Physicochemical Properties of Zero Amylose Hull-less Barley Starch

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ABSTRACT

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Zero amylose starch isolated from hull-less barley (HB) showed a typical A-type diffraction pattern. The X-ray analysis suggested that granules of zero amylose (SB94794) and 5% amylose (CDC Candle) HB starches had lower crystallinity than did commercial waxy corn starch. Differential scanning calorimetry showed lower transition temperatures and endothermal enthalpies for the HB starches than for the waxy corn starch. The zero amylose HB starch showed a Brabender pasting curve similar to that of waxy corn starch, but with lower pasting and peak temperatures and a higher peak viscosity. Noteworthy characteristics of zero amylose HB starch were its low pasting temperature and high paste clarity and freeze-thaw stability, which make this starch useful for many food and industrial applications.

Hull-less barley (HB) contains 60–75% starch (Bhatty 1997). Amylose content in HB starches may vary from 0 to 40% (Zheng and Bhatty 1998); such starches offer a wide potential for food and industrial applications. Bhatty and Rossnagel (1997) reported a HB line containing zero amylose starch that showed higher tolerance to freeze-thaw when compared to waxy HB (≈5% amylose) and corn (≈1% amylose) starches.

Clarity and freeze-thaw stability are important characteristics of starches used in various food applications such as fruit pie fillings. Although native potato and tapioca starches have higher paste clarity than corn, waxy corn, and wheat starches (Craig et al 1989), their freeze-thaw stability is very poor (Zheng and Sosulski 1998).Waxy corn starch is widely used in food applications in North America, partially because its cold storage stability is superior to that of normal corn or potato starches. However, its clarity and freeze-thaw stability need to be improved by etherification. Wu and Seib (1990) reported that a cross-linked and hydroxypropylated waxy barley starch containing ≈5% amylose showed a much higher freeze-thaw stability than similarly modified waxy corn and tapioca starches, indicating the potential of modifying waxy barley starches for food applications.

The importance of amylose-amylopectin ratio in starch functionality has been well documented. Sandhya Rani and Bhattacharya (1995) reported that swelling power and fragility of starch granules increased with decreased amylose content. Starches with lower amylose generally show lower pasting temperature, higher peak viscosity, and larger breakdown than starches with higher amylose contents. In starch-water systems, swelling and fragility of starch granules may also affect clarity of starch pastes (Craig et al 1989). Waxy barley starches containing 1–7% amylose have been investigated for physicochemical properties (MacGregor and Morgan 1984, Tester and Morrison 1990, Wu and Seib 1990, Vasanthan and Bhatty 1996). Although these starches showed some different characteristics from waxy corn starch, other properties such as paste clarity and freeze-thaw stability were basically similar to those of waxy corn starch (Wu and Seib 1990). Zero amylose HB starch showed lower pasting temperature and higher freeze-thaw stability than 5% amylose HB starch (Bhatty and Rossnagel 1997). The objectives of this study were to determine the physicochemical properties of zero amylose HB starch and assess its potential for food and industrial applications.

MATERIALS AND METHODS

Materials

SB94794, a two-rowed zero amylose HB, was developed at the Crop Development Center, University of Saskatchewan, from two waxy HB lines (Bhatty and Rossnagel 1997). Commercial waxy corn starch (National Starch and Chemical Co., Bridgewater, NJ), and starch isolated from CDC Candle, a registered Canadian waxy HB cultivar containing ≈5% amylose, were used for comparison. SB94794 and CDC Candle HB were both from the 1996 crop grown at the Kernen Crop Research Farm of the University of Saskatchewan, Saskatoon. The barleys were ground in a cyclone mill (Udy Corp., Fort Collins, CO) to pass a 0.5-mm sieve.

