Properties of Granular Cold-Water-Soluble Starches Prepared by Alcoholic-Alkaline Treatments[1]

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ABSTRACT

Granular cold-water-soluble (GCWS) starches were prepared from normal maize, Hylon V (HA5), Hylon VII (HA7), and waxy maize starches by treating the starches with mixtures of ethanol and NaOH solutions at a controlled temperature. No Maltese crosses appeared when the GCWS starches prepared by these treatments were examined under polarized-light microscopy, which indicated changes of crystalline structures. Gel-permeation chromatography analyses of the GCWS starches were identical with those of their native starch counterparts, which indicated there was no detectable degradation of starch molecules during the preparation. The treated GCWS starches showed V-type X-ray diffraction patterns for normal maize, HA5, and HA7 starches; the GCWS waxy maize starch pattern was amorphous. The GCWS starches showed fully swollen granules when dispersed in cold water and exhibited ~70–90% cold-water solubility. Most of the GCWS starches displayed higher viscosities and better freeze-thaw stabilities than those of their native starch counterparts.

Granular cold-water-soluble (GCWS) starches provide instant and greater viscosity and a smoother texture when compared with drum-dried pregelatinized starch. Therefore, GCWS starches are desirable as ingredients of instant foods. GCWS starches also have more processing tolerance than traditional pregelatinized starches (Light 1990). GCWS starches can be prepared by several methods: 1) spray-drying (Pitchon et al 1981); 2) heating native starch in aqueous monohydric alcohol at 149–177°C under elevated pressure (Eastman and Moore 1984); 3) heating native starch in aqueous polyhydric alcohol at atmospheric pressure (Rajagopalan and Seib 1991, 1992a); 4) treating a slurry of native starch and a monohydric alcohol with an alkaline solution (Jane and Seib 1991, Chen and Jane 1994).

The molecular structure and the properties of GCWS starch are of interest and have been investigated. GCWS starch prepared by high-temperature treatment of a starch-aqueous alcohol suspension displayed a V-type X-ray diffraction pattern (Jane et al 1986a,b). The mechanism proposed was that treating native starch with aqueous alcohol at high temperature converted the native double-helical structure into single helices. Removal of alcohol by drying left an empty cavity in the center of the helices, which resulted in starch granules that were metastable and cold-water-soluble. The GCWS starch is not chemically modified, but the processing caused a mild degradation of starch molecules. The paste viscosity of the GCWS starch is similar to that of its amylograph-cooked native starch counterpart.

Rajagopalan and Seib (1992b) reported a V-type X-ray pattern of GCWS starches prepared by heating native starches in aqueous propan-1,2-diol at atmospheric pressure, but the X-ray pattern of hydroxypropylated cross-linked wheat starch was amorphous. Thickening and gelling properties of the GCWS starches prepared by heating starches in aqueous propan-1,2-diol at atmospheric pressure were similar to those of their native starch counterparts.

Objectives of this study were to characterize the GCWS starches prepared by alcohol-alkaline treatments (Chen and Jane 1994) and to derive a mechanism of the GCWS starch formation. Molecular size distribution, viscosity, ash content, X-ray diffraction pattern, pasting property, and freeze-thaw stability of the resulting starches were investigated.

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MATERIALS AND METHODS

Materials

GCWS normal maize and high-amylose HA5 and HA7 (Hylon V and Hylon VII) starches were prepared by treating native starches with an aqueous ethyl alcohol and NaOH (3*M*) solution of starch, H_2O, absolute ethyl alcohol, and NaOH (3*M* at 1.0:4.2:2.8:5.0, w/w) at 35°C (Chen and Jane 1994). GCWS waxy starch was produced with a different proportion of starch to solvents (1.0:0.0:7.0:3.2) at 25°C (Chen and Jane 1994). Amyloglucosidase (EC 3.2.1.3) from *Rhizopus* mold was a product of Sigma Chemical Co. (St. Louis, MO). The enzyme activity was 11,600 units per gram of solid. One unit (U) of the enzyme is defined as the release of 1 mg of glucose from starch in 3 min at pH 4.5 and 55°C. The enzyme was used without further purification.

Gel-Permeation Chromatography

Molecular size distribution of GCWS starches was determined by gel-permeation chromatography on a Sepharose CL-2B column (Chen and Jane 1994).

