

# Functional Properties of Cross-Linked and Hydroxypropylated Waxy Hull-less Barley Starches

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## ABSTRACT

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Waxy hull-less barley (HB) starches containing 0 or 5% amylose were cross-linked with phosphorus oxychloride and the cross-linked starches were hydroxypropylated with propylene oxide. For comparison, waxy corn and potato starches were similarly modified. For all starches, cross-linking inhibited granule swelling and prevented swollen granules from disintegration, resulting in dramatic improvement in pasting properties and tolerance to cooking shear and autoclaving. Cross-linked waxy HB starches were more tolerant to cold storage and cooking shear than cross-linked waxy corn starch. Hydroxypropylation of the cross-linked starches reduced granule crystallinity and gelatinization temperature, and improved granule

swelling, paste clarity, and freeze-thaw stability. The double-modified waxy HB starches showed higher cold tolerance than similarly modified waxy corn and potato starches, as judged by freeze-thaw stability and clarity after cold storage. These results indicated that the cross-linked and double-modified waxy HB starches together may have a wide range of food applications. This study indicated that the behavior of granule swelling and disintegration of swollen granules played an important role in governing paste viscosity, clarity, and freeze-thaw stability of waxy HB starches.

Thickening and stabilization are important functional properties of food starches. Although waxy cereal starches are more tolerant to cold storage than nonwaxy cereal and root and tuber starches (Zheng and Sosulski 1998), the granules are readily ruptured during cooking, resulting in low paste viscosity and pastes with a “long” cohesive nature. Native starches are also sensitive to shear, high temperature, and acid treatment when cooked in water. Therefore, starches used in food industry are often modified to improve their functionality, and cross-linking is the most widely used technology for this purpose. Cross-linked waxy cereal starches generally show a “short” spoonable texture, higher paste stability, and resistance to cooking shear, temperature, and low pH as compared to native starches (Whistler and BeMiller 1997). However, cross-linking reduces paste clarity and stability to cold storage. These undesirable characteristics can be improved by further modifications such as esterification or etherification. Although waxy corn is used as the major modified food starch in North America, Wu and Seib (1990) reported that hydroxypropylated distarch phosphate from waxy barley showed higher freeze-thaw stability when compared to commercial samples of similarly modified waxy corn and tapioca starches. Bhatt and Rosnagel (1997) reported lines of waxy hull-less barley (HB) that were completely devoid of amylose. The zero-amylose HB starch showed higher paste clarity and freeze-thaw stability than waxy HB, corn, and potato starches (Zheng et al 1998). The zero-amylose HB starch may be modified by cross-linking to improve its paste properties while maintaining clarity and freeze-thaw stability.

This study reports the effect of cross-linking on the properties of waxy HB starches containing 0 or 5% amylose, and compares functionality of the modified waxy HB starches with those of similarly modified waxy corn and potato starches. Only a few studies have reported on modification of waxy HB starches (Vasanthan et al 1997), and as far as we are aware, none on zero-amylose HB starch, which has become available only recently (Bhatt and Rosnagel 1997). The ultimate goal of this study is to promote use of zero-amylose HB starch in food and industry applications.

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## MATERIALS AND METHODS

### Materials

Waxy HB SB94794 (0% amylose) and CDC Candle (5% amylose), obtained from B. Rosnagel, were grown in 1996 at the Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, SK. Commercial native and modified (cross-linked and hydroxypropylated) waxy corn starches were obtained from National Starch and Chemical Co., Bridgewater, NJ. Potato starch (S-4251, Lot 106-H112) was purchased from Sigma Chemical Co., St. Louis, MO.

### Starch Isolation and Modification

Starch was isolated from SB94794 and CDC Candle HB by the enzyme-assisted wet-extraction procedure described by Zheng and Bhatt (1998). The method of Wu and Seib (1990) was used to modify the starches. In preliminary experiments, paste viscosity of commercially cross-linked and hydroxypropylated waxy corn starch was used as the criterion to determine optimal conditions for cross-linking and hydroxypropylation. Subsequently, 0.005% or 0.01% of phosphorus oxychloride (POCl<sub>3</sub>) and 10% of propylene oxide were used to modify the starches.

For cross-linking, starch (200 g, db) was suspended in 370 mL of distilled water containing 10 g of Na<sub>2</sub>SO<sub>4</sub>. After adjusting to pH 10.5 with 0.1M NaOH, 10 or 20 µg of phosphorus oxychloride was added to the slurry using a microsyringe. The reaction mixture was stirred at 40°C for 2 hr, then adjusted to pH 6–6.5 with 1N HCl solution. Modified starch was recovered by centrifugation and washing with distilled water. It was then dried at ambient temperature.

