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Properties of Starch Subjected to Partial Gelatinization and β-Amylolysis

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The overall objective of this research is to understand the impact of partial gelatinization and β-amylase hydrolysis (β-amylolysis) on the physicochemical properties of starch. Three starches (normal corn, waxy corn, and wheat) were chosen as test examples and thermally treated at 40% moisture content to up to 95 °C and then subjected to β-amylolysis. The enzyme treatment resulted in over 10% maltose yield. Subsequent debranching analysis showed the production of chain stubs as short as having the degree of polymerization of 2 and 3, suggesting a thorough β-amylolysis at certain branch locations. For starch samples subjected to partial gelatinization, polarized light microscopy shows reduced intensity of birefringence and differential scanning calorimetry shows reduced enthalpy change associated with gelatinization. Both indicate the reduced chain organization due to the treatment. Further, a substantial transformation of initial A-type crystalline structure to B- and V-types upon treatments is noticed from X-ray powder diffraction measurements. In addition, the rapid viscosity analysis (RVA) indicated a drastic viscosity reduction, increased peak temperature, and improved stability of pasting behavior due to hydrothermal treatments and β-amylolysis. Overall, our results point out the possibility of obtaining modified starches having desirable stable pasting behavior by using a combined partial gelatinization and β-amylolysis approach.

KEYWORDS: Starch; partial gelatinization; β-amylolysis; chain organization; pasting behavior

INTRODUCTION

Starch is a primary carbohydrate polymer of higher plants. It comprises two D-glucose homopolysaccharides, amylose and amylopectin. Amylose is essentially a linear molecule with approximately 10^2-10^4 glucosyl units joined by α-1→4 linkages, whereas amylopectin is very large and highly branched with both α-1→4 and 1→6 linkages. Starches from major crops such as corn, wheat, rice, and potato constitute a significant proportion of commodity starches and play important roles in the food and nonfood industries. In many food applications, starch undergoes a gelatinization and retrogradation process so as to develop desirable properties such as viscosity and bulking, as well as providing nutritional value. Under most circumstances, particularly those in which starch is added to modulate the rheological properties of a food system, the stability of starch pasting behavior is critical for effective control of processing, storage, and sensory quality.

Chemical and genetic modifications are primary approaches to tailor starch functionalities. For example, substitutions by monofunctional groups such as succinate, acetate, phosphate, and octenyl succinate have been used to retard starch retrogradation or confer emulsification properties. On the other hand, distarch phosphates and distarch adipates have been prepared to introduce cross-linking to maintain the integrity of starch granules under shear and heat treatments. The most widely used genetically modified starches are waxy and high amylose starch. In addition, a variety of other mutations have been used to generate altered starch structure. For corn, these mutations include *su1*, *su2*, *du1*, and their combinations (*1*). In general, the properties of genetically modified starches differ greatly from those of normal starch (*2*−*5*).

Enzymatic modifications of starch have been proposed in order to reduce retrogradation (*6*−*9*), to modify rheological properties (*10, 11*), or to alter digestion properties (*9, 12*). The granules from different starch origins or genotypes may show substantially dissimilar susceptibility to enzymatic modification (*13*). Among different types of enzymes used, β-amylase is unique with its properties of exoacting and not being able to bypass branch points, which allows retention of the overall branch structure of amylopectin. Due to its capability to shorten the external chain length of amylopectin, β-amylase has been used to retard retrogradation of starch or starch-based foods (*6, 7*).

The conceptual difference between enzymatic modification and enzymatic conversion of starch should be considered. Enzymatic starch conversion includes the preparations of a variety of starch hydrolysates, such as maltodextrin, corn syrup, amylodextrin, and other starch-based α-glucans. These materials have low molecular weight, which differentiates them from starch-like material with high molecular weight and structure

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complexity for individual molecules. On the other hand, enzymatically modified starches are starch-based materials that retain, at least partially, the granular structure or molecular structure hierarchy, such as that found in amylopectin molecules.

Partial gelatinization of starch can result from hydrothermal treatments. However, most studies on hydrothermal treatments target annealing or heat-moisture treatment (*14*). For annealing, starch granules are incubated in excess or intermediate water content (>40%) at a temperature above the glass transition temperature but below the gelatinization temperature. For heat−moisture treatment, the moisture level is low (<35%) whereas the temperature is above the glass transition temperature and may reach up to 100 °C and result in partial gelatinization (*15*). Both annealing and heat−moisture treatment can lead to substantial change in starch properties. For rice starch, hydrothermal treatments lead to lower peak viscosity and setbacks and greater swelling consistency (*16*). However, annealing and heat−moisture treatment may have varying impacts on starch in terms of DSC gelatinization profile and X-ray crystalline structure (*16, 17*). Usually annealing leads to increased and narrower gelatinization temperature, whereas heat−moisture treatment results in broader ones. In addition, annealing and heat−moisture treatment at low moisture content typically has no impact on starch crystallinity, whereas heat−moisture treatment at higher moisture content leads to increased amorphous content (*16, 17*), which can be related to partial gelatinization. In a recent study, Shi (*18*) treated cereal starches using two-step and multistep annealing, and they found that there is always a starch-specific temperature ceiling for annealing without causing a decrease in crystallinity (that may lead to partial gelatinization).

While either hydrothermal treatment or enzymatic modification can be used to offer altered starch functionality, it has not been reported that these two approaches are used in coordination. Therefore, it could be a novel strategy to carry out starch modifications by combining hydrothermal and enzymatic treatments. For example, hydrothermal treatments may result in improved enzyme susceptibility while enzymatic modification may lead to an altered outcome for hydrothermal treatments. In this study, hydrothermal treatment and subsequent enzymatic modification are used. The objective is to enhance the efficiency of β-amylolysis via hydrothermal treatment-based partial gelatinization and to characterize the properties of starch materials thus prepared. It should be stressed that the aim of the hydrothermal treatment employed in this study is to enhance enzyme susceptibility; thus, the conditions are different from those of the annealing or heat−moisture treatment typically used in other studies.

MATERIALS AND METHODS

Materials. Normal corn starch and waxy corn starch were obtained from National Starch and Chemical (Bridgewater, NJ), and wheat starch was obtained from the Archer Daniels Midland Company (Decatur, IL). β-Amylase (Optimal BBA from Barley) was a gift from Genencor (Rochester, NY). The activity of β-amylase was 16,400 Betamyl units/mL, determined using a Betamyl method kit (Megazyme, Wicklow, Ireland). Our test showed that a low molecular weight starch hydrolysate other than maltose was negligible after the hydrolyzation by the β-amylase used, suggesting a negligible contamination of other amylases such as α-amylase.