Viscosity

Viscosity of starch pastes at a concentration of 6% (w/w, dsb) was measured by a Brabender Visco/Amylograph (model VA-5, 700 cm·g, C.W. Brabender, South Hackensack, NJ). Starch paste was prepared by mixing 27 g (dsb) of GCWS normal maize or waxy maize starch with sufficient water to make a total weight of 450 g. The mixture was gently stirred with a spatula, and the starch was quickly dispersed. The paste (400 g) was then transferred into an amylograph cup for viscosity measurement at 30°C and 75 rpm. The final viscosity, after stirring for 1 hr at 30°C, was compared with that of amylograph-cooked native starch pastes. Native starch pastes were prepared by a standard cooking procedure using the amylograph (Smith 1964a). The final viscosity was recorded after the temperature was cooled to 30°C. For high-amylose starch, a paste at a concentration of 3% (w/w, dsb) was used for viscosity measurement. Native high-amylose starch pastes were prepared in a high-pressure cooker (model 4522 benchtop reactor, Parr Instrument Co., Moline, IL) at 140°C for 30 min. GCWS high-amylose starch pastes were prepared by dispersing GCWS starches in distilled water with gentle stirring. Viscosity was measured by using a Brookfield viscometer (model LVF, Brookfield Engineering Laboratories, Stoughton, MA) with a No. 1 spindle at 30°C and 60 rpm.

Enzyme Susceptibility of GCWS Starch

GCWS normal maize starch was subjected to amyloglucosidase hydrolysis. The starch sample (0.1 g) was dissolved in 9 ml of

acetate buffer (0.1*M*, pH 4.5). Enzyme solution (1 ml in 0.1*M* acetate buffer, pH 4.5) containing 20 U of amyloglucosidase was added. The mixture was then incubated for 12 hr in a shaker bath (model 236, Versa-Bath S, Fisher Scientific) at 55°C, 100 strokes/min. The efficiency of the enzyme hydrolysis was determined by measuring the proportion of reducing sugars to total sugars. Reducing sugars were measured by a modified Park-Johnson's method (Hizukuri et al 1981, Jane and Chen 1992). Total sugars were determined by a phenol-sulfuric acid method (Dubois et al 1956).

Lipid Analysis

Total lipid content of native starches and GCWS starches were determined according to the method of Smith (1967).

X-Ray Diffraction

X-ray diffraction patterns of starches were recorded on a diffractometer (Simens D500) with a Cu X-ray tube and a nickel-foil filter operated at 40 kV and 25 mV. A step-scan was set at an angle of 0.05° per step with a counting of 2 sec.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to analyze starches on a Perkin-Elmer DSC-7 (Norwalk, CT) equipped with an intracooling I system. Starch (2.0 ± 0.1 mg, dsb) was weighed into an aluminum pan, and distilled water (~6 mg) was added to the starch sample. The pan was sealed and allowed to equilibrate for 2–3 hr at ambient temperature. The sample was then heated from 25 to 100°C at a rate of 10°C/min. An empty pan was used as a reference.