For hydroxypropylation, starch slurry was centrifuged after reacting with POCl<sub>3</sub>, and the solid was resuspended in 170 mL of distilled water containing 2 g of NaOH and 24 g of Na<sub>2</sub>SO<sub>4</sub> in a 500-mL polypropylene centrifuge bottle. After replacing the air with nitrogen gas, 20 g of propylene oxide was added to the mixture and the bottle was screw-capped. The mixture was then shaken in a 40°C water bath for 24 hr. Starch was recovered in the same manner as for cross-linked starch. Preliminary experiments indicated that cross-linked waxy HB starches showed acceptable freeze-thaw stability. Similarly modified waxy corn starch was used for comparison. Cross-linked potato starch showed poor freeze-thaw stability in a preliminary study. Therefore, potato starch was cross-linked and hydroxypropylated. Reagent usage for the modification of starches is given in Table I.

### Starch Paste Preparation at Various Cooking Conditions

*Amylograph pasting.* Starch slurries (6% db, w/v, 500 mL, pH 5.5) were heated in a Brabender ViscoAmylograph (C. W. Brabender

Instruments, Inc., South Hackensack, NJ) at a shear rate of 75 rpm and a torque of 700-cm/g, from 30 to 95°C at 1.5°C/min, held at 95°C for 30 min, cooled to 50°C at 1.5°C/min, and then maintained at 50°C for 30 min. Starch paste was collected after the completion of the cycle for determinations of free water and net syneresis after freezing and thawing.

**Normal cooking.** Starch slurry (100 mL, 6% db) contained in a sealed glass bottle was heated in a boiling water bath for 10 min. The slurry was continuously stirred using an overhead mixer at 250 rpm during heating. The cooked starch paste was cooled in a 30°C water bath for 30 min, and paste viscosity determined in a Brookfield viscometer (model LVF) at 30°C (before autoclaving). This paste sample was then subjected to autoclaving at 120°C for 20 min, and its viscosity was determined again after holding in a water bath at 30°C for 30 min (after autoclaving). The experiment was repeated twice.

**High-shear cooking.** Starch slurry (100 mL, 6% db) contained in a sealed 400-mL stainless steel chamber equipped with a mixing blade (Omni-Mixer, Ivan Sorvall, Norwall, CT) was heated in a boiling water bath for 10 min with a mixing speed of 2,700 rpm to generate mechanical cooking shear. The pastes were then treated in the same manner as for normal cooking. The experiment was also repeated twice.

**Analyses**

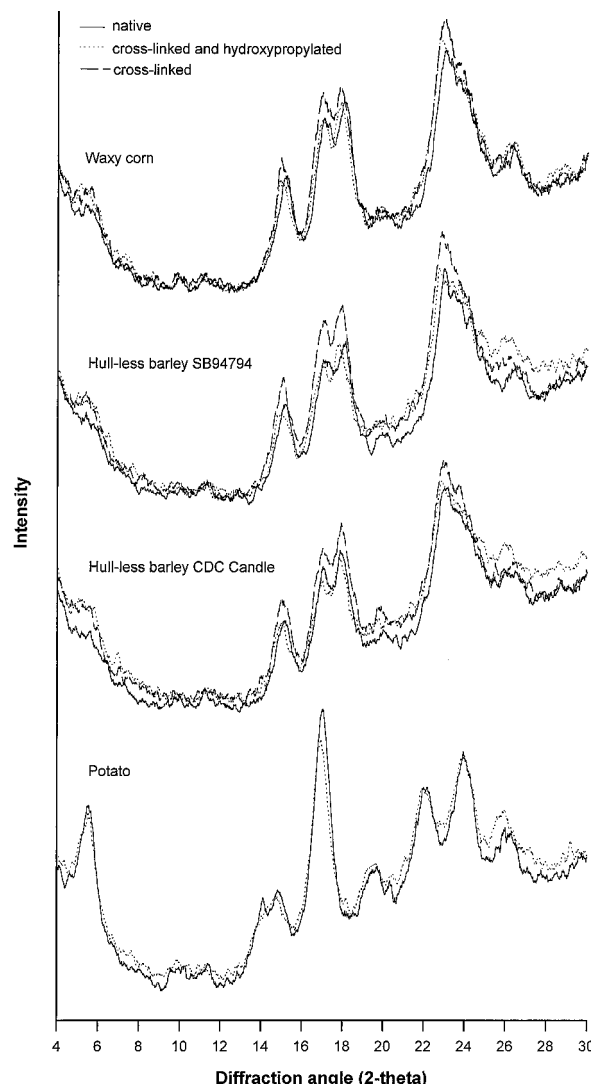
Total nitrogen in starch samples was determined according to Approved Method 46-11A (AACC 1995) for the calculation of protein ( $N \times 6.25$ ). Amylose was estimated by the method of Gibson et al (1997). Total starch was determined by the method of Holm et al (1986) after boiling the sample with 80% ethanol for 30 min and centrifuging at  $2,000 \times g$  for 10 min. Paste clarity was determined on 1–4% pastes either fresh or after one week of storage at 4°C by the method of Craig et al (1989) modified by Zheng et al (1998). Free water was determined on 2–2.5 g of fresh paste according to the procedure reported by Zheng and Sosulski (1998). The paste ( $\approx 60$  g) was then contained in 70-mL bottles and subjected to freeze-thaw procedures. Each freeze-thaw cycle consisted of a 16 hr of storage at  $-18^\circ\text{C}$  (freezer), followed by 2 hr of thawing at 40°C. After determination of net syneresis (Zheng and Sosulski 1998), the paste was refrozen and thawed to repeat the cycle up to 10 cycles. Differential scanning calorimetry (DSC) was performed (TA 400, Mettler) by heating starch samples (5.5 mg of starch and 21  $\mu\text{L}$  of water) from 20 to 120°C at 10°C/min (Zheng et al 1998). Wide-angle powder X-ray diffraction patterns of water-saturated starches were obtained with an X-ray diffractometer (model 42273, Phillips) (Zheng et al 1998). DSC, X-ray, and amylograph tests were performed in duplicate while all other determinations were done in quadruplicate. Results were subjected to analysis of variance, using Minitab statistical software (State College, PA).