Analyses

Chemical analyses. Starch was isolated from ground HB by an enzyme-assisted wet-extraction procedure described by Zheng and Bhatty (1998). Approved Methods were used for determinations of moisture (Method 44-15A), ash (Method 08-12), crude lipid (Method 30-25), total nitrogen (Method 46-11A), β-glucan (Method 32-23), and starch damage (Method 76-31) (AACC 1995). Starch was determined by the method of Holm et al (1986) after boiling the samples with 80% ethanol for 30 min and centrifuging at 2,000 × *g* for 10 min. Amylose content of the starches was determined by the method of Gibson et al (1997). Starch granule swelling power was determined at 50, 60, 70, and 80°C according to Leach et al (1959).

DSC and X-ray. Differential scanning calorimetry (DSC) was performed (TA400, Mettler) with samples at starch-to-water ratios of 5.5 mg to 21 µL. Samples were heated from 20 to 120°C at the heating rate of 10°C/min (Vasanthan and Bhatty 1996). Wide-angle powder X-ray diffraction patterns of dry (≈10% moisture) and water-saturated starches were obtained with an X-ray diffractometer (model 42273, Phillips). Water-saturated starches were prepared by suspending starches in double-distilled water at room temperature (≈23°C) for 16 hr followed by centrifugation at 5,000 rpm (3,000 × *g*) for 10 min as described by Nara and Komiya (1983). The samples (≈50% moisture) were scanned immediately after centrifugation. The method of X-ray diffractometry was described previously (Vasanthan and Bhatty 1996). Data were smoothed using an 11-point rolling mean technique. Relative crystallinity was calculated as the ratio of the areas of crystalline and amorphous regions of X-ray diffractograms (Nara and Komiya 1983).

Brabender amylograph. Starch slurries (6% db w/v, 500 mL, pH 6.0) 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. Breakdown and setback viscosities were calculated.

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Granule size distribution. Starch granules were sprinkled on slide glasses and observed under a light microscope (Leitz Laborlux K and D, Wetzler, Germany) at 200× magnification. Particle-size distribution of at least 1,000 starch granules was determined on an image analyzer (BioQuant System IV, Image Technology) equipped with an image acquisition and computer processor.

Determination of paste clarity. The procedure of Craig et al (1989) was modified for determination of starch paste clarity. Starch slurries (1–4% w/v, 25 mL of water, pH 6.5) contained in 50-mL screw-capped polypropylene tubes were cooked in a boiling water bath for 30 min. The tubes were vigorously mixed on a vortex mixer every 30 sec in the first 5 min then every 5 min during cooking. After cooling to room temperature, the starch pastes were poured into cuvettes, and air bubbles were removed on an ultrasonic cleaner. Light transmittance (%*T*) was read at 650 nm against a water blank in a spectrophotometer (Spectronic 1001+), after which the cuvettes containing starch pastes were stored at 4°C for 16 hr or one week and %*T* was determined again. Potato starch (Sigma Chemical Co., St. Louis, MO) was used for comparison.

Syneresis after freeze-thaw. Pastes were prepared by cooking starch slurries (5, 6, 8, and 10% w/w, pH 6.5) in a boiling water bath with continuously mixing at 250 rpm and cooling to room temperature (23°C). After determination of free water from the freshly prepared samples, the pastes were treated for 2, 4, 6, 8, and 10 freeze-thaw cycles. Syneresis was determined by the method of Zheng and Sosuski (1998). Each freeze-thaw cycle composed of 16 hr of freezing at –18°C and 2 hr of thawing at 40°C.

Data analysis. Determinations of chemical composition (Table I), swelling power (Table II), water separation (Table III), paste clarity, and syneresis were repeated four times for each sample. Data were subjected to analysis of variance using Minitab statistical software (Minitab, Inc., State College, PA). X-ray, DSC, and amylograph tests were performed in duplicate, and identical results were obtained from the duplicate determinations for each sample.

