

# Characteristics of Granular Cold-Water Gelling Starches of Cereal Grains and Legumes Prepared Using Liquid Ammonia and Ethanol

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## ABSTRACT

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Starches of wheat, corn, smooth and wrinkled peas, and chickpeas were modified to a free-flowing powder of granular cold-water gelling (GCWG) starch using liquid ammonia and ethanol at 23°C and atmospheric pressure. Amylose content of starches was 26.3% in wheat, 27.1% in corn, 35.4% in chickpeas, 43.2% in smooth peas, and 79.9% in wrinkled peas. The modified starches remained in granular form with an increased number of grooves and fissures on the surface of the granules compared with native starch, while the crystallinity was mostly lost, as shown by X-ray diffractograms and DSC endothermic enthalpies. Pasting viscosity of modified starches at 23°C was 171 BU and 305 BU in wheat and corn, respectively, and much higher in legume starches, ranging from

545 BU to 814 BU. Viscosities of modified legume starches at 23°C were at least twice as high as those of native starches determined at 92.5°C. Swelling power of modified starches at 23°C ranged from 8.7 g/g to 15.3 g/g, while swelling power of native starches heated to 92.5°C ranged from 4.8 g/g to 16.0 g/g. GCWG starches exhibited higher dextrose equivalent (DE) values of enzymatic hydrolysis, ranging from 25.2 to 27.0 compared with native starches (1.5–2.9). Modified starches from wheat, corn, smooth peas, and chickpeas formed weak gels without heat treatment and experienced no changes in gel hardness during storage, while native starch gels formed by heat treatment showed an increase in hardness by 1.1–7.5 N during 96 hr of storage at 4°C.

Starch is widely used in thickening agents, fillers, and binders in the food industry. When heated in aqueous conditions, the crystalline structure of starch granules is disrupted and hydrated, causing starch granules to swell and develop a viscous mass (Howling 1974). The disruption of starch granule crystallinity of 15–45% in various botanical sources (Zobel 1988) can be achieved by heating and other chemical and physical treatments. Because of convenience and versatility, the food industry is increasingly interested in modified starches that can be used without further heating to obtain gelling and pasting properties. Thermally gelatinized starches produce a viscous paste in cold water that often bears a graininess that creates a coarse texture on the palate, especially in high-amylose starches (Howling 1974).

Granular cold-water gelling (GCWG) starches form a gel with a smooth texture with the addition of water without applying energy in the form of heat (Chen and Jane 1994). Preparation of free-flowing GCWG starch involves exposing starch granules to conditions such as high temperature and pressure in aqueous alcohols (Eastman and Moore 1984), strong alkaline and ethanol (Jane and Seib 1991; Chen and Jane 1994), and 45–85% ammonia in methanol (Bernetti et al 1968), which disrupt the crystalline integrity of the starch granule but not to the extent that the granule bursts. In a previous study, we optimized the starch modification process for the production of GCWG starch from chickpea starch using liquid ammonia and ethanol (Jackowski et al 2002). In the current study, we prepared GCWG starches of diverse amylose content from various botanical sources and determined the chemical, physical, and functional properties of the modified starches as compared with the native starches.

## MATERIALS AND METHODS

Chickpea flour was provided by Blue Mountain Seeds, Inc. (Walla Walla, WA). Smooth peas (cv. Columbian) and wrinkled peas (cv. Bolero) were provided by Crites Co. (Moscow, ID) and milled to flour using a Buhler experimental roller mill (Buhler Inc., Braunschweig, Germany). Maize starch was provided by Staley

Starch Co. (Decatur, IL). Chickpea, smooth pea, and wrinkled pea starches were isolated from flours through repeated blending with water, centrifugation, and removal of solubles and tailings, according to the wet fractionation procedure of Czuchajowska and Pomeranz (1994). Wheat starch was isolated from a hard wheat flour according to the procedure of Czuchajowska and Pomeranz (1993).