**TABLE I**  
**Reagent Used to Prepare Modified Waxy Corn, Waxy Hull-less Barley (HB), and Regular Potato Starches**

Starch Modification	Reagent (% starch, dwb)	
	POCl <sub>3</sub>	Propylene Oxide
Waxy corn		
Cross-linking	0.005	...
Cross-linking and hydroxypropylation	0.005	10.0
HB SB94794		
Cross-linking	0.005	...
Cross-linking	0.010	...
Cross-linking and hydroxypropylation	0.010	10.0
HB CDC Candle		
Cross-linking	0.005	...
Cross-linking and hydroxypropylation	0.005	10.0
Potato		
Cross-linking and hydroxypropylation	0.005	10.0

**Physicochemical Properties**

All starch samples contained >98% starch and <0.4% protein ( $N \times 6.25$ ). Amylose content was 1.1% for waxy corn, 0% for SB94794, 4.5% for CDC Candle, and 22% for potato starch. X-ray diffractometry showed that waxy HB and waxy corn starches had typical A-type, whereas potato starch showed typical B-type polymorphic forms (Fig. 1). Cross-linking and hydroxypropylation had no effect on the X-ray diffraction patterns of the starches, indicating that the repeat distance of crystalline and amorphous lamellae of starch granules was not affected by the modifications. However, cross-linked starches showed more pronounced peaks than the native starches. When the cross-linked starches were hydroxypropylated, their peak intensities were reduced. Relative crystallinity, calculated as the ratio between crystalline and amorphous regions of X-ray diffractograms (Nara and Komiya 1983), was higher for cross-linked starches and lower for the double-modified starches than for the native starches (Table II). Muhrbeck et al (1991) reported that esterification (phosphorylation) reduced crystallinity of potato starch. The present study indicated that etherification (hydroxypropylation) lowered crystallinity in waxy HB, waxy corn, and normal potato starches. Unlike the monoderivatives, where single ester or ether groups (phosphoryl or hydroxypropyl) are attached to anhydro-



**Fig. 1.** Wide-angle powder X-ray diffraction pattern of water-saturated samples ( $\approx 50\%$  moisture) of native and modified waxy corn, waxy hull-less barley, and regular potato starches.

glucose units of starch molecules, cross-linking binds starch molecule chains together through double esterification. Jane et al (1992) reported that cross-linking preferentially occurred to amylopectin, the component responsible for crystalline structure of starch

granules (Zoble 1988). The reinforcement of amylopectin chains induced by cross-bonding may be partially responsible for the increase in crystallinity of cross-linked starches. Because the crystallinity was calculated based on the ratio between crystalline and amorphous regions of starch granules, a higher electron density contrast between the regions may also have resulted in higher crystallinity (Jacobs and Delcour 1998). There may be a need for detailed study to explain why simple etherification or esterification reduced crystallinity and cross-linking increased crystallinity of starch granules. Nevertheless, it is generally recognized that starch granules with higher crystallinity show correspondingly higher gelatinization temperature (Zobel 1988). DSC data indicated that cross-linked starches showed higher gelatinization temperatures and enthalpies when compared to native starches. Gelatinization temperature range of the cross-linked starches was similar to that of native starches. Compared to native starches, hydroxypropylated samples, after cross-linking, showed lower gelatinization temperature and endothermal enthalpy, and wider gelatinization temperature range.