Hydrothermal Treatment. The moisture content of 500 g (dry base) of each starch was adjusted to 40% by the addition of deionized water (pH 5.5). The starch was packed and sealed into 50-mL centrifugation tubes (VWR SuperClear) and submerged into a circulating water bath preheated to 60 °C followed by a stepwise heating procedure. From 60 °C the bath temperature was held for 5 min and then raised 5 deg and held another 5 min at the new temperature. The stepwise heating proceeded up to a temperature of 95 °C. At 95 °C, the sample was held for 180 min. Then the temperature was lowered 5 deg and held for 5 min followed by lowering the temperature another 5 deg until a bath temperature of 25 °C was reached. Upon reaching 25 °C, sample tubes were removed from the water bath, and starch from each tube was taken out.

***β*-Amylolysis.** A portion of solid material after hydrothermal treatment was mixed with 0.02 M sodium acetate buffer (pH 5.5) to make a 10% (dry base) starch suspension. The total amount was distributed into several groups. For normal corn starch (NCS), these groups contain NCS-1, NCS-2, NCS-3, NCS0-1, NCS0-2, and NCS0-3. NCS-1, NCS-2, and NCS-3 denote samples subjected to β-amylolysis for different time intervals, whereas NCS0-1, NCS0-2, and NCS0-3 denote their respective nonenzyme controls. The β-amylase doses for NCS-1, NCS-2, and NCS-3 were 0.5% (w/w dry starch base). All starch suspensions were subjected to incubation in a shaking water bath at 55 °C at 70 stokes per minute. After 2, 12, and 24 h, samples NCS-1 and NCS0-1, NCS-2 and NCS0-2, and NCS-3 and NCS0-3 were removed from the water bath, respectively. Upon removal from the bath, starch suspensions were centrifuged at 3000*g* for 5 min. The supernatant fraction was boiled and frozen for later maltose analysis. The starch precipitate was washed using five volumes of 80% (w/w) ethanol five times in a dispersing-filtration procedure to remove maltose. The starch samples were then dehydrated using anhydrous ethanol and dried in the hood. Dry starch samples were ground and sieved through an 80 mesh sieve (180 μm) before collection.

The labels for the waxy corn starch (WCS) group were as follows: WCS-1, WCS-2, and WCS-3 for those subjected to β-amylolysis for different time intervals, and WCS0-1, WCS0-2, and WCS0-3 for the controls of WCS-1, WCS-2, and WCS-3, respectively. In the same pattern, the labels for the wheat starch (WS) group were WS-1 and WS0-1, WS-2 and WS0-2, and WS-3 and WS0-3.

Maltose Yield Determination. Maltose released from β-amylolysis was determined using a 3,5-dinitrosalicylic acid (DNS) assay (*19*) and converted to the maltose yield based on dry starch.

HPSEC Analysis of Starch Fine Structure. To determine the fine structure of starch after enzymatic modification, high performance size-exclusion chromatography (HPSEC) was used. Five milligrams of starch was dispersed in 125 μL of 90% DMSO and heated in a boiling-water bath for 10 min. Sodium acetate buffer (0.02 M, 875 μL, 50 °C, pH 4.75) was added to the dispersion. The mixture was heated again in a boiling water bath for 10 min and cooled to 37 °C in a shaking water bath. Isoamylase solution (Megazyme, 5 U/mL, 50 μL in acetate buffer) was added to each mixture. The mixtures were incubated for 24 h at 37 °C with constant agitation and then boiled for 10 min to denature the enzyme. Moisture was removed using a centrifugal vacuum drier (Savant), and the volume of each sample was adjusted to 1 mL with 90% DMSO. After vortex and centrifugation to remove insoluble materials, a 20 μL aliquot was injected into to the HPSEC system. The HPSEC system contains two connected Zorbax gel PSM 60-S columns (6.2 mm × 250 mm, Agilent Tech., Santa Clara, CA) and has a flow rate of 0.5 mL/min with DMSO as the mobile phase. The elution was monitored with a Waters 2414 refractive index (RI) detector (Waters, MA). Glucose, maltose, malto-pentaose (DP5), and pullulan with molecular weights of 5900, 11800, 22800, 47300, 112000, and 212000 (Polymer Laboratories, Amherst, MA) were used for column calibration. For each starch, HPSEC data processing includes raw data exportation to an Excel spreadsheet and normalization of the chromatogram by the total area from the retention time of 10−20 min.

DSC Thermal Analysis of Starch. To examine the ordered structure of modified and native starch, differential scanning calorimetry (DSC) was used. The DSC unit used was a TA standard modulated DSC 2920. For each sample starch tested, 5 mg (dry weight) was added to a standard hermetic aluminum pan, and then deionized water was added, bringing the total sample weight to 16.6 mg and making a 30% solid dispersion. The sealed sample pan was allowed to equilibrate for 2 h at room temperature before being loaded to the DSC unit. DSC for each sample was performed, beginning with equilibration at 30 °C, held isothermal for 3 min. The temperature within the DSC unit cell

was then raised at 5 °C per minute up to 140 °C. Data was collected and evaluated by Universal Analysis software. Each starch sample was tested three to five times to obtain reproducible results.

Polarized Light Microscopic Imaging. Polarized light microscopy was used to identify the starch Maltese cross associated with the granular crystalline structure. A Leitz Labrolux 12 polarized light microscope was used with 10× magnification oculars and a 10× magnification objective lens for a total of 100× magnification. Sample starches were dispersed in deionized water and then mounted for viewing. Images of samples under standard light and a polarized filter were taken using a microscope mounted Kodak DC 290 200 M digital camera with a MDS universal adapter.

X-ray Powder Diffraction. About 500 mg of starch sample was packed in an aluminum holder and mounted on a Philips PW3710 diffractometer interfaced with Automated Powder Diffraction (APD) software. Ni-filtered Cu Kα radiation ($\lambda = 1.5418$ Å) was used, and the tube was operated at 40 kV and 25 mA. The intensity data were collected at room temperature at 0.01° intervals in the 2θ range 10−38°, and the time spent at each step was 4 s. The patterns were smoothed for further analysis by the PC-APD (version 3.6) software, and such a process resulted in 695 data points for the entire pattern. As the X-ray analysis reflects the average structure of a sample and shows highly reproducible results, a single test was conducted for each sample.