## Preparation of Granular Cold-Water Gelling Starches

GCWG starches were prepared using liquid ammonia and ethanol at –33°C and atmospheric pressure according to the procedure described by Jackowski et al (2002). Starches (10 g, db) were suspended in liquid ammonia (40 g) with continuous stirring for ≈15 sec. On formation of a thick slurry of liquid ammonia and starch mixture, ethanol (30 mL) was added. The ethanol, liquid ammonia, and starch mixture was removed and placed into a dish and dried for 8 hr at 23°C, allowing the excess liquid ammonia and ethanol to evaporate.

## Chemical Analyses

Moisture and protein contents of native and modified starches were determined according to Approved Methods 44-15A and 46-30 (AACC 2000). The amylose content of starch was determined by an iodometric method as described by Haase (1993), using the maize amylose and amylopectin (Sigma Chemical Co., St. Louis, MO) as references.

## Scanning Electron Microscopy (SEM)

A scanning electron microscope was used to examine the external structure and shape of the individual starch granules of both native and modified prime starches (Fig. 1). Starch granules were mounted on aluminum stubs and coated with gold using a Hummer sputter coater (Technics, Alexandria, VA). The coated starch granules were observed using a Hitachi S-570 scanning electron microscope (Hitachi, Japan).

## Wide-Angle X-ray Powder Diffraction

Starch crystallinity of both native and modified starches were evaluated using wide-angle X-ray powder diffraction (Sievert et al 1991). Prime starches were equilibrated to 16% moisture content and packed in an aluminum sample holder. X-ray diffraction patterns of the prime starches were recorded on a diffractometer (D500, Siemens, Madison, WI) operating at 35 kV, 30 mA. Diffractograms were obtained from 4° 2θ to 30° 2θ with a step size of 0.05° 2θ, with 4 sec on each step.

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### Differential Scanning Calorimetry (DSC)

Thermal behavior of both native and modified starches was determined using DSC (Pyris1, Perkin-Elmer Corp., Norwalk, CT). An indium standard was used for temperature and enthalpy calibration. Starch (10 mg, db) and distilled water (20  $\mu$ L) were placed in a stainless steel capsule, sealed, and allowed to equilibrate for 30 min at room temperature. The starch was then heated from 20 to 180°C at 10°C/min. A capsule with inert material (aluminum oxide) and water served as the reference. For each endotherm, onset temperature and peak temperatures were determined using Pyris Manager data processing software (Perkin Elmer). The transition enthalpy was calculated from the peak area by software and expressed as J/g of dry matter.

### Paste Viscosity

A microviscoamylograph (C. W. Brabender Instruments, Inc., South Hackensack, NJ) was used to determine the viscosity of starches. Native and modified starches (6.0 g, db) were dispersed in distilled water (100 mL) in the amylograph mixing bowl while stirring to prevent clumping, especially with modified starches. The starch slurries were stirred at 23°C for 13 min (just before heating), heated to 92.5°C at 7.5°C/min, and held at that temperature for 13 min. The starch was heated to 92.5°C to avoid boiling over of starch paste.

### Swelling Power

Swelling power of native starch was determined according to the procedure of Crosbie (1991). Native starch (1.0 g) was weighed into a 50-mL cylindrical centrifuge tube with distilled water (35 mL) and mixed immediately with a vortex mixer. The tube was placed in a hot water bath at 92.5°C and mixed by inverting twice in 20-sec intervals for the first 3 min, in 30-sec intervals for the next 2 min, every 1 min for the next 5 min, and then in 5-min intervals for 20 min, for a total time of 30 min. The tube was cooled in an ice water bath to  $\approx$ 23°C, inverted twice, and placed in a 23°C water bath for 5 min and then centrifuged at 1,000  $\times$  g for 20 min. The supernatant was removed, evaporated, and dried at 105°C for 5 hr. Swelling power was calculated as the weight of gel divided by the original dry weight of starch minus