Figure 2 shows amylograph pasting curves of native and modified starches. Potato starch is known for its high peak viscosity and rapid losses of viscosity on continued heating and at low pH levels (Zoble 1984). The peak viscosity of native potato starch, however, showed a considerable variation in the published data (Zoble 1984, Swinkels 1985, Wurzburg 1986, Kim et al 1993). The present study showed  $\approx 1,400$  BU for a 6% slurry at pH 6.5, which was lower than expected values. The variation in peak viscosity is probably due to the differences in the content of phosphate-monoesters, which varies considerably according to potato varieties and growing conditions (Bay-Smidt et al 1994). Phosphorylation is known to largely affect paste viscosity, and therefore, potato starch with different degrees of phosphorylation may show different pasting curves.

It was generally recognized that cross-linking increased, and hydroxypropylation decreased pasting temperature at which 10 BU of viscosity was observed (Fig. 2). Compared to native starches, peak viscosity of waxy corn and CDC Candle starches was increased and that of zero-amylose HB starch was decreased after cross-linking. All native starches had a large breakdown of viscosity on heating at 95°C for 30 min, which was diminished after cross-linking. On amylograph heating, starch granules undergo the process of swelling and disintegration of swollen granules. For a given starch, this process varies considerably among individual granules, and the peak viscosity observed in amylography indicates when the majority of starch granules reach their maximum swelling, after which the swollen granules collapse. Our previous study (Zheng

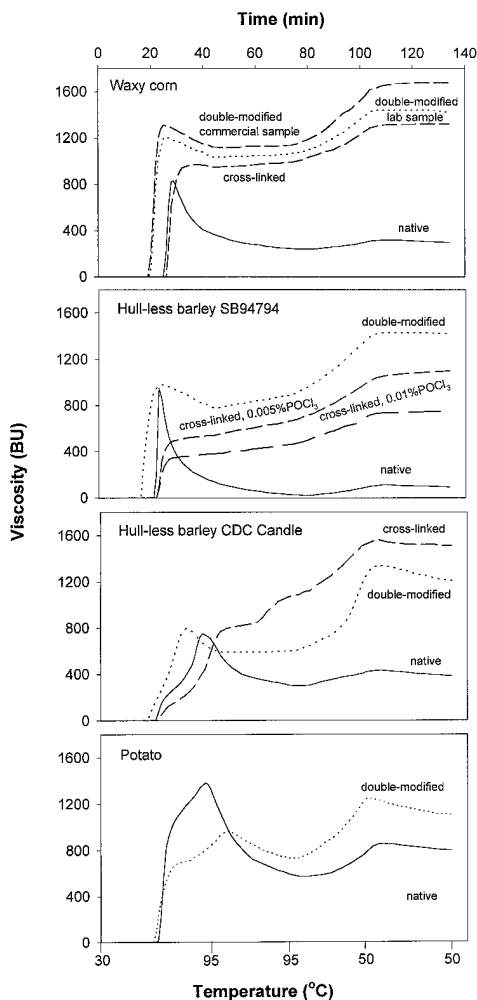


Fig. 2. Amylograph of native and modified waxy corn, waxy hull-less barley, and regular potato starches.

TABLE II  
X-ray and Differential Scanning Calorimetry<sup>a</sup> Characteristics of Modified Waxy Corn, Waxy Hull-less Barley (HB), and Regular Potato Starches

Starch	Relative Crystallinity (%)	$T_p$	$T_o$	$T_c$	$T_c - T_o$	Enthalpy (J/g)
Waxy corn						
Native	40	69.8	62.0	82.0	20.0	17.0
Cross-linked	41	71.5	63.0	82.0	19.0	17.3
Cross-linked and hydroxypropylated	39	65.8	56.1	82.0	25.9	15.0
LSD <sup>b</sup>	0.8	0.8	0.7	0.1	0.5	0.4
HB SB94794						
Native	37	63.2	57.0	77.0	20.0	13.0
Cross-linked (0.005% POCl <sub>3</sub> )	39	64.9	56.0	76.9	20.9	16.6
Cross-linked (0.010% POCl <sub>3</sub> )	41	65.3	56.5	78.7	22.2	16.1
Cross-linked and hydroxypropylated	32	59.1	51.0	74.4	23.4	9.8
LSD	0.8	0.7	0.6	0.8	0.6	0.5
HB CDC Candle						
Native	35	63.5	57.5	77.5	20.0	14.0
Cross-linked	40	65.0	57.0	78.0	21.0	15.6
Cross-linked and hydroxypropylated	31	58.7	51.0	73.2	22.2	11.4
LSD	0.7	0.9	0.8	0.7	0.7	0.8
Potato						
Native	42	65.0	55.8	79.0	23.2	19.5
Cross-linked and hydroxypropylated	39	59.6	51.0	78.0	27.0	14.1
LSD	0.8	0.8	0.5	0.5	0.7	1.2

<sup>a</sup> Gelatinization temperatures:  $T_p$  (peak),  $T_o$  (onset), and  $T_c$  (completion).