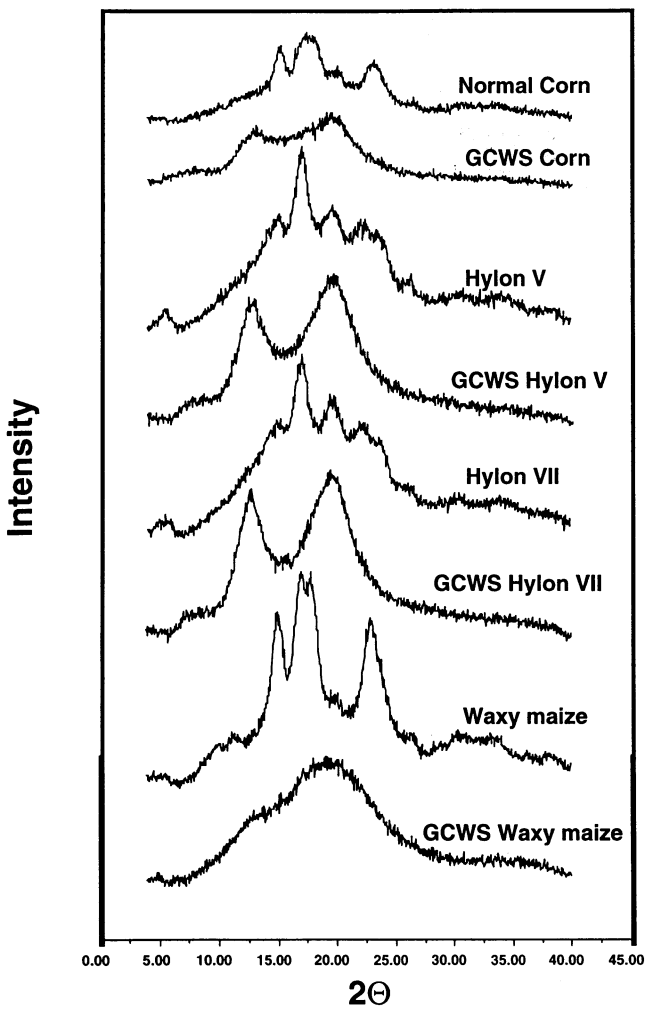

Fig. 1. X-ray diffraction profiles of granular cold-water-soluble (GCWS) starches and their native starch counterparts. GCWS starches showed V-type X-ray diffraction patterns; the GCWS waxy maize starch was amorphous.

Determination of Ash Contents

Ash content of starches was determined by the method of Smith (1964b).

Freeze-Thaw Stability

To measure freeze-thaw stability, normal maize and waxy maize starch pastes (6%, w/w) were prepared according to a standard cooking process by using the Brabender Visco/Amylograph. GCWS normal maize and waxy maize starch pastes were prepared by dispersing an adequate amount of GCWS starches in distilled water at room temperature. The mixtures were gently stirred with a spatula until the starches were completely dispersed. The pastes were then transferred to the amylograph operated at 30°C, 75 rpm for 1 hr. Portions (15 g) of each starch paste were transferred into each of 10 disposable petri dishes (60 × 15 mm). The petri dishes were covered and sealed with adhesive tape to prevent moisture loss while frozen at −20°C. On each freeze-thaw cycle, samples were frozen at −20°C for 24 hr and thawed at 25°C for 3 hr. The thawed samples were then subjected to a vacuum filtration after 1, 2, 3, 5, and 10 freeze-thaw cycles (Lim 1990). Water of syneresis from duplicate gel samples was collected and weighed. Freeze-thaw stability was expressed by the percentage of water lost.

RESULTS AND DISCUSSION

None of the GCWS starch granules prepared in this study showed Maltese crosses when examined under polarized-light microscopy. The X-ray diffraction of the GCWS starches showed V-type patterns (single helical conformation), except for GCWS waxy maize starch, which gave an amorphous pattern (Fig. 1). The single-helical amylose and amorphous starch were reported to be water soluble at 25°C (French and Murphy 1977). DSC thermograms also confirmed the cold-water-soluble property in that none of the GCWS starches in this study gave any gelatinization endotherm between 25 and 100°C (Fig. 2).

In characterizing GCWS starch prepared by superheating in aqueous ethanol solution under pressure, Jane et al (1986a) proposed a mechanism for the transformation of those commercial GCWS normal maize and wheat starches. They proposed that amylopectin, as well as amylose, formed a V-complex with the alcohol when the native starch double-helical structure was dissociated by heating. Removal of the alcohol leaves the starch in a metastable state and soluble in cold water.

The mechanism can be applied for the formation of the GCWS starches prepared by alcoholic-alkaline treatment. Starch is a weak ion exchanger (Oosten 1982). When starch molecules were placed in a strong alkaline solution, protons of the -OH group were dissociated and left negative charges on starch molecules. The repulsion between negative charges resulted in swelling of starch granules. The swelling of the granules exerted a tension on neighboring crystallites of starch molecules and tended to distort them (French 1984). Further swelling led to uncoiling or dissoci-