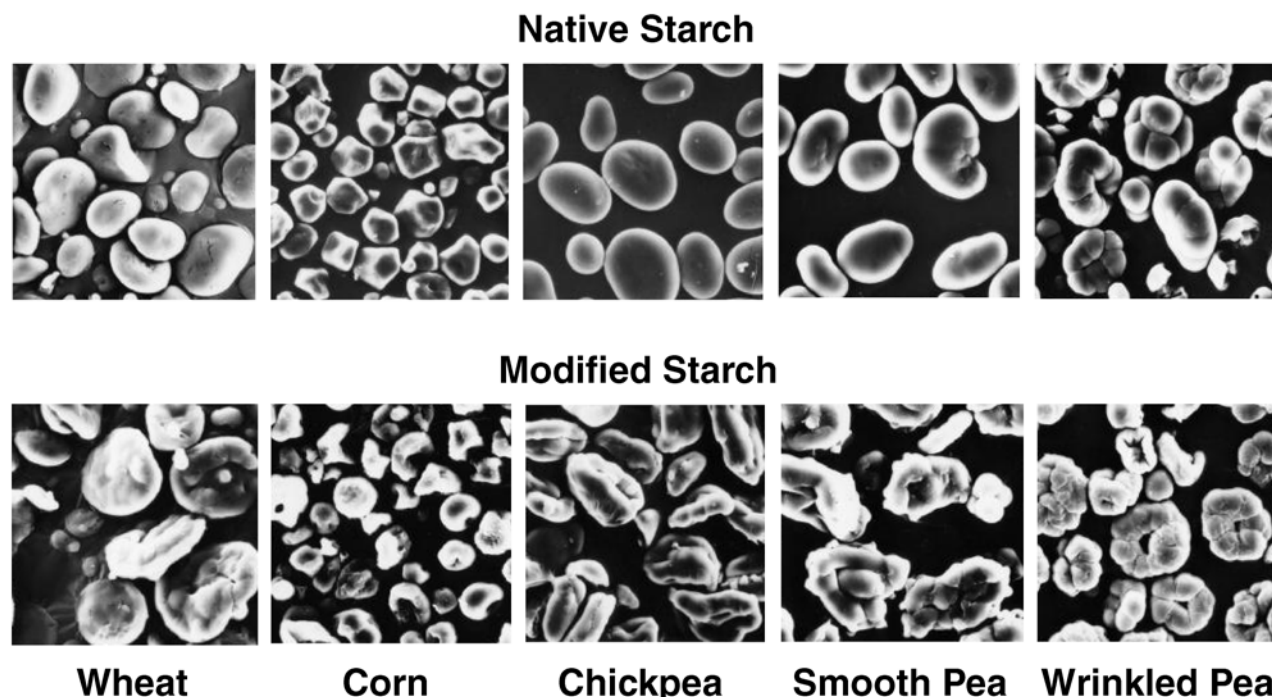
soluble dry matter. Swelling power of modified starches was determined as for native starches, with the exception of placing the starch suspension in a water bath at 23°C instead 92.5°C.

### Enzyme Digestibility of Modified Starches

Enzyme digestibility of modified starches was compared with that of native starch. To test enzyme digestibility, native and modified starches (0.5 g) were suspended separately in 0.05M phosphate buffer solution (50 mL, pH 6.9) and treated with pancreatic  $\alpha$ -amylase solution (10 mL) at 37°C for 5 min with continuous shaking.  $\alpha$ -Amylase from porcine pancreas was purchased from Sigma Chemical and prepared by suspending 5 g of enzyme in 100 mL of phosphate buffer (pH 6.9) under vigorous stirring and subsequent centrifuging and filtering. The reducing power of the hydrolysates was analyzed as described in Bernfield (1955). A 3,5-dinitrosalicylic acid (DNS) solution was prepared by dissolving sodium hydroxide (8.0 g) and DNS (5 g) in distilled water (100 mL), combining with a 60% sodium potassium tartrate (w/v) in distilled water, and bringing the total volume to 500 mL by adding distilled water. DNS solution (2 mL) was added to the hydrolysate (1 mL) and boiled for 5 min to develop color, then diluted with distilled water (15 mL). The optical densities of the solutions were observed with a spectrophotometer at 546 nm against a reagent blank. A standard curve was prepared using maltose. The degree of starch hydrolysis was expressed as dextrose equivalents.