In order to calculate the crystallinity of each starch sample, initially around 450 data points in the nonpeak regions were selected as background intensities. A ninth order polynomial was fitted using the Origin 7.5 Evaluation version, generating the background profile. Further, the cubic spline interpolation methodology was used for estimating the background intensity at each measured diffraction angle (2θ). The background profile was scaled such that it abuts the semicrystalline pattern. The percentage of crystallinity was calculated as follows:

$$\% \text{ crystallinity} = (\text{total area} - \text{background profile area}) \times 100/\text{total area}$$

Swelling Power of Modified Starch. Swelling power was measured using a suspension-centrifugation procedure. For each starch, 150 mg of sample (dry basis) and 1500 mg deionized water was added to a 2.5-mL microtube. After full dispersion, centrifugation was conducted at 22 °C and 3000 × g for 5 min. Supernatant was removed followed by weighing the precipitate. The swelling power (SP) was calculated as: SP = starch precipitate (mg)/dry starch (mg)

Rapid Viscosity Analysis of Modified Starch. Rapid viscosity analysis (RVA) was conducted using a Newport Scientific RVA unit with Thermocline control and data collecting software. For each starch sample, 3.0 g of material (dry weight) was added to an RVA sample canister. The total weight was brought to 30.0 g by adding deionized water to create a 10% dispersion of sample. After this the canister with mixing paddle were loaded into the RVA unit. The thermal and shearing protocol begins with a boot idle temperature of 50 °C and an initial shearing speed of 960 rpm for the first 10 s, then a constant 160 rpm for the remainder of the test. One minute from initial start of the test, the temperature began ramping 12 °C per minute from 50 to 95 °C. The temperature was held at 95 °C for 2.5 min then reduced at 12 °C per minute back to 50 °C concluding the test. For each type of starch, duplicate tests were conducted with the RVA curve of both duplicates given in the graphs.

RESULTS

Partial β-Amylolysis and Starch Fine Structure. Figure 1 shows the β-amylolysis maltose yields of hydrothermally treated normal corn starch (NCS), wheat starch (WS), and waxy corn starch (WCS). For WCS the maltose yield almost reaches a plateau after only 2 h. After 24 h, the maltose yield is 20.7%. On the other hand, NCS and WS both display increased maltose yield along with reaction time, reaching 11.9% and 14.1%, respectively, at 24 h. Note that β-amylolysis releases much less maltose for native starch controls that receive no hydrothermal treatment. Interestingly, the maltose yield of nontreated WS

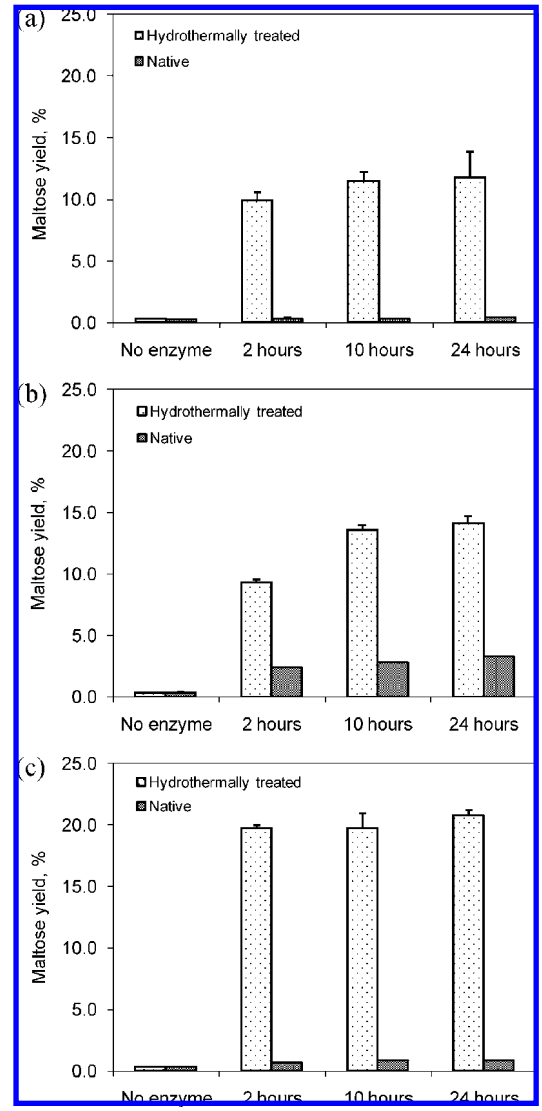

Figure 1. Maltose yield (dry starch based) after β-amylolysis. Comparisons are made between hydrothermally treated and native nontreated normal corn starch (**a**), wheat starch (**b**), and waxy corn starch (**c**).

reaches 3.3% at 24 h of β-amylolysis, much higher than those for NCS (0.44%) and WCS (0.88%). This can be attributed to the presence of damaged starch granules for WS which are more susceptible to enzymatic hydrolysis. It has been reported that the limit of β-amylolysis (i.e., maximal maltose yield) for most starches ranges from 50 to 60% (*20*). Therefore, while the β-amylolysis achieved is remarkable, it is far from reaching the limit.

Figure 2 shows the HPSEC analysis of starch subjected to hydrothermal treatment and β-amylolysis. In general, it appears that the impact of treatment on the overall chain length profiles of starch preparations is minor. However, for those subjected to β-amylolysis (samples labeled 1, 2, and 3), the presence of very short chains (DP2, 3, and 4) is evidenced. These very short chains originate directly from the consecutive maltose removal during β-amylolysis. Specifically, the creation of DP2 and DP3 stubs indicates an exhaustive cleavage of selected chains by β-amylase. It is noticed that the amount of maltosyl stubs of NCS and WS is stabilized after only 2 h of β-amylolysis, whereas, for WCS, the amount of maltosyl stubs at the 2-h stage is much less than that for the 10- and 24-h stages.