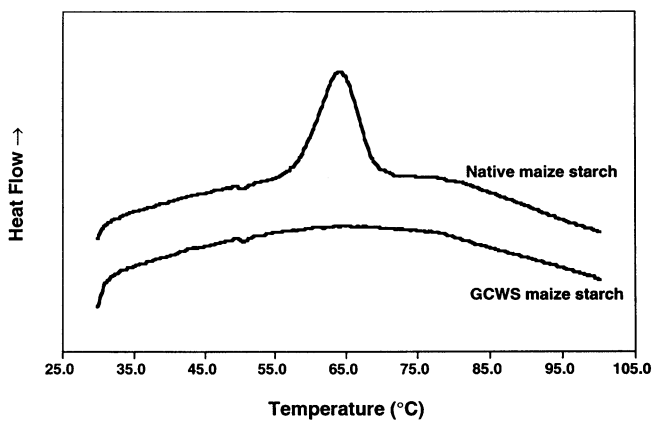

Fig. 2. Differential scanning calorimetry thermograms of granular cold-water-soluble (GCWS) normal maize and its native starch counterpart. Samples (25%, w/w) were heated from 25 to 100°C at a rate of 10°C/min.

ation of double-helical regions and the breakup of crystalline structure. As a result, the order of crystallites was destroyed, but the entanglement of amylose with amylopectin molecules inside the granules retained the swollen granules in one entity (Jane et al 1986a, 1992, 1993). After neutralization of the treated starches, the starch molecules formed single-helical complexes with ethanol (V-complex). GPC profiles of the GCWS starches showed no detectable degradation of starch molecules (Fig. 3). The HA5 profile showed a higher blue value at the amylopectin peak, indicating a longer branch-chain length. This was consistent with the results of high-performance anion-exchange chromatography (Chen and Jane 1994).

The function of alcohol in the reaction mixture was not only to restrict the swelling of starch granules by decreasing the effective water concentration, but also to serve as a complexing agent to stabilize the dissociated starch chains. Rajagopalan and Seib (1992b) reported that the X-ray diffraction pattern of the treated starches was amorphous immediately after heating starches in aqueous polyhydric alcohol. After solvent exchange with ethanol, the diffraction pattern changed to a V-type pattern.

Enzyme hydrolysis showed no significant differences between GCWS normal maize starch and its native starch counterpart (data not shown), indicating that GCWS starch was not chemically modified, because modified starch would interfere with enzyme hydrolysis (Chan et al 1984). It confirmed that the cold-water solubility was rendered by a physical change of crystalline structure of starch from double helix (A-type) to single helix (V-type).

Paste viscosities of the GCWS normal maize, waxy maize, HA5, and HA7 starches, and their native starch counterparts are shown in Table I. GCWS waxy maize, HA5, and HA7 starches exhibited viscosities higher than those of their native starch counterparts. The viscosity of GCWS normal maize starch, however, was lower.

Figure 4 shows typical pasting curves for GCWS normal maize and waxy maize starches (Fig. 4a) and their native starch counterparts (Fig. 4b) at 6% (w/w) concentration. The viscosities of the GCWS starches reached ~300 BU instantly, and then they gradually increased to 400 BU after 15 min of stirring at 30°C. The viscosity reached a plateau and changed little over 1 hr with continuous stirring at 75 rpm. GCWS normal maize showed a better stability than did GCWS waxy maize.

Dispersion of the GCWS starch granules prepared by alcoholic-

TABLE I
Viscosity of Various Starch Pastes

	Viscosity[a]	
Sample	**Native**	**GCWS**
Waxy maize[b]	320 ± 28 BU	375 ± 7 BU
Normal maize[b]	570 ± 28 BU	405 ± 7 BU
HA5[c]	9.5 ± 0.7 cps	11.0 ± 0.7 cps
HA7[c]	5.5 ± 2.1 cps	10.0 ± 2.8 cps

[a] Data are mean ± standard deviation of duplicate samples.
[b] Viscosity measured by Brabender Visco Amylograph at 6% (w/w) starch concentration and recorded at the end of the measurement.
[c] Viscosity measured by Brookfield Viscometer at 3% (w/w) starch concentration with No. 1 spindle at 30°C, 60 rpm.

