasticized amorphous regions in starch granules. When the temperature is high enough, sufficient stress is created to cause the double helices within crystallites to dissociate. It seems that T_o can be increased by annealing, but when the subsequent holding (annealing) temperature is close to the original T_o of a starch before annealing, the swelling of the amorphous regions leads to the disruption of crystallites in starch granules even though the holding temperature is now far below the T_o of the annealed starch (Tables 5–8).

Annealing seems to be an interplay between a more efficient packing of crystallites in starch granules and swelling of plasticized amorphous regions. It increases the mobility of part of the amorphous phase and leads to a more efficient packing of crystallites in starch granules. Two- or multi-step annealing is an effective way to achieve the efficient packing of crystallites in starch granules in a short time. Without annealing, double helices in starch granules are not well packed and can be disassociated to a larger extent, compared with the starch that is annealed (Tables 5 and

6). However, when a second- or third-step annealing temperature is high enough, it can cause a decrease in crystallinity, even though the holding temperature remains below the onset gelatinization temperature of the previously annealed starch. These results support that gelatinization is a nonequilibrium process and that dissociation of double helices is driven by the swelling of amorphous regions. There is always a temperature ceiling that can be used to anneal a starch without causing a decrease in crystallinity. That temperature ceiling is starch-specific, dependent on the structure of a starch, and is lower than the original onset gelatinization of a starch.

ACKNOWLEDGMENT

I am grateful to Dr. Paul Seib for his advice and reviewing the manuscript, Dr. Yijun Sang for repeating the small-scale annealing experiments on wheat starch, and Shiva Garimella for his help in preparing the figures.

LITERATURE CITED

- (1) French, D. Organization of starch granules. In *Starch Chemistry and Technology*, 2nd ed.; Whistler, R. L., BeMiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 182–242.
- (2) Zobel, H. F. Starch crystal transformations and their industrial importance. *Starch* **1988**, *1*, 1–7.
- (3) Cooke, D.; Gidley, M. J. Loss of crystalline and molecular order during starch gelatinization: origin of the enthalpic transition. *Carbohydr. Res.* **1992**, *222*, 103–112.
- (4) Wunderlich, B. Crystal Nucleation, Growth, Annealing. In *Macromolecular Physics, Vol. 2 – Crystal Nucleation, Growth, Annealing*; Academic Press: New York, 1976.
- (5) Brydson, J. A. The glass transition, melting point and structure. In *Polymer Science*; Jenkins A. D., Ed.; North Holland Publishers: Amsterdam, The Netherlands, 1972; pp 194–249.
- (6) Slade, L.; Levine, H. Recent advances in starch retrogradation. In *Recent Developments in Industrial Polysaccharides*; Stivala, S. S., Crescenzi, V., Dea, I. C. M., Eds.; Dordon and Breach Science: New York, 1987; pp 387–430.
- (7) Slade, L.; Levine, H. Non-equilibrium melting of native granular starch: part I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. *Carbohydr. Polym.* **1988**, *8*, 183–207.
- (8) Waigh, T. A.; Kato, K. L.; Donald, A. M.; Gidley, M. J.; Clarke, C. J.; Riekel, C. The phase transformations in starch during gelatinization: a liquid crystalline approach. *Starch* **2000**, *52*, 450–460.
- (9) Donald, A. M. Plasticization and self assembly in the starch granule. *Cereal Chem.* **2001**, *78*, 307–314.
- (10) Perry, P. A.; Donald, A. M. SANS study of the distribution of water within starch granule. *Int. J. Biol. Macromol.* **2000**, *28*, 31–39.
- (11) Waigh, T. A.; Gidley, M. J.; Komanshek, B. U.; Donald, A. M. Side-chain liquid-crystalline model for starch. *Carbohydr. Res.* **2000**, *328*, 165–176.
- (12) Biliaderis, C. G. Non-equilibrium phase transitions of aqueous starch system. In *Water Relationships in Foods*; Levine, S., Slade, H., Eds.; Plenum Press: New York, 1991; pp 251–273.
- (13) Biliaderis, C. G.; Page, C. M.; Maurice, T. J.; Julian, B. O. Structures and phase transitions of starch in food systems. *J. Agric. Food Chem.* **1986**, *34*, 6–14.
- (14) Biliaderis, C. G. Thermal characterization of rice starches: a polymeric approach to phase transitions of granular starch. *Food Technol.* **1992**, *6*, 98–109.
- (15) Maurice, T. J.; Slade, L.; Sirett, R. R.; Page, C. M. Polysaccharide–water interactions – thermal behavior of rice starch. In *Properties of Water in Foods*; Simatos, D., Multon, J. L., Eds.; Martinus Nijhoff Publishers: Dordrecht, The Netherlands, 1985; pp 211–217.
- (16) Zelezak, K. J.; Hosney, R. C. The glass transition in starch. *Cereal Chem.* **1987**, *64*, 121–124.
- (17) Yost, D. A.; Hosney, R. C. Annealing and glass transition of starch. *Starch* **1986**, *38*, 289–292.

