

# Physical Properties and Enzymatic Digestibility of Phosphorylated *ae*, *wx*, and Normal Maize Starch Prepared at Different pH Levels<sup>1</sup>

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## ABSTRACT

Cereal Chem. 76(6):938–943

Phosphorylated starches were prepared with sodium triphosphate (STPP) at pH 6, 8, and 10 from waxy (*wx*, 3.3% amylose), normal (22.4% amylose), and two high-amylose (*ae*, 47 and 66% amylose) maize starches. After phosphorylation, the gelatinization peak temperature ( $T_p$ ) decreased and pasting peak viscosity (PV) increased for all the starches except *wx*, which showed a slight increase in gelatinization temperature. There was a substantial effect of phosphorylation pH on paste viscosity. More cross-linking was found in *ae* starches with phosphorylation at pH 10. Sodium ions apparently decreased PV of all the phosphorylated starches while only slightly affecting PV of native starches. Phosphorylation increased swelling power of some of the starches, with maximum swelling power at phosphorylation pH 8 and minimum at pH 10. Maximum swelling power for *wx* starch after preparation was at pH 8 and minimum at pH 6. After phos-

phorylation, the clarity and freeze-thaw stability of all the starches was greatly increased compared with the native starches. Phosphorylation increased digestibility of *ae* starches but had little effect on *wx* and normal starches. After phosphorylation, the adhesiveness, springiness, and cohesiveness of all starch gels generally increased, the hardness of 47% *ae* and *wx* starches increased, and that of normal starches decreased. Enthalpy of gelatinization decreased after phosphorylation with the greatest decrease observed for *ae* starches. When the phosphorylation pH increased from 6 to 10, the brightness ( $L^*$ ) of all the phosphorylated starches decreased, while  $a^*$  and  $b^*$  of all the phosphorylated starch increased. Scanning electron micrographs showed some erosion on the surface of starch granules after phosphorylation.

Chemical modifications are often made to starches to provide improved or specific properties to extend their usefulness in food or industrial applications. Chemically modified starches can have markedly altered physicochemical properties as compared with their parent starches (Rutenberg and Solarek 1984). Phosphorylation is a widely used method for starch modification in which repulsion between adjacent starch chains caused by the introduction of negatively charged phosphate groups reduces interchain associations and facilitates starch hydration. There are small amounts (<0.1%) of naturally occurring phosphate in root, tuber, and cereal starches. Phosphates are present as esters covalently linked to starch in root and tuber starches. However, in cereal starches, most phosphate is present as phospholipids (Posternak 1951, Hizukuri et al 1970, Tabata et al 1975, Morrison 1978, Kasemsuwan and Jane 1996).

Phosphorylated starches may be either monostarch phosphates or distarch phosphates that form a phosphate cross-link. During phosphorylation, pH plays a major role in determining the ratio of monoester bonds to diester bonds. Monoesters are formed at a higher level of substitution at <pH 7, and diesters at >pH 8 (Lim and Seib 1993).

The phosphorylation of starch with phosphate salts has been investigated by several authors (Paschall 1964, Nierle 1969, Lim and Seib 1993). After phosphorylation, starches gave clear pastes with high viscosity and had good freeze-thaw stability.

Mutations affecting starch synthesis can influence both the total starch content and the amylose-to-amylopectin ratio. The *ae* mutant of maize is associated with a high amylose content of the endosperm starch, whereas *wx* starch has essentially no amylose. The special properties of different starches from mutant plants such as thermal behavior during gelatinization and altered starch structures have been described (Sanders et al 1990, Wang et al 1992). In normal starch, there is no difference in the susceptibility of amylose or amylopectin to cross-linking (Jane et al 1992), but the potential for differential phosphorylation in other starches has not been examined.

The objectives of this study were to compare the phosphorylation of *ae*, *wx*, and normal maize starches with sodium triphosphate (STPP) at different pH levels and to evaluate the effect of phosphorylation on physical properties and enzymatic digestibility of these starches.