### Starch Gel Hardness

Native starch slurries (6%, w/v) were heated from 23°C to 92.5°C at a heating rate of 7.5°C/min using a microviscoamylograph and held at 92.5°C for 15 min. The slurry was poured into three cylindrical steel molds (3.5 cm height and 3.5 cm diameter) and covered with a plastic cap to prevent film formation on the surface. Modified starch slurries (6%, w/v) were prepared at 23°C and poured into three cylindrical steel molds. The molds of native and modified starch slurries were held at 4°C for 0, 48, and 96 hr. Gel hardness was determined in duplicate using a TA-XT2 texture analyzer (Stable Micro System, Hasselmeres, England). Gel hardness was determined by placing the cylindrical molds on a steel



**Fig. 1.** Scanning electron microscopy of native and modified starch granules of wheat, corn, chickpeas, smooth peas, and wrinkled peas. Starch (10 g) was modified using liquid ammonia (40 g) and ethanol (30 mL).

platform, followed by compressing the gels with a plastic probe (2.0 cm diameter) at a rate of 1.0 mm/sec. The probe compressed into the mold of the gel, determining the force required to perforate 30% into the body of the gel.

### Statistical Analyses

At least two replicates were made for chemical and physical measurements. Data were analyzed with a computer software package (SAS Institute, Cary, NC) using analysis of variance and Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level.

## RESULTS AND DISCUSSION

### Composition and Appearance of Starches

The amylose content of starch was 26.3% in wheat, 27.1% in corn, 35.4% in chickpeas, 43.2% in smooth peas, and 79.9% in wrinkled peas. The modified starches exhibited nitrogen contents higher by 0.02–0.04% than that of the native starches, due to the remnant of liquid ammonia used for modification.

Scanning electron microscopy (SEM) detailed the appearance of individual starch granules. Starch was present as discrete granules after the modification process using liquid ammonia and ethanol, and maintained a free-flowing powder character. Modified starches of wheat, corn, and wrinkled peas retained an appearance similar to native starch granules, while starches of chickpeas and smooth peas mostly lost the granular shape of the native starch due to the modification, probably due to differences in integrity of starch granules of various botanical sources. Compared with native starches, the modified starch exhibited granules with more grooves and fissures on the surface of the starch granules.

### X-ray Diffraction

Both wheat and corn starch, in native form, gave A-type X-ray diffraction patterns, while the three legume starches exhibited C-type patterns, which is considered to be a mixture of A- and B-type patterns (Biliaderis 1991). Modified starches of wheat, corn, and chickpea showed no X-ray diffraction peaks, indicating that the crystalline integrity of the starch granule was altered to amorphous structure during the modification process. However, the modified starch of smooth and wrinkled peas exhibited two distinct peaks at  $2\theta$  sec of  $13.0^\circ$  and  $20.2^\circ$ , although peak intensities were considerably lower than in the native starches. Much higher starch amylose content of smooth peas and wrinkled peas compared with starches of wheat, corn, and chickpeas is probably responsible for

the two remaining X-ray diffraction peaks in smooth and wrinkled peas. Overall, regardless of starch type, a majority of the crystalline structure of starch granules disappeared as a result of the modification process.