It has been reported that the β-amylolysis of amylopectin proceed in two stages: first, a rapid progress up to 52%

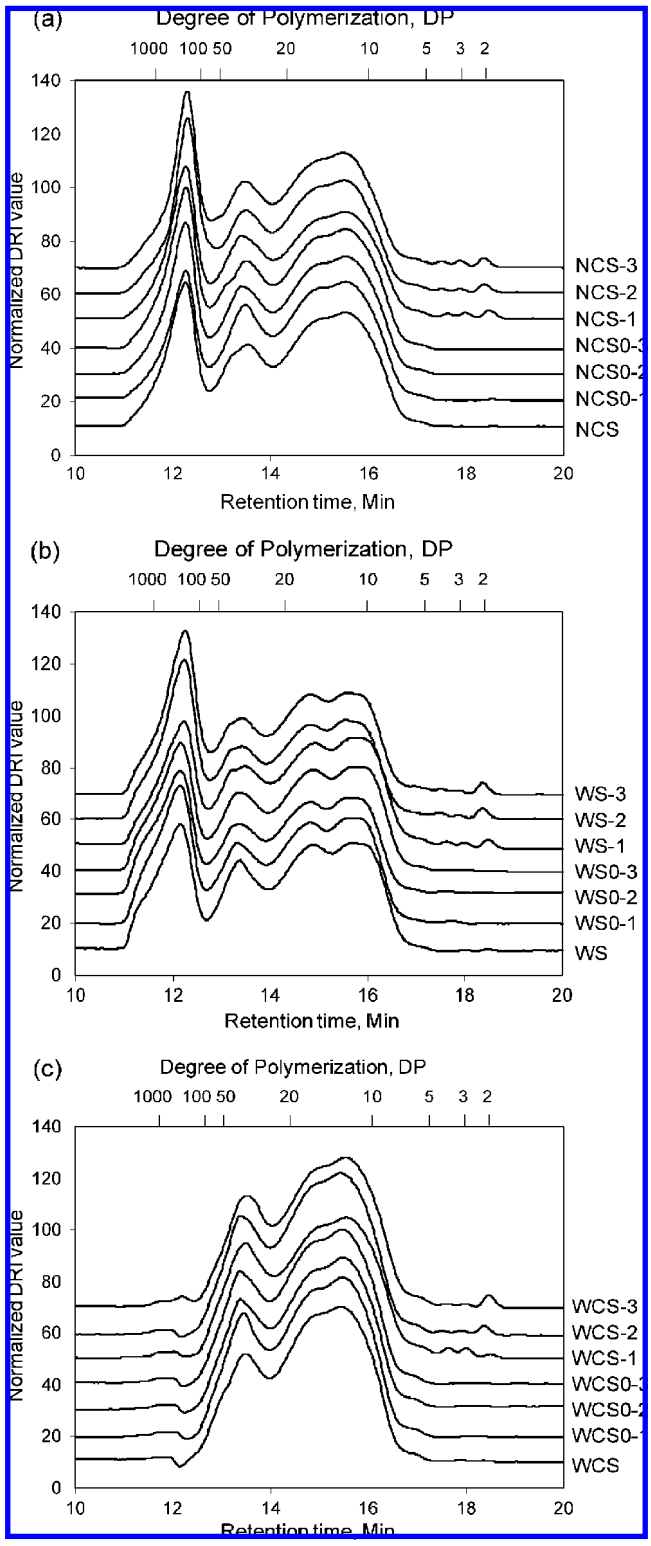

Figure 2. Chain length distribution of starches subjected to hydrothermal treatment and β-amylolysis. Starch samples related to normal corn starch (**a**), wheat starch (**b**), and waxy corn starch (**c**) are compared. Starch type is labeled for each chromatogram.

hydrolysis, and second, a slow progress to completion (*21*). In the first stage, chains are shortened to maltotetraosyl residues (DP4), which are further hydrolyzed to maltosyl residues in the second but slow stage (*21*). For this study, it appears that the second stage for the chains susceptible to β-amylolysis is nearly completed in 2 h (**Figure 2**) for NCS and WS. In contrast, for WCS it is far from being completed in 2 h. The rationale for such a behavior of β-amylolysis is not clear from the current data. Perhaps the β-amylolysis of hydrothermally treated starch is affected by both the overall accessibility of starch granules and the microenvironment of individual chains, which is related to the partial gelatinization and hydrolysis of starch. **Table 1** shows that retained chain organization (quantified by DSC enthalpy change and X-ray crystallinity) after hydrothermal treatment is greater for WCS than for NCS and WS. Perhaps this leads to a lower rate of β-amylolysis at individual chains for WCS. However, WCS granules could be more accessible to β-amylase due to its lack of amylose association or retrogradation, which results in a higher maltose release.

Partial Gelatinization of Starch Characterized by Polarized Light Microscopy, DSC, and X-ray Powder Diffraction. **Figure 3** shows the granular Maltese cross of treated starches. In the case of NCS and WCS (**Figure 3a**, **c**), the hydrothermal treatment and following β-amylolysis have not demolished the chain organization of starch granules. In addition, the birefringence intensity is similar among treated samples, and β-amylolysis seems to have negligible impact on granular birefringence. In contrast, for WS the hydrothermal treatment removes most of the birefringence indicated by an almost undetectable level of Maltese cross (**Figure 3b**).

The hydrothermal and enzymatic treatments have high impact on the DSC thermal behavior of starches (**Table 1**). The endothermic enthalpy change value, which reflects the amount of ordered structure associated with amylopectin, was partially retained after treatments. Overall, all treated samples show substantially reduced enthalpy change compared with their native counterparts. Specifically, the enthalpy changes of treated NCS (<3.6 J/g) and WS (<1.9 J/g) are much lower than that of WCS (<7.6 J/g). This is related to the observation of intense birefringence for WCS treated samples compared with that of NCS and WS. However, a well-defined impact from additional β-amylolysis has not been observed in the present case.

The impact of hydrothermal treatment on gelatinization temperature is quite evident. For NCS, WS, and WCS, the onset temperatures increased from 65.5 to up to 80.3 °C, from 58.1 to up to 77.9 °C, and from 64.3 to up to 77.4 °C, respectively. Further, the peak temperature is also found to increase from 70.6 to 83.9 °C, from 62.4 to 81.6 °C, and from 69.9 to 81.6 °C, respectively, for NCS, WS, and WCS. The increase of gelatinization temperature suggests the degree of reorganization or perfection of starch crystallites upon hydrothermal treatment. Interestingly, β-amylolysis has a negligible effect on the onset and peak temperature, suggesting that the trimming of certain amylopectin external chains has a negligible impact on the amount of retained ordered structure.

The X-ray powder diffraction patterns of NCS, WS, and WCS along with their treated samples are shown in **Figure 4**. In general, the patterns are indicative of the semicrystalline nature of the samples. In the case of native starches, the diffraction patterns are mainly comprised of characteristic peaks observed at 11.2°, 15.0°, 17.0°, 18.8°, 20.0°, and 23.1° of 2θ. These reflections are indicative of A-type starch diffraction (*22*). It should be noted that these five peak intensities as well as the profile shapes are different for NCS, WS, and WCS, suggesting that the relative crystalline amount present in each of them is different. This observation is reflected by the calculated crystallinity values of 16.4, 13.2, and 20.4% for NCS, WS, and WCS, respectively (**Table 1**).