## MATERIALS AND METHODS

### Starch Samples

All native starch samples were supplied by Starch Australasia Ltd. (Lane Cove, Australia), and amylose contents were determined with an amylose-amylopectin assay kit (Megazyme Pty Ltd., Bray, Ireland). Samples were two high-amylose (*ae*) maize starches (Hi-Maize) with 66% amylose and Gelose 50 with 47% amylose; one waxy starch Mazaca 3401X (*wx*) with 3.3% amylose; and one normal starch Maize Cornflour 3401C with 22.4% amylose.

### Preparation of Starch Phosphates

Starch was phosphorylated by the procedure described by Lim and Seib (1993). STPP (2.5 g) was dissolved in 50 mL of water containing 2.5 g of sodium sulfate. Starch (50 g, db) was dispersed in the solution, and the final weight was brought to 111.2 g by adding water. The starch concentration of the final dispersion was 45% (w/v). The solution was adjusted to pH 6, 8, or 10 by adding 10% (w/v) HCl or NaOH. The slurry was stirred for 1 hr at room temperature, then dried to 10–15% moisture at 40°C. The dried starch was incubated for 2 hr at 130°C. After cooling to room temperature, the reaction mixture was dispersed in distilled water (100 mL), adjusted to pH 6.5, centrifuged at  $1,700 \times g$  for 5 min, and the starch pellet was washed with water ( $3 \times 600$  mL).

### Viscoamylography

A Rapid Visco Analyser (RVA) (model 3-D, Newport Scientific Pty. Ltd., Warriewood, Australia) was used to determine the pasting properties of starch samples. Starch (3.0 g, db) and a weighed amount of distilled water were combined and stirred in the aluminum RVA sample canister to make a 10.7% starch suspension (w/w). In a programmed heating and cooling cycle, sample was held at 50°C for 1 min, heated to 95°C in 7.5 min, held at 95°C for 5 min, cooled to 50°C in 8.5 min, and then held at 50°C for 3 min. Triplicate tests were made in each case. Values recorded were peak viscosity (PV), holding or hot paste viscosity (HPV, minimum vis-

<sup>1</sup> Presented in part at the AACC 83rd Annual Meeting, Minneapolis, MN, September 1998.

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cosity during stirring at 95°C), final or cool paste viscosity (CPV, viscosity at the end of the cycle at 50°C), breakdown (BD, PV – HPV), and setback (SB, CPV – HPV). To examine the influence of ionic concentration on paste consistency, aliquots of 0.01M and 0.05M NaCl were added to starch phosphates prepared at pH 6 to give a 10.7% (w/w) starch suspension.

### Differential Scanning Calorimetry

Thermal analysis was performed with a Mettler DSC 20 instrument (Mettler, Nänikon-Uster, Switzerland) equipped with a Mettler TC 11 data analysis station. Starch (2.5 mg, db) was weighed directly into a 40-μL pan and then 7.5 mg of deionized water was directly added to the pan by a microsyringe and mixed. After sealing, the pan was left for 1 hr to allow the sample to equilibrate. The sample was then heated from 30 to 120°C at a heating rate of 10°C/min. An empty pan was used as a reference.

### Swelling Power

The swelling power of starches was determined as described by Subramanian et al (1994) using a modified temperature. The 0.25% suspension (w/w) of starch and distilled water was heated in a controlled temperature bath for 30 min at 70°C for wx and normal starch samples, and 80°C for the ae starch samples. Lump formation was prevented by thorough stirring. The mixture was centrifuged

at 2,000 × g for 15 min, the supernatant removed, and the swollen starch sediment weighed. Swelling power was the ratio in weight of the wet sediment to the initial weight of dry starch.