### Differential Scanning Calorimetry

The DSC characteristics of native and modified starches are summarized in Table I. The native cereal starches exhibited two prominent endothermic transitions. The first transition, with a peak temperature at  $63.6^\circ\text{C}$  for wheat and  $73.0^\circ\text{C}$  for corn, corresponded to endotherms of starch gelatinization. The second endothermic transition at  $104.5^\circ\text{C}$  for wheat and  $101.5^\circ\text{C}$  for corn corresponded to the melting of amylose-lipids complex. The three legume starches displayed only one gelatinization peak of amylopectin, but the size, shape, and enthalpy of the peak differed depending on the type of legume. Prime starch of smooth peas exhibited a broad peak range from  $50.2$  to  $94.7^\circ\text{C}$  with a gelatinization enthalpy of  $16.2$  J/g. Chickpea starch displayed a sharper enthalpy peak with a gelatinization enthalpy of  $14.7$  J/g, while starch from wrinkled peas showed a much smaller enthalpy peak ( $7.3$  J/g) compared with smooth pea and chickpea starches, as a result of lower amylopectin content. Even though corn and wheat starches were similar in amylose content, corn starch ( $15.3$  J/g) displayed higher enthalpy values than wheat starch ( $11.6$  J/g), which may reflect a lower crystallinity in the latter. Our findings agree with Ward et al (1994), who reported that the degree of crystallinity in native corn starch is higher than that in native wheat starch. The gelatinization enthalpy of starch decreased by 95% in wheat and 99% in corn by the modification, while the melting enthalpy for the amylose-lipid complex remained at 61% in wheat and 56% in corn after the modification of starch. The enthalpy values for starch gelatinization decreased substantially  $<0.8$  J/g for modified cereal and wrinkled pea starches, while modified chickpea and smooth pea starch had enthalpy values of  $2.3$  and  $4.3$  J/g, respectively. The modified starches exhibited lower onset temperatures of  $6.3$ – $14.5^\circ\text{C}$  than the native starches. These results indicate that starch modification process using liquid ammonia not only destroys most of the crystalline structure of starch granules but also makes the remaining crystalline structure unstable compared with the crystalline structure of native starch.

### Viscosity of Starches

Native starches are not able to swell and form a viscous mass without heating. When heated in water, a starch suspension forms a viscous paste. The viscosity profiles of native and modified

TABLE I  
Differential Scanning Calorimetry Characteristics of Native and Modified Cereal and Legume Starches<sup>a,b</sup>

Source of Starch	Starch Gelatinization			Amylose-Lipid Complex		
	$T_o$ ( $^\circ\text{C}$ )	$T_p$ ( $^\circ\text{C}$ )	$\Delta H$ (J/g)	$T_o$ ( $^\circ\text{C}$ )	$T_p$ ( $^\circ\text{C}$ )	$\Delta H$ (J/g)
Wheat						
Native	58.3a	63.6a	11.6a	97.5a	104.5b	1.3a
Modified	52.0b	58.8b	0.4b	108.5b	110.0a	0.8b
Corn						
Native	67.0a	73.0a	15.3a	93.5a	101.5a	1.3a
Modified	52.5b	56.2b	0.1b	98.5b	101.5a	0.7b
Chickpea						
Native	61.6a	66.5a	14.7a	nd <sup>c</sup>	nd	nd
Modified	51.1b	60.1b	2.3b	nd	nd	nd
Smooth Pea						
Native	60.9a	67.6a	16.1a	nd	nd	nd
Modified	50.2b	62.7b	4.3b	nd	nd	nd
Wrinkled Pea						
Native	58.3a	79.8a	7.0a	nd	nd	nd
Modified	51.7b	63.1b	0.8b	nd	nd	nd

<sup>a</sup> Onset temperature ( $T_o$ ); peak temperature ( $T_p$ ); transition enthalpy ( $\Delta H$ ).

<sup>b</sup> Mean values in the same column with different letters within each source of starch are significantly different ( $P < 0.05$ ).

<sup>c</sup> Not detected.