- (18) Liu, H.; Lelievre, J.; Ayoung-Chee, W. A study of starch gelatinization using differential scanning calorimetry, X-ray, and birefringence measurements. *Carbohydr. Res.* **1991**, *210*, 79–87.
- (19) Liu, H.; Lelievre, J. Phase and state transitions in granular starches studies by dynamic differential scanning calorimetry. *Carbohydr. Res.* **1991**, *219*, 23–32.
- (20) Noel, T. R.; Ring, S. G. A study of the heat capacity of starch/water mixtures. *Carbohydr. Res.* **1992**, *227*, 203–213.
- (21) Liu, Y.; Shi, Y.-C. Phase and state transitions in granular starches studies by dynamic differential scanning calorimetry. *Starch* **2006**, *58*, 433–442.
- (22) Cheng, S. Z. D.; Wu, Z. Q.; Wunderlich, B. Glass transition and melting behavior of poly(thio-1,4-phenylene). *Macromolecules* **1987**, *20*, 2802–2810.
- (23) Cheng, S. Z. D.; Cao, M. Y.; Wunderlich, B. Glass transition and melting behavior of poly(oxy-1,4-phenyleneoxy-1,4-phenylene-carbonyl-1,4-phenylene). *Macromolecules* **1986**, *19*, 1868–1876.
- (24) Suzuki, H.; Grebowicz, J.; Wunderlich, B. Heat capacity of semicrystalline, linear poly(oxyethylene) and poly(oxyethylene). *Makromol. Chem.* **1985**, *186*, 1109–1119.
- (25) Suzuki, H.; Grebowicz, J.; Wunderlich, B. Glass transition of poly(oxyethylene). *Br. Polym. J.* **1985**, *17*, 1–3.
- (26) Krishnaswamy, R. K.; Geibel, J. F.; Lewis, B. J. Influence of semicrystalline morphology on the physical aging characteristics of poly(phenylene sulfide). *Macromolecules* **2003**, *36*, 2907–2914.
- (27) Kuge, T.; Kitamura, S. Annealing of starch granules – warm water treatment and heat-moisture treatment. *J. Jpn. Soc. Starch Sci.* **1985**, *32*, 65–83.
- (28) Blanshard, J. M. V. Starch granular structure and function: physicochemical approach. In *Starch: Properties and Potential*; Galliard, T., Ed.; Wiley: New York, 1987; pp 16–54.
- (29) Stute, R. Hydrothermal modification of starches: the difference between annealing and heat/moisture treatment. *Starch* **1992**, *44*, 205–214.
- (30) Jacobs, H.; Delcour, J. A. Hydrothermal modification of granular starch, with retention of the granular structure: a review. *J. Agric. Food Chem.* **1998**, *46*, 2895–2905.
- (31) Tester, R. F.; Debon, S. J. J. Annealing of starch—a review. *Int. J. Biol. Macromol.* **2000**, *27*, 1–12.
- (32) Chiu, C.-W.; Shi, Y.-C.; Sedam, M. Process for producing amylase resistant starch granular starch. U.S. Patent 5,902,410, 1999.
- (33) Hoover, R.; Vasanthan, T. The effect of annealing on the physicochemical properties of wheat, oat, potato, and lentil starches. *J. Food Biochem.* **1994**, *17*, 305–325.
- (34) Tester, R. F.; Ansell, R.; Snape, C. E.; Yusuph, M. Effects of storage temperatures and annealing conditions on the structure and properties of potato (*Solanum tuberosum*). *Int. J. Biol. Macromol.* **2005**, *26*, 1–8.
- (35) Beshtein, V. A.; Egorov, V. M. Differential scanning calorimetry of polymers. In *Physics, Chemistry, Analysis, Technology*; Kep, T. J., Ed.; Ellis Horwood: New York, 1994; pp 1–253.
- (36) Kiseleva, V. I.; Genkima, N. K.; Tester, R.; Wasserman, L. A.; Popov, A. A.; Yuryev, V. P. Annealing of normal, low and high amylose starches extracted from barley cultivars grown under different environmental conditions. *Carbohydr. Polym.* **2004**, *56*, 157–168.
- (37) Gough, B. M.; Pybus, J. N. Effect on Gelation temperature of wheat starch granules of prolonged treatment with water at 50 °C. *Starch* **1971**, *23*, 210–212.
- (38) Tester, R. F.; Debon, S. J. J.; Karkalas, J. J. Annealing of wheat starch. *J. Cereal Sci.* **1998**, *28*, 259–272.
- (39) Jacobs, H.; Eerlinger, R. C.; Spaepen, H.; Grobet, P. J.; Delcour, J. A. Impact of annealing on the susceptibility of wheat, potato and pea starches to hydrolysis with pancreatin. *Carbohydr. Res.* **1998**, *305*, 193–207.