Though these three starches belong to A-type, the intrinsic variation in their amylose content culminates marked differences in the overall crystalline pattern upon hydrothermal treatment.

Table 1. Swelling Power, DSC Enthalpy Change, Onset and Peak Temperature, and X-ray Crystallinity of Treated Starches

			DSC			
		swelling power[a]	enthalpy change (J/g)	onset temp (°C)	peak temp (°C)	X-ray crystallinity (%)
normal corn starch						
native		2.0 ± 0.0 e	13.5 ± 2.1 a	65.5 ± 0.6 b	70.6 ± 0.4 b	16.4
2 h	NCS0-1	4.4 ± 0.0 b	3.6 ± 0.4 b	79.8 ± 0.1 a	83.9 ± 0.3 a	9.2
	NCS-1	4.4 ± 0.1 b	3.4 ± 0.4 b	79.8 ± 0.1 a	83.4 ± 0.3 a	9.7
10 h	NCS0-2	4.5 ± 0.0 c	3.0 ± 0.7 b	80.1 ± 0.3 a	83.5 ± 0.6 a	8.8
	NCS-2	4.3 ± 0.0 a	2.8 ± 0.4 b	80.3 ± 0.5 a	83.9 ± 0.6 a	10.5
24 h	NCS0-3	4.5 ± 0.0 d	2.4 ± 0.2 b	80.1 ± 0.4 a	83.4 ± 0.3 a	8.9
	NCS-3	4.0 ± 0.0 a	3.6 ± 0.2 b	79.8 ± 0.5 a	83.7 ± 0.5 a	10.0
wheat starch						
native		2.1 ± 0.0 d	5.8 ± 0.3 a	58.1 ± 0.5 d	62.4 ± 0.7 b	13.2
2 h	WS0-1	4.3 ± 0.0 c	1.5 ± 0.0 bc	77.2 ± 0.0 bc	81.0 ± 0.0 a	8.5
	WS-1	4.1 ± 0.0 a	1.9 ± 0.1 b	76.6 ± 0.4 c	81.4 ± 0.1 a	8.7
10 h	WS0-2	4.3 ± 0.0 bc	1.5 ± 0.4 bc	77.4 ± 1.0 ab	81.1 ± 0.4 a	8.5
	WS-2	4.1 ± 0.1 a	1.3 ± 0.2 c	77.0 ± 0.3 bc	81.2 ± 0.4 a	8.9
24 h	WS0-3	4.3 ± 0.0 b	1.1 ± 0.1 c	77.9 ± 0.5 a	81.1 ± 0.6 a	9.0
	WS-3	4.2 ± 0.0 a	1.4 ± 0.1 c	77.3 ± 0.4 ab	81.6 ± 0.1 a	8.5
waxy corn starch						
native		2.4 ± 0.1 e	11.9 ± 1.4 a	64.3 ± 0.7 d	69.9 ± 0.4 b	20.4
2 h	WCS0-1	3.7 ± 0.1 c	5.0 ± 0.2 d	77.4 ± 0.5 a	81.6 ± 0.4 a	12.2
	WCS-1	3.4 ± 0.1 b	7.6 ± 0.5 b	76.2 ± 0.2 c	81.6 ± 0.4 a	15.1
10 h	WCS0-2	4.0 ± 0.2 c	4.2 ± 0.2 de	77.4 ± 0.1 a	81.6 ± 0.0 a	12.3
	WCS-2	3.4 ± 0.0 a	6.2 ± 0.4 c	76.6 ± 0.1 bc	81.6 ± 0.2 a	14.7
24 h	WCS0-3	3.8 ± 0.0 d	4.1 ± 0.0 de	77.3 ± 0.1 ab	81.5 ± 0.2 a	12.4
	WCS-3	3.2 ± 0.1 ab	3.6 ± 0.4 e	77.2 ± 0.0 ab	81.2 ± 0.2 a	15.1

[a] Different letters show the significance of variance within columns for each starch type ($\alpha<0.05$) ($n = 3$).

For NCS and WS, a new peak around 13.0° has been observed. In addition, the 20.0° peak intensity has increased significantly. These two intensity bands mainly correspond to the V-type starch, as observed in several amylose−ethanol or amylose−lipid complexes (*23*, *24*). Lack of amylose in WCS seems to preclude the formation of V-type starch (**Figure 4c**). It appears that V-areas of treated normal corn starch are comparatively less than those of wheat starch, whereas the A-areas show an opposite trend. Overall, hydrothermal treatment substantially reduces the amount of A-type crystallites with a concomitant increase of V-type in amylose-containing starches.

The crystallinity of three starches is greatly affected by the hydrothermal treatment and β-amylolysis (**Figure 5** and **Table 1**). The crystallinity decreased by about 45%, 35%, and 60%, for NCS, WC, and WCS, respectively, upon hydrothermal treatment. Subsequent β-amylolysis increases the WCS crystallinity by about 25%, while the enzyme effect is somewhat mild in the case of NCS with an about 10% increase. On the other hand, WS did not show appreciable crystallinity variation upon β-amylolysis. All these effects could be intricately related to the amylose content and amylopectin fine structure in each starch type.

The DSC and X-ray analyses demonstrate the impact of hydrothermal treatment on the physical change of starches. The enthalpy change originates from both short-range ordered structure (e.g., double helices) and long-range ordered structure (e.g., crystallites), whereas the X-ray diffraction detects crystallinity. It has been established that for retrograded starches the level of crystallinity was much different from that of double helical order (*25*). By comparing the abundance of the retained DSC enthalpy change and crystallinity during processing, the nature of chain reorganization can be further understood. As shown in **Figure 5**, such a comparison indicates that the ratio of retained crystallinity (RRC) is always higher than the ratio of retained enthalpy change (RRE). For NCS, the RRE ranges from 17.8 to 26.7%, whereas the RRC ranges from 53.7 to 64.0%. For WS these values are from 19.0 to 32.8% and from 64.4 to 68.2%, for enthalpy change and crystallinity, respectively. Similarly, for WCS, they range from 30.3 to 63.9% and from 59.8 to 74.0%, respectively, in the same order. These results clearly indicate that the noncrystalline ordered structures in starch, such as the noncrystalline double helices, are preferentially removed by the hydrothermal treatment.