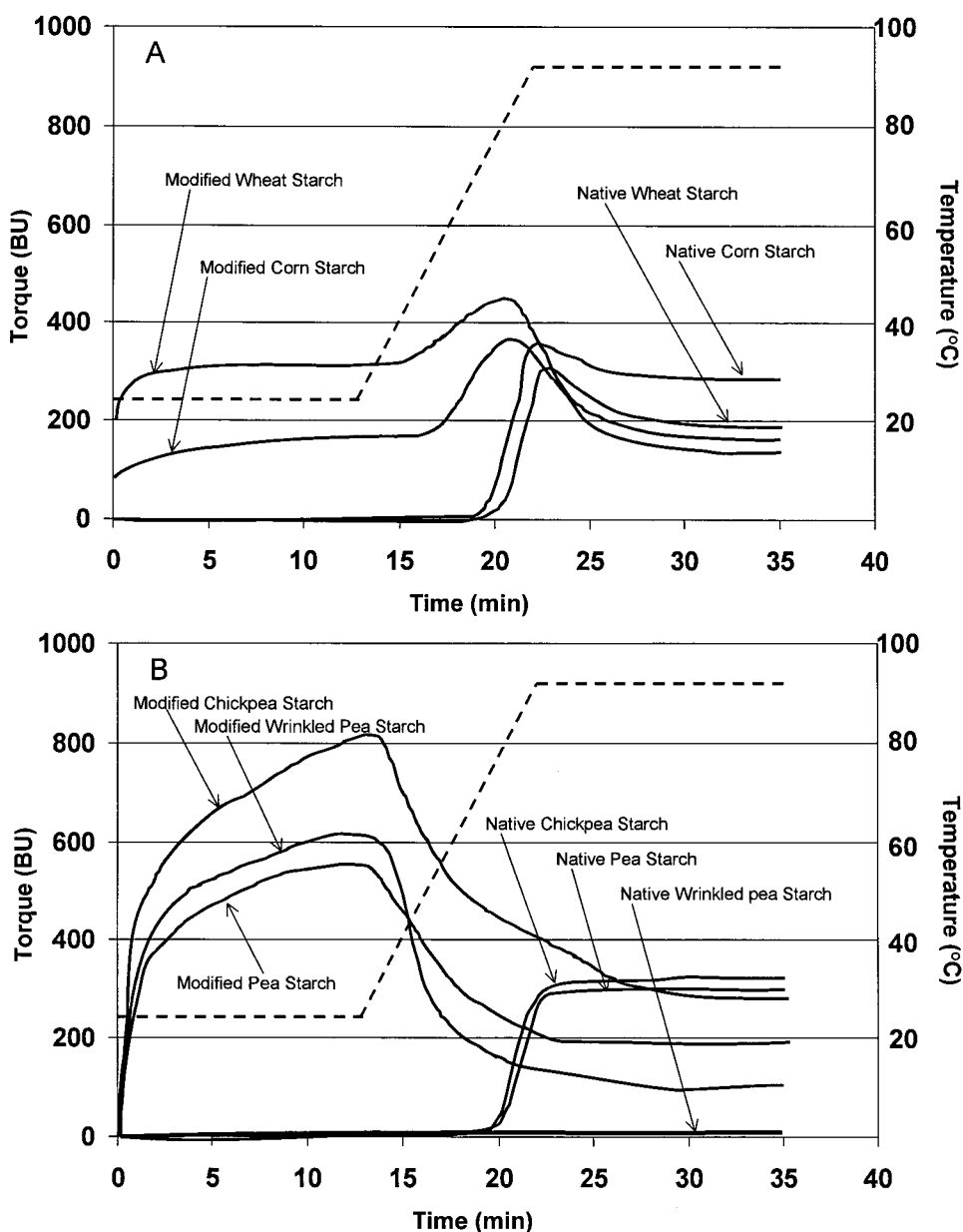
starches are shown in Fig. 2A for wheat and corn and Fig. 2B for legumes. Upon heating to 92.5°C, native wheat and corn starches formed a viscous mass and exhibited peak viscosity of 311 and 362 BU, respectively. Native chickpea and smooth pea starches had viscosity values of 322 and 284 BU, respectively. Wrinkled pea starch did not exhibit any viscosity during heating to 92.5°C, which is consistent with starches containing high concentrations of amylose with strong intragranular association (Schoch and Maywald 1968).