<sup>b</sup> Least significant difference ( $P < 0.05$ ).

et al 1998) showed that granule swelling of native waxy corn and CDC Candle starches occurred in a much wider temperature range than zero-amylose HB starch, indicating that a larger variation existed among the granules of waxy corn and CDC Candle starches than in the zero-amylose HB starch. It could be assumed that some granules of waxy corn and CDC Candle starches swelled and ruptured before reaching peak viscosity, and cross-linking minimized the rupture of these granules, resulting in increased peak viscosities. Decreased peak viscosity of cross-linked zero-amylose HB starch was indicative of inhibited swelling of the majority of its granules. For zero-amylose HB starch, the inhibition of swelling was proportional to the amount of cross-linking reagent used.

Hydroxypropylation further increased peak viscosity in waxy corn starch. The peak viscosity of the double-modified HB starches was similar to that of native starches (Fig. 2). This result suggested that hydroxypropylation enhanced granule swelling of waxy corn starch but had little effect on waxy HB starches. In contrast, the double-modified potato starch showed lower peak viscosity than its native parent, indicating that hydroxypropylation partially improved granule swelling of cross-linked potato starch. Compared to cross-linked starches, hydroxypropylation slightly reduced the integrity of swollen granules, resulting in increased breakdown of viscosity. These results suggested that the behavior of granule swelling and disintegration of swollen granules during heating played an important role in governing pasting properties of the starches. Compared to native starches, the modified starches showed higher setback viscosities, indicating higher degrees of starch molecule reassociation during cooling. Pastes of the modified starches showed a "short" texture, while all native starches exhibited a "long" cohesive texture. The improvement of paste texture was of particular importance in food applications (Wurzburg 1986).

### Paste Clarity

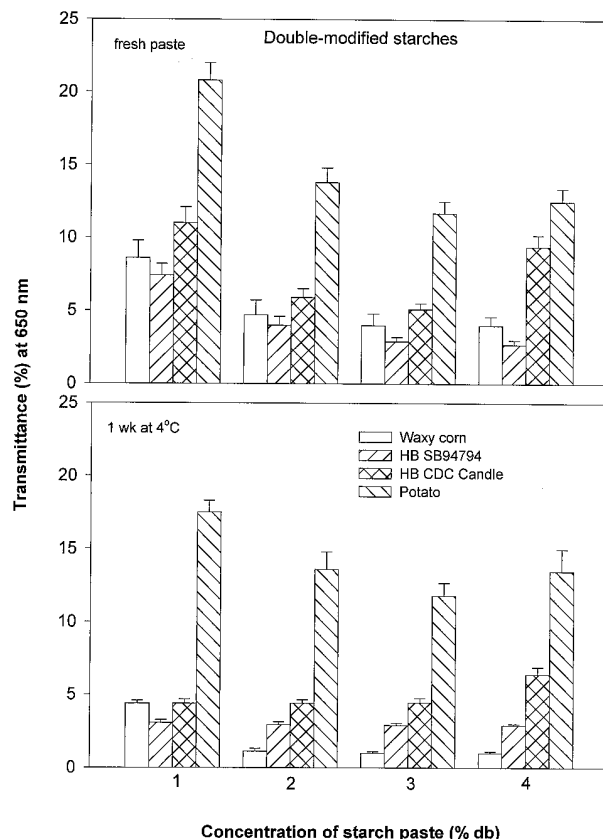
In a previous study, it was found that paste clarity of native waxy HB starches, particularly the zero-amylose HB starch, was higher than those of waxy corn and potato starches, especially after cold storage (Zheng et al 1998). Because paste clarity was the result of rupture of swollen starch granules (Craig et al 1989) and cross-linking improved the integrity of swollen granules, this modification markedly reduced paste clarity of waxy HB and corn starches (Table III). Despite of a large variation observed in fresh pastes of the native starches, little difference was found among cross-linked waxy starches. Hydroxypropylation improved paste clarity of cross-linked waxy HB starches (compare Fig. 3 and Table III). Paste clarity of double-modified HB CDC Candle and potato starches was higher than those of zero-amylose HB and waxy corn starches. This could be explained by the fragility of swollen granules. Craig et al (1989) reported that starch pastes with more disintegrated granules gave higher light transmittance than those with more remnants of granules. On amylograph pasting (Fig. 2), double-modified CDC Candle