TABLE I
Chemical Composition of Waxy Hull-less Barley and Waxy Corn Starches (% db)

Starch	Amylose	Protein	Ash	β-Glucan	Starch Damage
SB94794	0	0.2	0.1	0.2	0.6
CDC Candle	4.5	0.3	0.2	0.1	0.8
Waxy corn	1.1	0.4	0.1	0	1.7
LSD[a]	0.6	0.1	0.1	0.1	0.3

[a] Least significant difference ($P < 0.01$).

TABLE II
Swelling Power of Waxy Hull-less Barley and Waxy Corn Starches (g/g db)

	Temperature (°C)			
Starch	50	60	70	80
SB94794	1.4	8.9	27.2	nd[a]
CDC Candle	1.2	7.5	10.4	17.4
Waxy corn	1.2	2.4	11.1	55.9
LSD[b]	0.1	0.3	2.0	8.5

[a] Not determined due to starch granule breakdown.
[b] Least significant difference ($P < 0.01$).

TABLE III
Free Water (% paste weight) from Freshly Cooked Waxy Hull-less Barley and Waxy Corn Starch Pastes

	Free Water (concentration %)			
Starch	5	6	8	10
SB94794	7.5	5.1	2.5	1.8
CDC Candle	8.4	5.5	2.1	1.9
Waxy corn	15.8	7.8	2.2	1.8
LSD[a]	2.2	1.8	0.5	0.3

[a] Least significant difference ($P < 0.01$).

RESULTS AND DISCUSSION

Chemical analysis showed that the starch isolated from SB94794 had 0% amylose (Table I), confirming results based on iodine-amylose reaction reported previously (Bhatty and Rossnagel 1997). CDC Candle and waxy corn starches contained 5 and 1% amylose, respectively. Compared to waxy corn, CDC Candle and zero amylose HB starch had lower starch damage. The starches contained 0.2–0.4% protein (N × 6.25) and <0.2% β-glucan. No surface lipids were detected in any of the starches.

SB94794 and CDC Candle starches had generally similar bimodal granule size distribution ranging from 2 to 30 μm, whereas waxy corn starch showed a relatively narrower granule size distribution of 2–20 μm (Fig. 1). Large granules (≥8 μm in diameter) accounted for ≈30% of the total starch granules in SB94794 and CDC Candle. Granule size distribution of zero amylose HB starch was similar to that reported for the nonwaxy barley starches by MacGregor and Fincher (1993).

Wide-angle X-ray diffraction pattern of SB94794 was similar to that of CDC Candle and waxy corn starches (Fig. 2), showing typical A-type polymorphic form (Zobel 1988). Compared to the dry samples, water-saturated starches exhibited sharper peaks for all the starches. Nara and Komiya (1983) reported that relative crystallinity of starch granules was low and variable at low moisture content, while that of moistened samples was high and constant.