Contrary to native starches, modified starches formed a viscous paste at 23°C, indicating that they are able to imbibe water and swell without sources of energy in the form of heat. Even though modified starches absorbed water rapidly and produced a viscous mass, the viscosity profiles depended on the type of starch. Modified wheat and corn starches showed paste viscosity of 305 and 171 BU, respectively, with 13 min of stirring at 23°C. Viscosity of modified starch increased further with heating to 450 BU in wheat and 365 BU in corn at ≈82°C. Further heating modified starch paste of wheat and corn to 92.5°C, and holding decreased

viscosity to <200 BU. The increase in viscosity during heating of modified cereal starches suggests that amylose and small molecular weight amylopectin is exuded into the intragranular phase more readily when heat is applied, resulting in an increase in viscosity. Paste viscosity of modified legume starches increased rapidly within the first 2 min of stirring at 23°C and then at a slower rate, to 814, 619, and 545 BU in chickpeas, wrinkled peas, and peas, respectively. In contrast to cereal starches, heating the modified legume starches resulted in a rapid decrease in paste viscosity. The decrease in viscosity of modified legume starch during heating may be due to the destruction of starch granular structure and shear thinning of starch molecules.

#### Swelling Power and Enzyme Digestibility of Starches

Swelling powers of both native wheat and corn starches were 14.0 g/g, while swelling powers of native legume starches ranged from 4.8 g/g for wrinkled peas to >16.0 g/g for chickpeas and smooth peas (Table II). The low swelling power of starch in wrinkled peas was probably due to the restricted swelling of starch



**Fig. 2.** Amylograph pasting profiles of native and modified cereal (A) and legume (B) starches. Pasting viscosity of starches (6 g, db) in distilled water (100 mL) was determined using a microviscoamylograph.

TABLE II  
Swelling Power, Dextrose Equivalent, and Gel Hardness of Native and Modified Cereal and Legume Starches<sup>a</sup>

Starch	Swelling Power <sup>b</sup>	Dextrose Equivalent <sup>c</sup>	Gel Hardness <sup>d</sup> (N)		
			0 hr	48 hr	96 hr
Wheat					
Native	14.0a	2.9b	2.1a	3.2a	3.3a
Modified	11.5b	26.5a	0.2b	0.3b	0.4b
Corn					
Native	14.0a	2.7b	2.6a	4.0a	4.1a
Modified	10.6b	25.7a	0.1b	0.2b	0.3b
Chickpea					
Native	16.0a	2.6b	6.0a	11.4a	11.7a
Modified	9.7b	25.2a	0.3b	0.5b	0.5b
Smooth Pea					
Native	16.5a	1.9b	8.5a	13.5a	16.0a
Modified	8.7b	27.0a	0.4b	0.5b	0.5b
Wrinkled pea					
Native	4.8b	1.5b	0.2b	0.5b	0.6b
Modified	15.3a	25.5a	2.1a	5.0a	6.5a

<sup>a</sup> Mean values with different letters in the same column within botanical source of starch are significantly different ( $P < 0.05$ ).

<sup>b</sup> Swelling power of starch was determined at 92.5°C in native starches and at 23.0°C in modified starch.

<sup>c</sup> Dextrose equivalent of starch determined after treating starch with  $\alpha$ -amylase.