and potato starches showed larger viscosity breakdown during heating at 95°C, as well as during the maintaining period at 50°C, than did similarly modified zero-amylose HB and waxy corn starches. This result indicated that the swollen granules of double-modified CDC Candle and potato starch were more fragile than those of double-modified zero-amylose HB and waxy corn starches. The difference in fragility of swollen granules among the modified starches may be related to their different amylose contents. Jane et al (1992) reported that cross-linkage between amylopectin and amylose could also occur although amylopectin chains were preferentially cross-linked. Because granule swelling, and therefore the disintegration of swollen granules, are primarily the properties of amylopectin molecules, and amylose acts as a diluent (Tester and Morrison 1990), it is reasonably hypothesized that the amylose-amylopectin linkage in CDC Candle and potato starch does not impart the same force of holding granules unbroken during heating as the amylopectin-amylopectin linkage in zero-amylose HB and waxy corn starches. The cross-linkage between amylose and amylopectin may also explain the slower deterioration of clarity during cold storage for double-modified potato and CDC Candle starches than for similar zero-amylose HB and waxy corn starches. For potato and CDC Candle starches, amylose reassociation was the primary reason for the deterioration of clarity (Zheng et al 1998). Cross-linking between amylose and amylopectin reduced the chance for amylose molecules to reassociate during cold storage, therefore they maintained relatively high clarity (Fig. 3). For starches lacking amylose, the degree of amylopectin reassociation was dependent on the branched chain length (Ring et al 1987). Average chain length was smaller for barley starches (MacGregor and Morgan 1984) than corn starches (Hizukuri 1985), therefore, the reassociation of amylopectin molecules of double-modified zero-amylose HB starch was slower than that of similar waxy corn starch, giving higher paste clarity after cold storage.

**TABLE III**  
Clarity (% light transmittance at 650 nm) of Fresh Pastes of Native and Cross-Linked Waxy Corn and Hull-less Barley (HB) Starches

Starch	Concentration (% db)			
	1	2	3	4
Native				
Corn	28.7	32.0	42.0	42.5
HB SB94794	25.0	47.2	60.2	76.0
HB CDC Candle	18.1	15.7	13.2	11.2
LSD <sup>a</sup>	3.0	3.9	3.8	2.3
Cross-linked				
Corn (0.010% POCl <sub>3</sub> )	7.2	3.4	2.6	2.4
HB SB94794 (0.005% POCl <sub>3</sub> )	5.7	3.2	2.6	2.1
HB SB94794 (0.010% POCl <sub>3</sub> )	4.3	2.6	1.9	1.5
HB CDC Candle (0.010% POCl <sub>3</sub> )	7.7	3.8	2.5	2.2
LSD	0.6	0.5	0.3	0.3

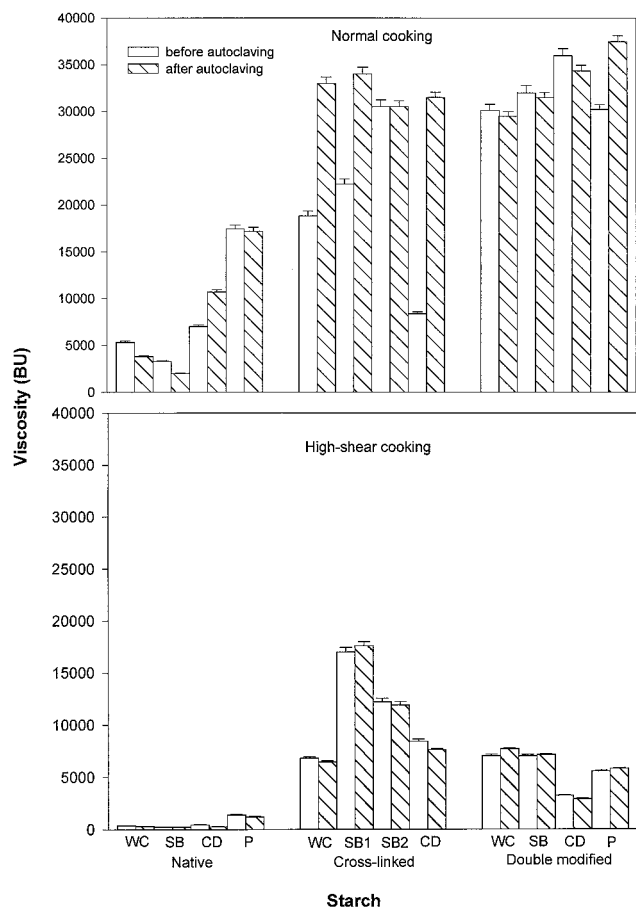
<sup>a</sup> Least significant difference ( $P < 0.05$ ).



**Fig. 3.** Clarity of freshly cooked or refrigerated waxy corn, waxy hull-less barley, and regular potato starches after cross-linking and hydroxypropylation.

## Viscous Properties After Different Cooking Treatments

**Normal cooking and autoclaving.** As shown in Fig. 4, native potato starch had the highest viscosity after normal cooking while native zero-amylose HB starch exhibited the lowest viscosity after normal cooking. This was in agreement with the amylograph data



**Fig. 4.** Brookfield viscosity of native and modified waxy corn (WC), waxy hull-less barley SB94794 (SB) and waxy hull-less barley CDC Candle (CD), and potato (P) starches prepared by normal cooking or high-shear cooking before and after autoclaving. SB1, HB SB94794 cross-linked with 0.01%  $\text{POCl}_3$ ; SB2, HB SB94794 cross-linked with 0.005%  $\text{POCl}_3$ .