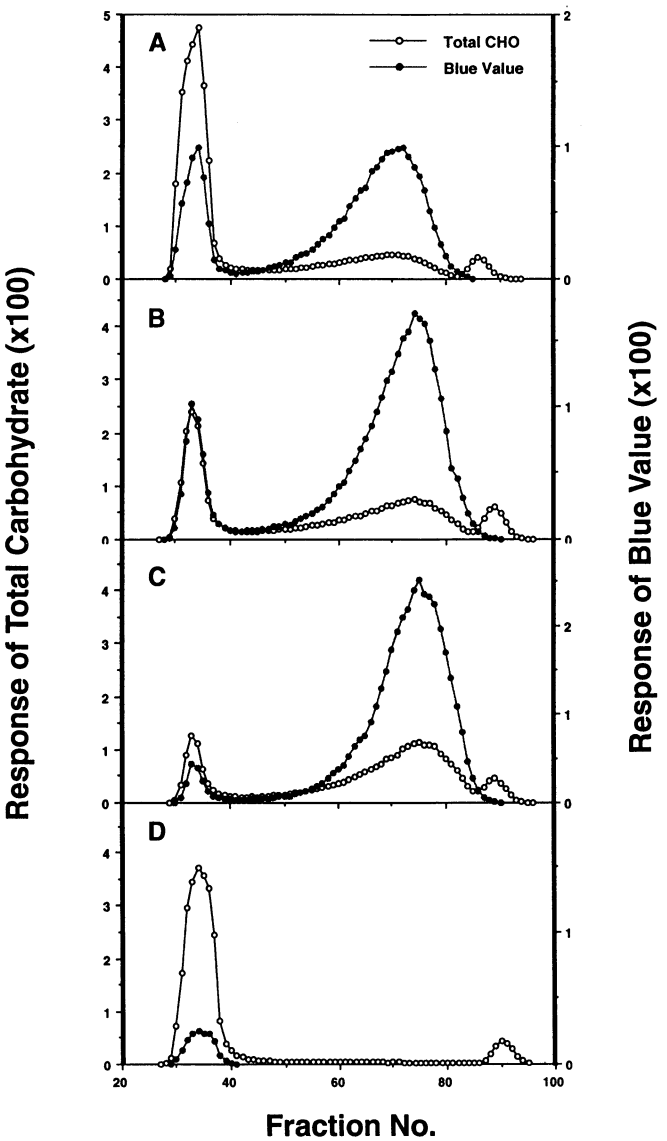

Fig. 3. Sepharose CL-2B gel-permeation chromatograms of granular cold-water-soluble starches. **A,** normal maize starch; **B,** HA5 starch; **C,** HA7 starch; **D,** waxy maize starch. No degradation of starch molecules was found.

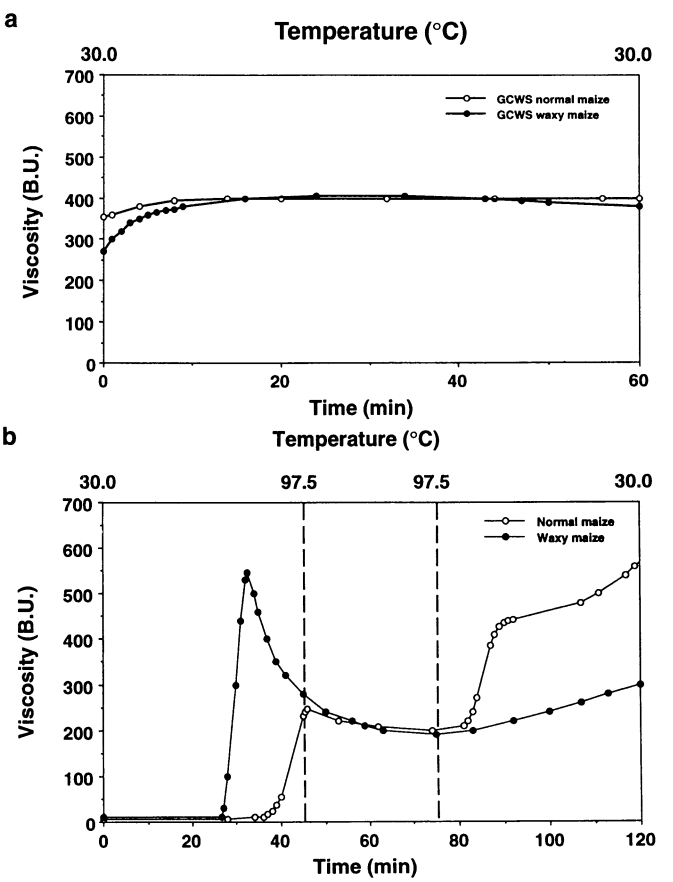

Fig. 4. Amylograms of granular cold-water-soluble (GCWS) normal maize and waxy maize starches and their native starch counterparts (6%, w/w). **a,** Pastes prepared by dispersing starches in cold water and transferring to amylograph operated at 30°C, 75 rpm for 1 hr. **b,** Pastes subjected to regular cooking process.

alkaline treatment in water did not produce lumps as did the paste of GCWS starch prepared by heating starch in aqueous alcohol under pressure. The smoothness of the texture and the easy preparation of paste were attributed to the trace amount of salt residues in the GCWS starch granules. Ash contents of the starches were measured as a reference of salt residues in the GCWS starch granules. The ash contents of the GCWS starches produced by this method were two to five times greater than those of their native starch counterparts (Table II). The lipid contents of the GCWS starches were reduced ~20–40% (Table II).