<sup>d</sup> Gels of native starches were prepared by heating starch to 92.5°C. Modified starch gels were prepared at 23°C without heating.

granules during heating at 92.5°C for 30 min. Even without heat treatment, however, swelling power of modified starches ranged from  $\approx 8.7$  g/g to 15.3 g/g, indicating that modified starches at 23°C are able to swell, imbibe, and hold water under centrifugal force. The swelling power of the modified wheat, corn, chickpea, and smooth pea starches at 23°C were lower than the swelling power of the respective native starches at 92.5°C. On the other hand, modified wrinkled pea starch had swelling power three times higher than that of native wrinkled pea starch. This result indicates that the starch modification process contributed more severe effects on the crystalline structure of starch granules than heating at 92.5°C for 30 min, resulting in a significant increase in swelling and gelatinization of the wrinkled pea starch. Czuchajowska et al (1998) reported that wrinkled pea starch (8%) developed no viscosity during 30 min of heating at 93.5°C and retained easily visible intact starch granules in a gel network. Therefore, the wrinkled pea starch granules were not able to fully swell and undergo gelatinization with heat treatment at 93.5°C (Schoch and Maywald 1968; Stute 1990).

The dextrose equivalent (DE) values of native and modified starches treated with  $\alpha$ -amylase are shown in Table II. Regardless of starch type under the conditions of the assay, the native starch was hydrolyzed by  $\alpha$ -amylase to a lesser extent than the modified starches, with DE values of 1.5–2.9. The extent of hydrolysis was greatest for the modified starches with DE values of 26.5, 25.7, 25.2, 27.0, and 25.5 for wheat, corn, chickpeas, smooth peas, and wrinkled peas, respectively. The modification process altered the crystalline structure of starch granules, allowing for a greater extent of enzymatic hydrolysis compared with native starch.

#### Hardness of Native and Modified Starch Gels

Hardness of native starch gels prepared at 92.5°C and modified starch gels prepared at 23°C are presented in Table II. Hardness values of native wheat and corn prime starch gels determined immediately on cooling (0 hr) were 2.1 and 2.6 N, respectively. Ward et al (1994) explained that the recrystallization in native corn starch was greater than in native wheat starch. Native chickpea and smooth pea starch gels showed larger hardness values than the native cereal starches, with gel hardness values  $>6.0$  N at 0 hr, indicating that the higher amylose legume starches form strong rigid gels upon cooling. Native wrinkled pea starch at 0 hr displayed a significantly lower hardness value of 0.2 N because of incomplete gelatinization of the starch. After 96 hr of storage at 4°C, the native cereal starches exhibited an increase in hardness of 1.1–1.5 N, while chickpea and smooth pea starch gels showed

$>5.5$  N increase in hardness. Wrinkled pea starch showed a negligible increase in gel hardness over 96 hr stored at 4°C. Modified wheat, corn, chickpea, and smooth pea starch gels displayed hardness of 0.2, 0.1, 0.3, and 0.4 N at 0 hr, respectively. Hardness of gels after 96 hr of storage showed negligible increases. The high viscosity values and weak gel formation of modified starches indicates that the modification process inhibits the formation of molecular junction zones and retrogradation of starch over a period of 96 hr in wheat, corn, chickpea, and smooth pea starches. Modified wrinkled pea starch, however, had a hardness of 2.1 N at 0 hr and of 5.5 N over 96 hr of storage. The modification process disrupted the molecular order of wrinkled pea starch granules, which did not swell during the heating process, allowing granules to swell and amylose molecules to leach out of the starch granules. Leached amylose molecules formed molecular junction zones, leading to the formation of gels.

#### CONCLUSIONS

Modified starches of wheat, corn, smooth and wrinkled peas, and chickpeas retained the free-flowing granular form of the native starch but exhibited more grooves and fissures on the surface of the granules. The modification process mostly destroyed the crystalline structure of starch granules and let water penetrate into the granules, which led to increases in swelling power and viscosity, even at 23°C.

GCWG starches of wheat, corn, chickpeas, and smooth peas swelled rapidly in contact with water without application of heat and produced a homogeneous gel, which exhibited a minimum retrogradation of starch during storage. Starch modification using liquid ammonia and ethanol may impart more severe changes to the crystalline structure of starch granules than heating at 92.5°C for 30 min, as evidenced by high starch digestibility and paste viscosity of modified starch.

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