Swelling Power and Pasting Behavior. Table 1 shows the swelling power of treated starches and their native counterparts. The swelling powers of native NCS, WS, and WCS are 2.0, 2.1, and 2.4, respectively. The hydrothermal treatments and β-amylolysis have increased the swelling power to a range from 4.0 to 4.5 for NCS, from 4.1 to 4.3 for WS, and from 3.2 to 4.0 for WCS. Overall, the swelling is somewhat lower with β-amylolysis than that of hydrothermal treatment only. Perhaps the β-amylolysis reduces the water-retaining capability of external chains in amorphous regions.

The RVA pasting behavior of all these treated starches is compared in **Figure 6**. The negligible viscosity development for treated starches at the initial stage is consistent with the low swelling power of these materials. In addition, the phase for undetectable viscosity of treated starches, or their pasting temperature, is extended compared with that of native starches. For NCS, the pasting temperature of treated material is around 87 °C, 10 deg higher than that of native starch, 77 °C. For treated WCS, the pasting temperature is around 86 °C, 16 deg higher than native starch, 70 °C. For WS, the pasting temperature is around 89 °C, 15 deg higher than native starch, 74 °C. Before the pasting point, the viscosity is undetectable. For all starches subjected to hydrothermal treatment, the temperature for the peak viscosity is higher than that of native starch. In addition, more stabilized pasting behaviors are observed for modified NCS and WS compared with their native starches, which is reflected by their much reduced breakdown and setback. In contrast, for WCS, the reduction of breakdown is proportional to the reduction of peak viscosity, and the setback is negligible for both treated and untreated samples, which can be attributed to the lack of amylose.

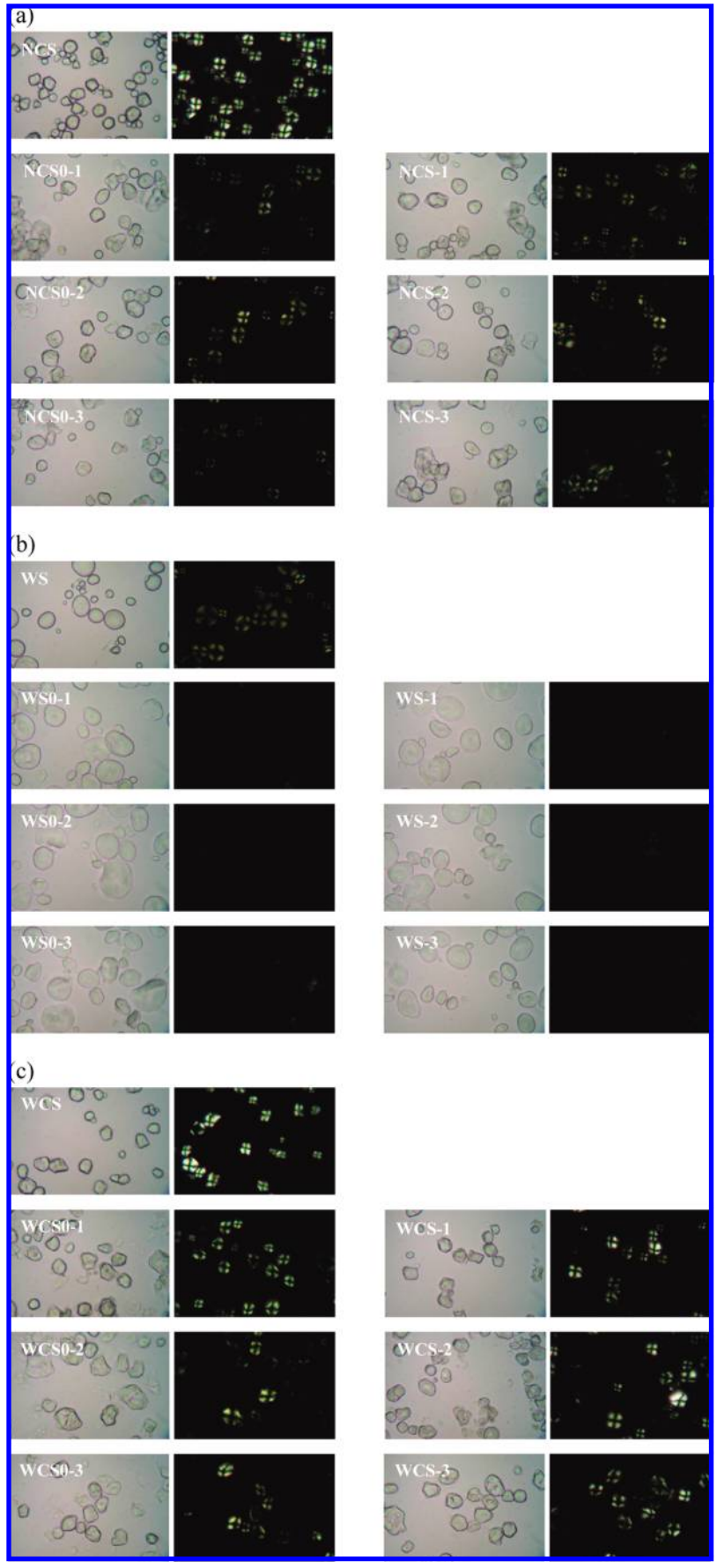

Figure 3. Regular optical microscopic images (labeled) and the same-view polarized light microscopic images (on the right side of the labeled images) for individual starch samples (native and treated) of normal corn starch (**a**), wheat starch (**b**), and waxy corn starch (**c**).