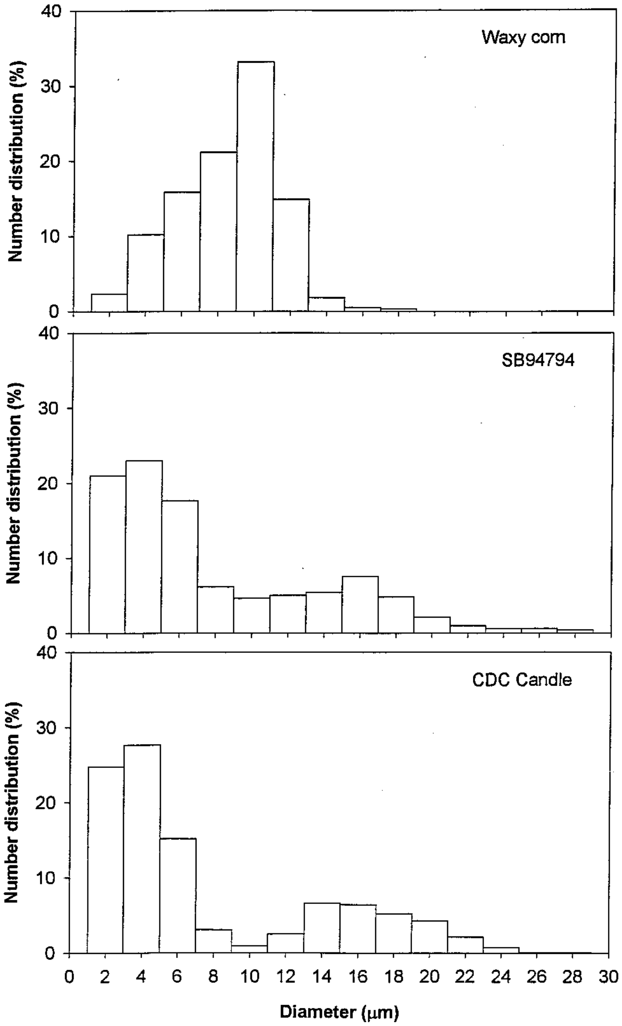

Fig. 1. Granule size distribution of waxy hull-less barley and waxy corn starches.

In the present study, dry samples showed 36% (waxy corn and SB94794) to 37% (CDC Candle) relative crystallinity. However, relative crystallinity of water-saturated samples was 37 and 35% for SB94794 and CDC Candle, respectively, both lower than that for waxy corn starch (40%) (Fig. 2). Shi and Seib (1992) also reported a lower relative crystallinity for a waxy barley starch as compared to waxy corn starch. In general, more crystalline samples show correspondingly higher gelatinization temperatures, particularly for A-type starches (Zobel 1988, Shi and Seib 1992). DSC analysis revealed that the transition temperatures of SB94794 were similar to those of CDC Candle, but waxy corn starch showed much higher gelatinization temperature than waxy HB starches (Fig. 3). Waxy HB starches also showed lower endothermal enthalpy than waxy corn starch.

The amylograph (Fig. 4) of zero amylose HB starch showed a pasting pattern similar to that of waxy corn starch. Both starches produced high peak viscosities as a result of rapid starch granule swelling, followed by a sharp granule breakdown. Little setback was observed in either zero amylose HB or waxy corn starches during the cooling period. Zero amylose HB starch had a lower pasting temperature and higher peak viscosity than waxy corn starch. Although X-ray diffractometry and DSC analyses showed little differences between SB94794 and CDC Candle starches (Figs. 2, 3), the amylograms of the starches were quite different. Compared to zero amylose HB and waxy corn starches, CDC Candle had a lower peak viscosity, a higher peak temperature, and a higher setback viscosity (Fig. 4). CDC Candle showed a unique pasting curve in which viscosity started to increase at a relatively low temperature (63°C) with a slow increase in the first 15 min (63–85°C), followed by a sharp increase before reaching the maximum (Fig. 4). The shoulder observed in the amylograph of CDC Candle starch was also present in the data reported by Wu and Seib (1990) on a waxy barley starch containing ≈5% amylose. The pasting behavior of CDC Candle suggested a slow swelling of the starch granules at the early stage of pasting. This was supported by the swelling power (SP) test. As temperature increased from 60 to 70°C, SP of zero amylose HB and waxy corn starches increased 300 and 460%, respectively, whereas that of CDC Candle increased only 140% (Table II). Setback viscosity of CDC Candle was 92 and 67% higher than waxy corn and zero amylose HB starches, respectively. This was probably due to its higher amylose content (Pomeranz 1991).