(Fig. 2). Autoclaving the cooked starch paste had little effect on paste viscosity in native potato starch (Fig. 4). This stability may be due to a higher content of phosphate-monoesters than that of the waxy cereal starches (Jane et al 1996). For native CDC Candle HB starch, autoclaving increased paste viscosity by 53%, indicating an improvement of granule swelling due to autoclaving. In contrast, autoclaving reduced paste viscosity by 28 and 39% in native waxy corn and zero-amylose HB starches, showing that native waxy corn and zero-amylose HB starches were not resistant to autoclaving. Compared to the native starches, cross-linking significantly ( $P < 0.05$ ) increased paste viscosity particularly for waxy corn and zero-amylose HB starches. Autoclaving further increased viscosity of cross-linked waxy corn and HB starches. For nonautoclaved sample, hydroxypropylation increased viscosity of the cross-linked starches by 60% for waxy corn, 44% for SB94794, and 330% for CDC Candle HB starches. Viscosity of double-modified potato starch was 73% higher than native potato starch. Autoclaving had little effect on viscosity for double-modified waxy corn and HB starches and increased viscosity in similarly modified potato starch. The increased viscosity after cross-linking could be explained by reinforcement of starch molecule chains which prevented swollen granules from breakdown (Fig. 2). In contrast, autoclaving improved swelling of inhibited granules in the cross-linked starches, resulting in higher paste viscosity. Increased viscosity in hydroxypropylated samples, however, was ascribed to the introduction of hydrophilic groups (hydroxypropyl molecules) which helped granule to swell. When the normally cooked pastes were subjected to autoclaving, all modified (cross-linking alone or in combination with hydroxypropylation) samples showed virtually similar viscosities (Fig. 4).

**High-shear cooking and autoclaving.** Compared to normal cooking (Fig. 4) high-shear cooking significantly ( $P < 0.01$ ) reduced paste viscosity in all starches. All native starches lost  $\approx 90\%$  or more of their viscosities after being cooked with high shear. These results were indicative of fragility of swollen native starch granules that easily broke down under mechanical shear. Cross-linking effectively inhibited breakdown of swollen granules, resulting in much higher viscosities in the modified starches than in the native starches. Cross-linked waxy HB starches showed higher viscosities than similarly modified waxy corn starch. A negative correlation ( $r^2 = 0.89$ ,  $P < 0.05$ ) was found between paste viscosity and amylograph peak viscosity (Fig. 2) for cross-linked starches, suggesting that the resistance of swollen granules to cooking shear was highly correlated to the degree of inhibition of granule from swelling. Hydroxypropylation reduced shear resistance of cross-linked waxy HB starches but showed little

**TABLE IV**  
Net Syneresis (% gel weight) of Native and Modified Waxy Corn, Hull-less Barley (HB), and Regular Potato Starches After Four Freeze-Thaw Cycles

Starch	Amylograph	Normal Cooking		High-Shear Cooking	
		Before Autoclaving	After Autoclaving	Before Autoclaving	After Autoclaving
Native					
Waxy corn	52.1	... <sup>a</sup>	40.1	...	...
HB SB94794	5.4	23.7	11.8	...	...
HB CDC Candle	26.7	45.4	11.6	...	...
Potato	...	...	...	...	...
LSD <sup>b</sup>	10.7	12.6	10.4	...	...
Cross-linked					
Waxy corn	52.6	-	48.0	...	34.9
HB SB94794 (0.005% $\text{POCl}_3$ )	15.8	50.5	24.2	13.5	13.1
HB SB94794 (0.010% $\text{POCl}_3$ )	23.7	56.7	30.6	19.7	14.2
HB CDC Candle	33.3	...	34.7	15.9	15.0
LSD	9.8	7.5	8.8	9.1	8.4
Cross-linked and hydroxypropylated					
Waxy corn	5.5	11.2	12.2	23.5	13.8
HB SB94794	3.6	8.6	11.0	15.1	18.6
HB CDC Candle	6.5	12.8	11.8	27.1	29.7
Potato	15.2	29.5	10.0	53.4	17.4
LSD	4.8	7.9	4.4	8.7	9.1

<sup>a</sup> Syneresis exceeded 60%.

<sup>b</sup> Least significant difference ( $P < 0.05$ ).

effect on cross-linked waxy corn starch (Fig. 4). Autoclaving after high-shear cooking had negligible additional effect on paste viscosity for all starch samples, native or modified.