Freeze-thaw stability studies revealed that the pastes of the GCWS starches prepared by the alcoholic-alkaline treatments had improved freeze-thaw stabilities (Fig. 5). The paste of GCWS normal maize starch did not reach maximum syneresis during five freeze-thaw cycles; the paste of native normal maize starch reached maximum syneresis after the first freeze-thaw cycle. The water loss of GCWS waxy maize starch was not detectable until the second cycle. Both GCWS normal and waxy maize starches displayed better freeze-thaw stabilities than those of their native starch counterparts. This could be attributed to the integrity of the swollen granules that did not completely disperse. Consequently, the tendency of starch retrogradation was decreased.

CONCLUSIONS

GCWS starches prepared by alcoholic-alkaline treatment exhibited V-type X-ray patterns, except for GCWS waxy maize starch, which showed an amorphous type. This was attributed to the changes of the crystalline structures from double to single helices. Molecular size distributions of the GCWS starches for Sepharose CL-2B chromatography were identical with those of their native starch counterparts, indicating no detectable degradation of starch molecules. Results of enzyme hydrolysis indicated that the GCWS starch was not chemically modified. None of the GCWS starch granules prepared in this study showed Maltese crosses when examined under polarized-light microscopy. The GCWS starches swelled instantly when rehydrated in cold water, and most of the pastes had better viscosities and freeze-thaw stabilities. The smooth texture and easy preparation of the pastes are attributed to the trace amount of salt residues in the GCWS starch granules.

TABLE II
Ash Contents and Lipid Contents of Various Starches

Sample	Ash Content, %[a]		Lipid Content, %[a]	
	Native Starch	GCWS[b] Starch	Native Starch	GCWS Starch
Waxy maize	0.09 ± 0.02	0.40 ± 0.07	0.05 ± 0.01	0.04 ± 0.01
Normal maize	0.08 ± 0.01	0.14 ± 0.00	0.46 ± 0.04	0.35 ± 0.01
HA5	0.16 ± 0.02	0.81 ± 0.00	0.74 ± 0.06	0.45 ± 0.02
HA7	0.22 ± 0.01	0.81 ± 0.08	0.86 ± 0.06	0.64 ± 0.01

[a]Data are mean ± standard deviation of duplicate samples.
[b]Granular cold-water-soluble starch.

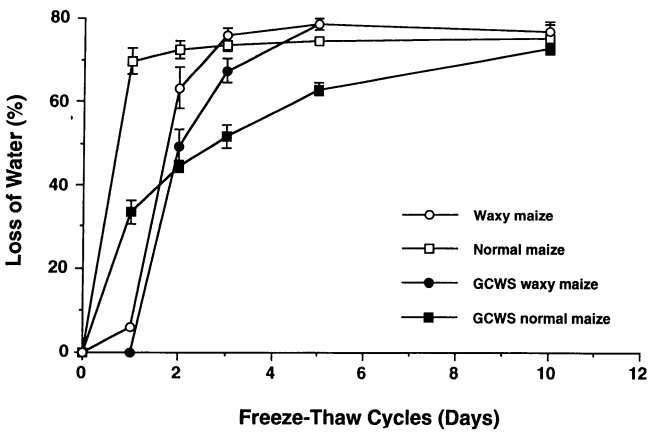

Fig. 5. Freeze-thaw stabilities of granular cold-water-soluble (GCWS) normal maize and waxy maize starches and their native starch counterparts. Pastes (6%, w/w) of GCWS starches were prepared by dispersing starches in distilled water at room temperature and transferring to amylograph operated at 30°C, 75 rpm for 1 hr. Pastes (6%, w/w) of native starches were prepared by a regular amylograph cooking cycle. Samples (15 g) were taken for study following preparation. Data were means of four replicates.

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