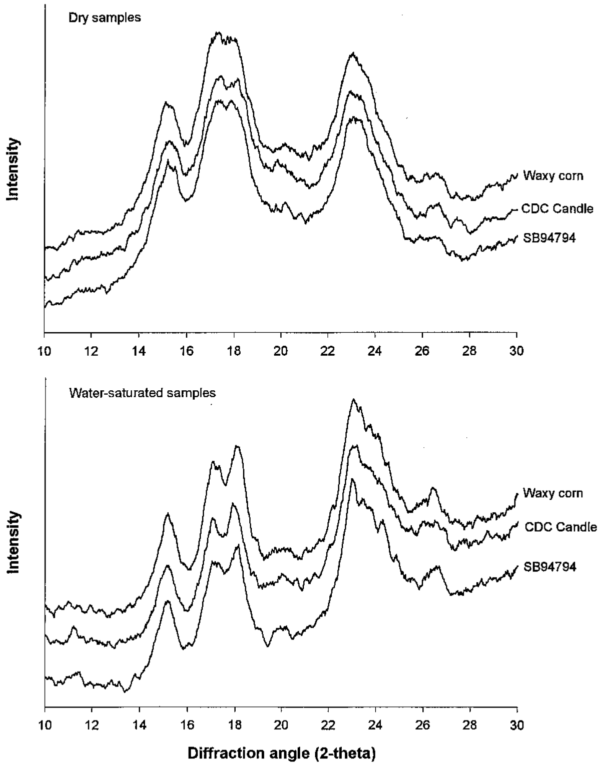

Fig. 2. Wide-angle powder X-ray diffraction patterns of dry and water-saturated samples of waxy corn, CDC Candle, and SB94794 hull-less barley starches.

Freshly cooked potato starch was clear, giving 92–84% light transmittance for 1–4% pastes (Fig. 5). This was in agreement with data reported by Craig et al (1989). Compared to potato starch, fresh pastes of other starches showed lower transmittance. For example, 1% pastes of zero amylose HB, waxy corn, and CDC Candle gave only 25, 29, and 18%*T* respectively. These starches might have lost granular structure but formed large numbers of junction zones in the dispersed phase after pasting, which in turn reflected or scattered light, resulting in low %*T* (Graing et al 1989).

An apparent characteristic of zero amylose HB starch paste was high clarity, even after cold storage. The %*T* of zero amylose HB starch increased almost linearly with increase of paste concentration. To a lesser extent, the waxy corn starch showed the same trend (Fig. 5). This could be explained by granule fragility of low amylose starches. Sandhya Rani and Bhattacharya (1995) reported that low amylose starch granules are fragile so that they swell and disintegrate easily. In dilute pastes, the granules swell with little disruption. The transmitted light passing through swollen granules was refracted or scattered, giving low clarity (Craig et al 1989). In concentrated paste, however, the greatly swollen granules disintegrate by mutual crowding and shear, resulting in increased light transmission. The %*T* of zero amylose HB starch increased more pronouncedly with the increase of concentration than did waxy corn starch (Fig. 5), suggesting that the granules of zero amylose HB starch were more fragile than those of waxy corn starch. In contrast to zero amylose HB and waxy corn starches, the %*T* of potato and CDC Candle decreased with the increased starch concentration. This may be due to the interaction between amylose leaching and granule swelling. It is well known that amylose leaches out from starch granules during cooking. Leached amylose forms a three-dimensional network (Tester and Morrison 1990), and swollen granules are embedded in such a continuous matrix (Ring 1985). This may interrupt disintegration of swollen granules. Although potato starch granules were fragile (Craig et al 1989), decreased clarity in the concentrated pastes suggested that the fragility of potato starch granules was reduced, probably due to the formation of amylose matrix.

In food applications such as fruit pie fillings, a stable paste clarity after repeated freeze-thaw is highly desired (Schoch 1968).

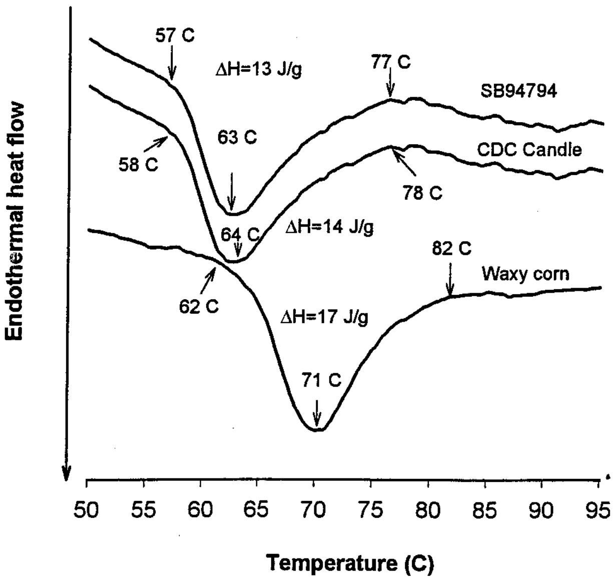

Fig. 3. Differential scanning calorimetry thermal curves of waxy hull-less barley and waxy corn starches.