### Freeze-Thaw Stability

Free water detected in fresh pastes varied from 4 to 9%, and little difference was found among the samples (data not shown). This was expected because all pastes were prepared at 6% concentration where free water separation from fresh paste was limited (Zheng and Sosulski 1998). After freeze-thaw treatment, however, net syneresis (NS) showed a broad range of variation among the starches. Cooking condition also affected NS. For native starches collected from the amylograph bowl, potato starch showed 61% NS after two freeze-thaw cycles (FT cycles), indicating little resistance to freeze-thaw. Although native waxy corn starch was more tolerant to freeze-thaw than potato starch, it showed 52% NS after four FT cycles, suggesting it only had a limited cold resistance. Compared to waxy corn starch, waxy HB starches were highly tolerant to freeze-thaw; they showed negligible NS after two FT cycles and their NS values were only 5 and 27% after four FT cycles (Table IV). Cross-linking increased NS for waxy HB starches but did not affect NS for waxy corn starch. NS was much lower for cross-linked waxy HB starches than for cross-linked waxy corn starch, suggesting higher freeze-thaw resistance of cross-linked waxy HB starches than cross-linked waxy corn starch. A dosage effect was observed for cross-linked SB94794, the higher the dosage of cross-linking reagent resulted in higher level of NS. Hydroxypropylation greatly improved freeze-thaw stability of the cross-linked starches. This was correlated to the improvement of granule swelling (Fig. 2) and was due to the introduction of hydrophilic hydroxypropyl groups. Such starches showed significantly ( $P < 0.05$ ) lower levels of NS after FT cycles than did native starches. The double-modified waxy corn and HB starches had <20% NS after 10 FT cycles (data not shown). However, similarly modified potato starch was less tolerant to FT cycles and had >60% NS after 10 FT cycles.

Starch pastes prepared by normal cooking showed higher NS after FT cycles than samples collected from amylograph, indicating that cooking condition affected water-holding capacity of starch pastes during freeze-thaw. Water-bonding ability of starch is largely dependent on the degree of granule swelling and molecule dispersion during cooking. Kim et al (1993) reported that starch pastes collected before the completion of amylograph cycle had higher syneresis than those collected at the completion of the cycle. Nevertheless, the order of freeze-thaw resistance of the native and double-modified starches was similar to that found in amylograph test, zero-amylose HB >> CDC Candle HB, waxy corn >> potato starches (Table IV). The cross-linked starches prepared by normal cooking showed 89% (SB94794, 0.005% POCl<sub>3</sub>) to 127% (waxy corn, 0.01% POCl<sub>3</sub>) increase in NS over amylograph test after two FT cycles and all samples had >50% NS after four FT cycles. In normal cooking, autoclaving improved granule swelling (Fig. 4) and therefore reduced NS values, particularly for the cross-linked starches.

Net syneresis data (Table IV) indicated that native starches lost their water-holding capacity after high-shear cooking because their granules were readily destroyed by shear (Fig. 4). High-shear cooking increased NS for the double-modified starches when compared to normal cooking, also due to increased breakdown of swollen granules. For cross-linked starches, however, high-shear cooking showed lower NS than normal cooking. Possible explanation for this result was that the reinforced hydrogen bonds in cross-linked starch were weakened by shear, resulting in more complete hydration of starch granules, which in turn increased water-holding capacity of the starch matrix.

### CONCLUSIONS

Our previous study indicated that native zero-amylose HB starch had unique properties such as high freeze-thaw stability and clarity

(Zheng et al 1998). Like other waxy starches, this HB starch also showed some undesirable characteristics such as breakdown of viscosity and a cohesive texture. As expected, cross-linking dramatically improved pasting properties of also waxy HB starches as reported for other starches. Cross-linked waxy HB starches were much more tolerant to freeze-thaw, high-shear cooking and autoclaving than similarly cross-linked waxy corn starch. This makes cross-linked waxy HB starches more suitable for food uses than cross-linked waxy corn starch. Hydroxypropylation further improved freeze-thaw stability and paste clarity of the cross-linked waxy HB starches. Although the double-modified waxy HB starches showed paste viscosity and freeze-thaw stability similar to that of similarly modified waxy corn starch, they had higher clarity after cold storage than similarly modified waxy corn starch. Compared to cross-linked starches, the double-modified starches were more tolerant to freeze-thaw but less tolerant to high-shear cooking. Therefore, cross-linked and double-modified waxy HB starches together may have a very wide spectrum of food applications. Double-modified potato starch was less tolerant to freeze-thaw than similarly modified waxy HB and waxy corn starches.

Rheological studies (Lii et al 1996, Tsai et al 1997) have suggested that pasting and gelling properties of starches are largely affected by granular structure which is manipulated by the content, structure, and packing of amylopectin and amylose molecules. The present study indicated that the behavior of granule swelling and disintegration of swollen granules during cooking was responsible for pasting properties and paste viscosities of the starches. However, detailed studies are needed to reveal molecular basis that causes the differences in the behavior of starch granules to various cooking conditions as well as to modifications.

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