- (40) Jacobs, H.; Mischenko, S.; Koch, M. H. J.; Eerlinger, R. C.; Delcour, J. A.; Reynaers, H. Evaluation of the impact of annealing on gelatinization at intermediate water content of wheat and potato: a differential scanning calorimetry and small-angle X-ray scattering study. *Carbohydr. Res.* **1998**, *306*, 1–10.
- (41) Vermeylen, R.; Goderis, B.; Delcour, J. A. An X-ray study of hydrothermally treated potato starch. *Carbohydr. Polym.* **2006**, *64*, 364–375.
- (42) Collado, L. S.; Corke, H. Heat–moisture treatment effects on sweetpotato starches differing in amylose content. *Food Chem.* **1999**, *65*, 339–346.
- (43) Nakazawa, Y.; Wang, Y.-J. Acid hydrolysis of native and annealed starches and branch-structure of their Naegeli dextrins. *Carbohydr. Res.* **2003**, *338*, 2871–2882.
- (44) Hoover, R.; Vasanthan, T. Effect of heat–moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches. *Carbohydr. Res.* **1994**, *252*, 33–53.
- (45) Gunaratne, A.; Hoover, R. Effect of heat–moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydr. Polym.* **2002**, *49*, 425–437.
- (46) Brumovsky, J. O.; Thompson, D. B. Production of boiling-stable granular resistant starch by partial acid hydrolysis and hydrothermal treatment of high-amylose maize starch. *Cereal Chem.* **2001**, *78*, 680–689.
- (47) Juliano, B. O. Rice starch: production, properties, and uses. In *Starch: Chemistry and Technology*, 2nd ed.; Whistler, R. L., BeMiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 507–528.
- (48) Wu, Y.; Seib, P. A. Acetylated and hydroxypropylated distarch phosphates from waxy barley. Paste properties and freeze–thaw stability. *Cereal Chem.* **1990**, *67*, 202–208.
- (49) Shi, Y.-C.; Seib, P. A. The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.* **1992**, *227*, 131–145.
- (50) Komiya, T.; Nara, S. Changes in crystallinity and gelatinization phenomena of potato starch by acid treatment. *Starch* **1986**, *38*, 9–13.
- (51) Seow, C. C.; Vasanti-Nair, C. K. Sub- T_g annealing of granular rice starch: effects on enthalpy relaxation and starch–sucrose interactions. *Carbohydr. Res.* **1994**, *261*, 307–316.
- (52) Lorenz, K.; Kulp, K. Steeping of barley starch. Effects on physicochemical properties and functional characteristics. *Starch* **1984**, *36*, 116–121.
- (53) Perry, P. A.; Donald, A. M. The role of plasticization in starch granule assembly. *Biomacromolecules* **2000**, *1*, 424–432.
- (54) Shi, Y. C.; Seib, P. A.; Bernardin, J. E. Effects of temperature during grain-filling on starches from six wheat cultivars. *Cereal Chem.* **1994**, *71*, 369–383.
- (55) Larsson, I.; Eliasson, A.-C. Annealing of starch at an intermediate water content. *Starch* **1991**, *43*, 227–231.
- (56) Ridout, M. J.; Parker, M. L.; Hedley, C. L.; Bogracheva, T. Y.; Morris, V. J. Atomic force microscopy of pea starch: granule architecture of the *rug 3-a*, *rug 4-b*, *rug 5-a* and *lam-c* mutants. *Carbohydr. Polym.* **2006**, *65*, 64–74.
- (57) Chinachoti, P.; Vittadini, E.; Chatakanonda, P.; Vodovotz, Y. Characterization of molecular mobility in carbohydrate food systems by NMR. *Modern Magn. Reson.* **2006**, *3*, 1681–1690.
- (58) Jenkins, P. J.; Donald, A. M. Gelatinization of starch: a combined SAXS/WAXS/DSC and SANS study. *Carbohydr. Res.* **1998**, *308*, 133–147.
- (59) Donovan, J. W. Phase transitions of the starch–water system. *Biopolymers* **1979**, *18*, 443–446.
- (60) Donovan, J. W.; Mapes, C. J. Multiple phase transitions of starches and Nägeli amylopectins. *Starch* **1980**, *32*, 190–193.
- (61) Evans, I.; Haisman, D. The effect of solutes on the gelatinization temperature range of potato starch. *Starch* **1982**, *34*, 224–231.

Received for review August 14, 2007. Revised manuscript received November 19, 2007. Accepted November 30, 2007. This is Contribution 07-52-J from the Kansas Agricultural Experiment Station, Manhattan, KS.