However, the clarity of potato starch pastes decreased rapidly after 16 hr of refrigeration except for the 1% paste (Fig. 5). For example, the %*T* of 2, 3, and 4% pastes decreased by 23, 44, and 84% respectively. The %*T* of waxy corn starch also decreased after 16 hr of refrigeration by 19, 23, 32, and 39% in the 1, 2, 3, and 4% pastes, respectively. Prolonged refrigeration further reduced the clarity of pasted potato and waxy corn starches (Fig. 5). Amylose reassociation was the primary reason responsible for the deterioration in clarity of potato starch (Pomeranz 1991), whereas recrystallization of amylopectin molecules was responsible for deterioration in clarity in waxy corn starch (Ring et al 1987). Compared to waxy corn and potato starches, the zero amylose HB starch paste exhibited a very stable clarity under refrigerated conditions. No significant change was observed in %*T* of 1–4% zero amylose HB starch pastes even after four weeks of refrigeration (data not given). Although %*T* of CDC Candle pastes also showed a decrease after refrigeration, it was more stable than that of waxy corn starch. The average branch chain length of amylopectin was 12–13 for waxy barley (MacGregor and Morgan 1984) compared to 15 for waxy corn (Hizukuri 1985). Amylopectin with smaller chain length was less prone to crystallization than amylopectin with larger chain length (Ring et al 1987). After one week of refrigeration, a 19% water separation was detected from the 1% paste of waxy corn starch, whereas no water separation was observed in the waxy HB starches.

When a cooked starch paste is stored at low temperatures, amylose chains reassociate and water is released (syneresis). Pastes of waxy starches may remain very stable for weeks to months under refrigerated conditions, but the stability breaks down quickly after freeze-thaw (Schoch 1968). Under certain cooking and freeze-thaw conditions, slurry concentration is the major variable affecting freeze-thaw stability of a starch paste (Zheng and Sosulski 1998). Therefore, the syneresis test was conducted at different concentrations in this study. Free water from the freshly cooked starch pastes decreased with the increase of slurry concentration, and was higher for waxy corn than for the waxy HB starches (Table III), suggesting that waxy HB starches had higher water-holding capacity than waxy corn starch. Net syneresis excludes free water in the fresh paste from water released after cold storage, giving true values of water separation. Percent net syneresis values were then used as indices of freeze-thaw stability in this study. When subjected to freeze-thaw treatment, a 5% waxy corn starch paste gave 24% net syneresis after one freeze-thaw cycle; this increase was threefold higher than that of CDC Candle and zero amylose HB starches (Fig. 6). However, none of the starches showed stability after two cycles of freeze-thaw at 5% concentration. Increasing paste concentration did not improve freeze-thaw stability of native waxy corn starch, although its net syneresis values after two cycles decreased with an increase in starch concentration. If 20% net syneresis value is used as the breakdown point of freeze-thaw stability, CDC Candle was stable for four cycles at 6 and 8%, and for six cycles at 10%. Compared to waxy corn and CDC Candle, zero amylose HB starch showed a high freeze-thaw stability: six cycles at 6%, eight cycles at 8%, and >10 cycles at 10%. Wu and Seib (1990) reported that a hydroxypropylated and cross-linked waxy barley starch showed a much higher freeze-thaw stability than did similarly modified waxy corn starch. The undesirable characteristic of viscosity breakdown in zero amylose HB starch could be improved by a simple cross-linking at low level. Functional properties of zero amylose HB starch after various modifications are currently under investigation.