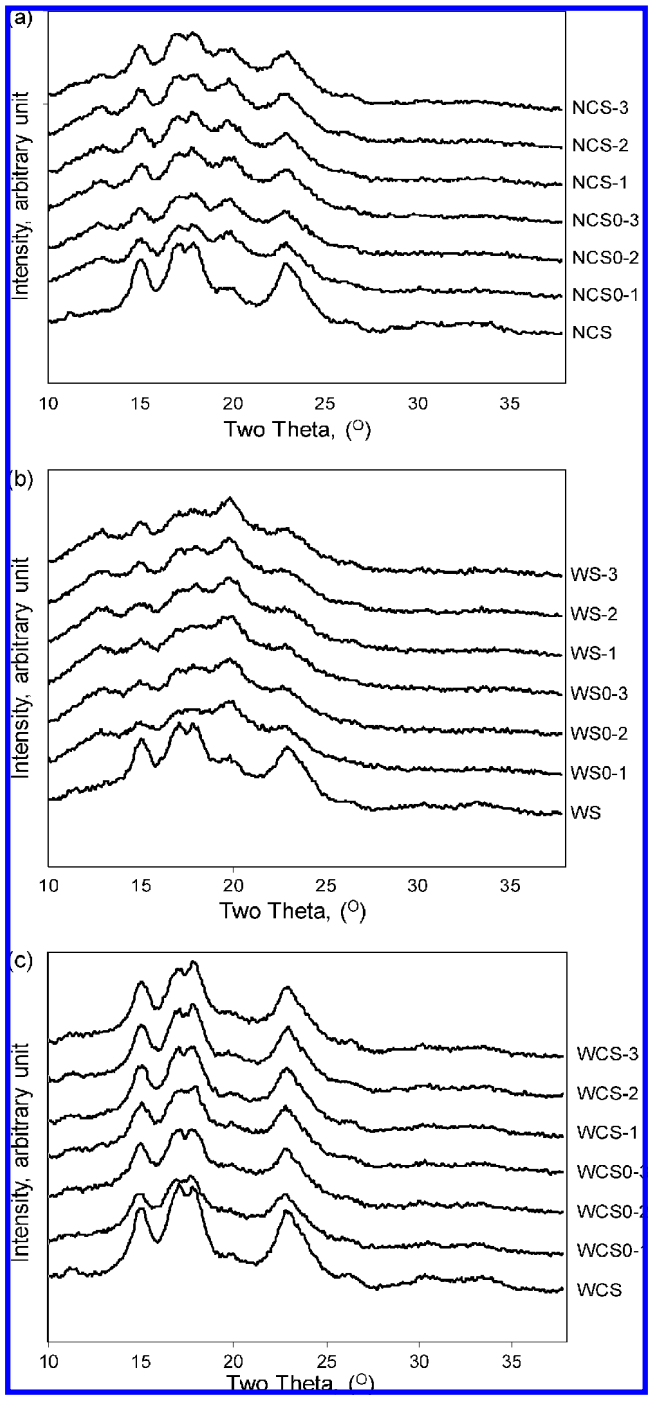

Figure 4. X-ray powder diffraction patterns of native and treated normal corn starch (**a**), wheat starch (**b**), and waxy corn starch (**c**).

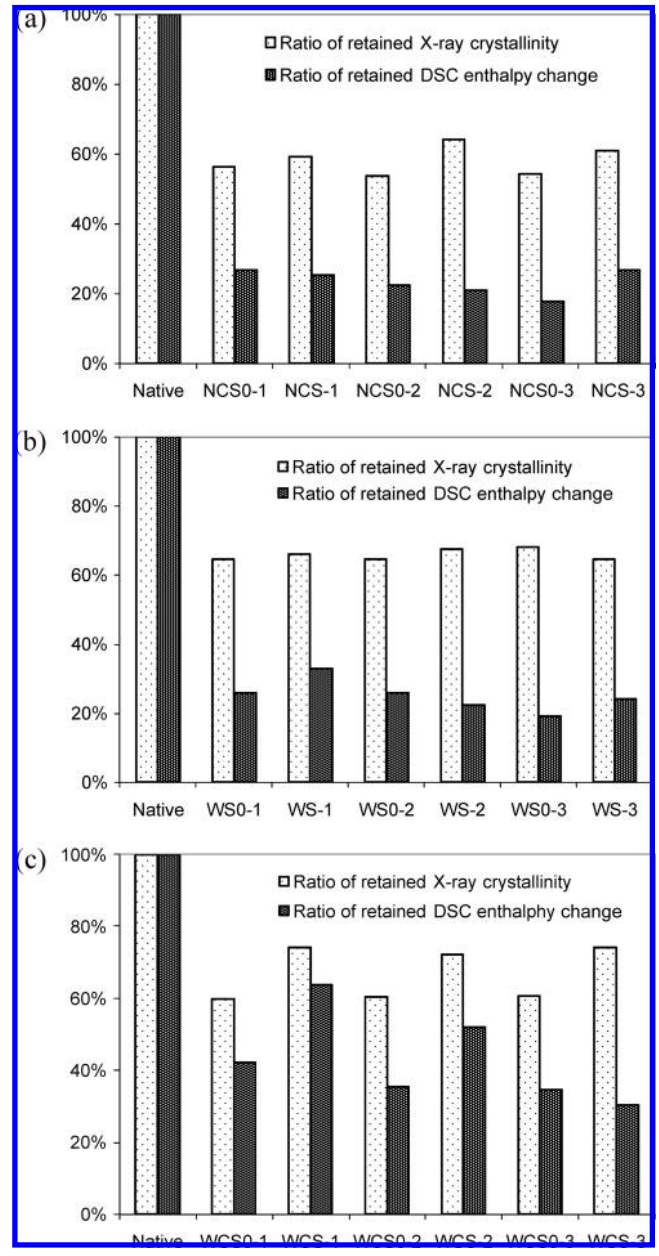

Figure 5. Ratio of retained X-ray crystallinity and DSC enthalpy change after hydrothermal and enzymatic treatment of normal corn starch (**a**), wheat starch (**b**), and waxy corn starch (**c**).

DISCUSSION

Most works of hydrothermal treatments have been related to annealing and heat−moisture treatments (*14*, *26*). Both treatments have been broadly studied; however, there is no report on using hydrothermal treatment to deliver partial gelatinization for enzymatic starch modification. In this study, the conditions (gradual heating up to 95 °C at 40% moisture content) applied in the hydrothermal treatment have been employed to improve starch susceptibility to β-amylase. The treatment was delivered to ensure partial gelatinization while maintaining the granular integrity of starch.

The hydrothermal treatment applied in this study led to a reduced pasting viscosity for starch. Such a reduction has been observed in the presence or absence of amylose, and the pasting behavior of amylose-containing starches seems to be more sensitive to hydrothermal treatments (**Figure 6a**, **b**). For WCS, which lacks amylose, the hydrothermal treatment leads to reduced pasting viscosity (**Figure 6c**), reduced DSC enthalpy change and crystallinity (**Figure 5c**), as well as increased gelatinization temperatures (**Table 1**). These results suggest an increased resistance of WCS granules to heat and shear even at reduced chain organization. Such a behavior suggests that the hydrothermal history applied might have resulted in more effective intragranular interactions due to a reorganization or redistribution of retained A-type crystallites.