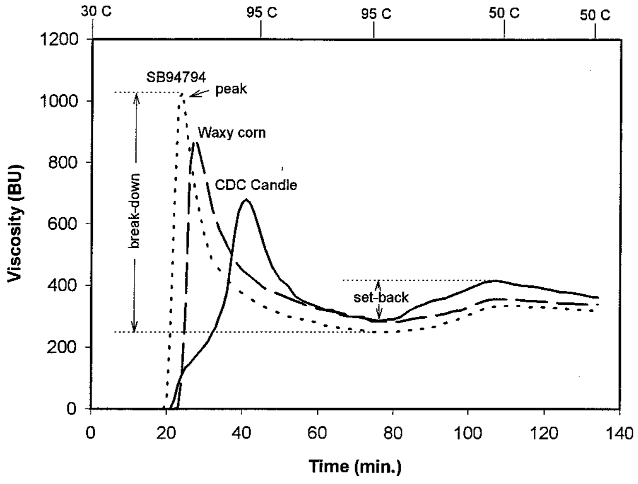

Fig. 4. Amylograph pasting patterns of waxy hull-less barley and waxy corn starches.

CONCLUSION

Zero amylose HB starch granules showed easier swelling and higher fragility as indicated by lower pasting temperature, higher peak viscosity, higher paste clarity, and freeze-thaw stability, when compared to CDC Candle HB starch (5% amylose) and waxy corn starch (1% amylose). These properties may be due to highly branched amylopectin or shorter amylopectin chain lengths. High and stable paste clarity and freeze-thaw stability of zero amylose HB starch make it uniquely suitable for use in frozen foodstuffs.

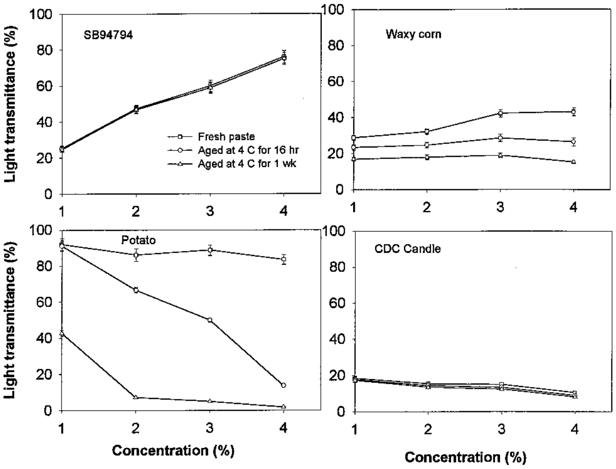

Fig. 5. Paste clarity of waxy hull-less barley and waxy corn starches. Vertical bars indicate standard error.

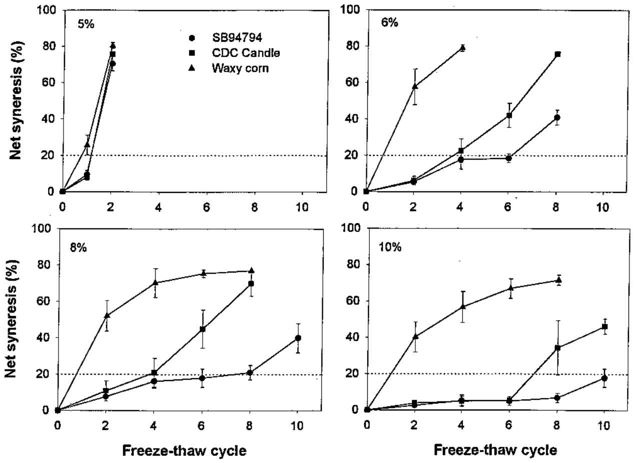

Fig. 6. Net syneresis of waxy hull-less barley and waxy corn starches. Vertical bars indicate standard error.

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