The presence of amylose in NCS and WS adds to the impact of hydrothermal history on pasting behaviors. Similar to WCS, the hydrothermal treatment reduces the DSC enthalpy change and X-ray crystallinity associated with amylopectin and leads to more effective glucan chain interactions inside the granules. A more important factor, however, is related to the retrogradation of amylose. As shown in **Figure 4a**, **b**, the reduction of

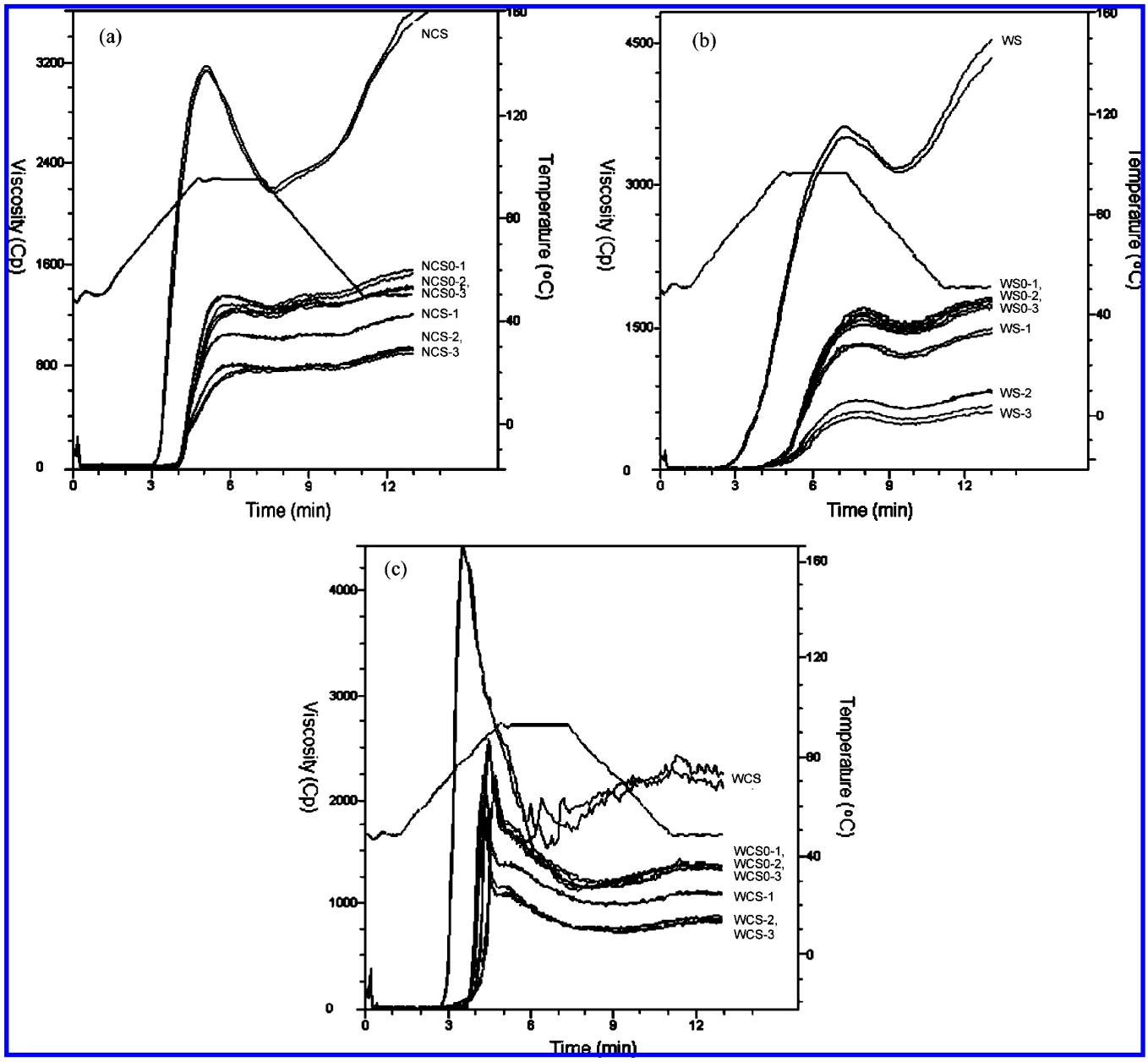

Figure 6. RVA amylograph of treated normal corn starch (**a**), wheat starch (**b**), and waxy corn starch (**c**) as compared with their native counterparts.

A-type structure is accompanied by an increased V-type amount, which clearly indicates the formation of amylose-based crystallinity. This effect is more prominent for WS, in which the A-type structural organization is almost lost due to hydrothermal treatment.

We consider that the granule-strengthening effect of junction zones related to amylose crystalline structure is longer in range compared with that of amylopectin. Such an effect may lead to a considerable increase of granular integrity against heat and shear during pasting. The much reduced breakdown observed for hydrothermally treated NCS and WS is shown in **Figure 6a**, **b**, highlighting the importance of amylose-related glucan association on granular resistance to heat and shear. In addition, the setback for both NCS and WS was much reduced, suggesting a reduced interaction among starch granules, their disrupted pieces, and leached amylose during cooling. Conceivably, the intergranular amylose chain association occurs far less intensively, simply due to an already formed amylose network inside the granules.

Compared to hydrothermal treatment, additional β-amylolysis does not seem to change the RVA pasting temperature. However, it leads to a further reduction of viscosity all through the RVA profile. We believe that the shortening of amylopectin external chains is the primary factor for such reduction, as previously indicated by Dahle et al. (*27*). In the case of WCS, the generation of short stubs by β-amylolysis reduces the interaction among individual granules or their disrupted pieces. For all types of starch, the prolonged β-amylolysis results in further reduced viscosity. It is interesting that a small population of short stubs (**Figure 2**) can lead to such a drastic change of viscosity. Perhaps the short stubs mostly distribute on the granule surface due to a preferential β-amylolysis on the surface of partially gelatinized starches, and these short stubs could have much greater impact on intergranular interactions than those distributed inside the granules.

Our study shows that hydrothermal treatment can be used to enhance the susceptibility of starch to enzymatic modifications for altered starch structure and functionality. To tailor the

properties of starch materials, the complexity involved in a variety of hydrothermal and enzymatic modifications should be understood, and the current study will shed some light on the relationship among the structure, chain organization, and pasting behavior of starch-based materials. It also provides a methodology of using nonchemical modifications to realize stabilized pasting behavior, i.e. reduced breakdown and setback for more predictable behaviors of starch.

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