### Texture Analysis

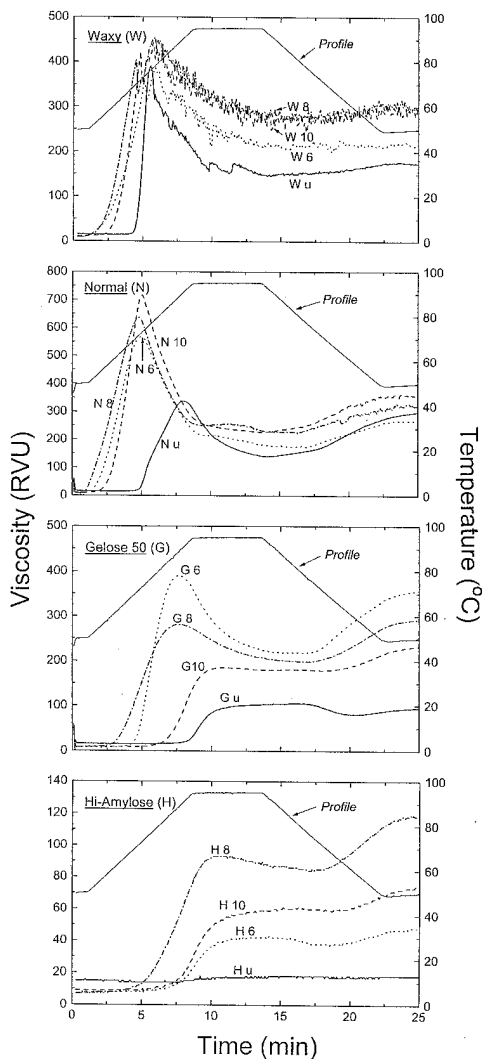
After RVA testing, the starch pastes were covered and kept at 25°C for 4 hr. The texture of the gels (10.7%) was determined using a texture analyzer (TA-XT 2, Stable Micro Systems, Godalming, England). The gel was compressed at a speed of 1.0 mm/sec over a distance of 10 mm with a cylindrical flat-ended probe (5 mm diameter). The peak height at 10 mm compression was termed hardness, and the negative area of the curve during retraction of the probe was termed stickiness. Native wx maize and phosphorylated wx maize were also tested with a larger probe (20 mm diameter).

### Clarity

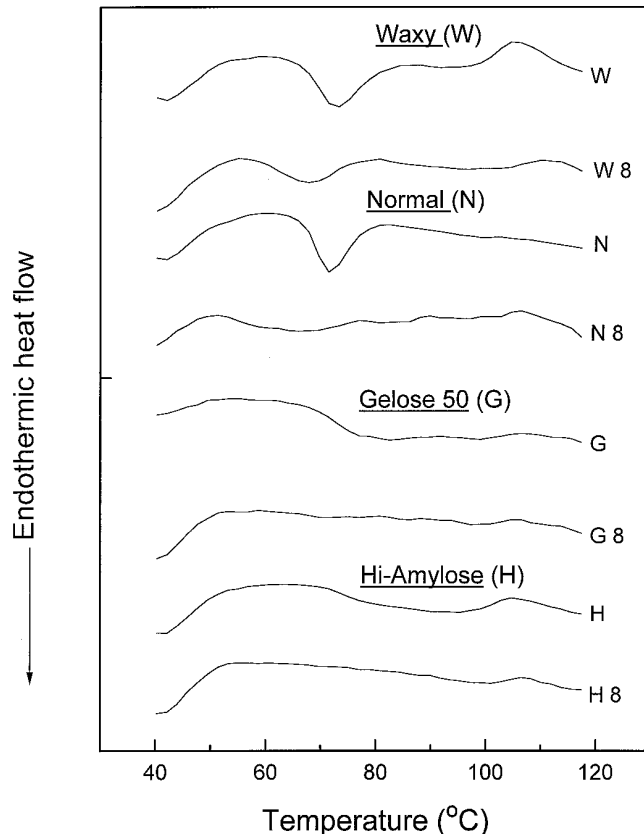
The clarity of starches was determined as described by Wu and Seib (1990). Starch paste (1%) was heated in a boiling water bath for 30 min and cooled to 25°C. The clarity was evaluated using percent transmittance at 650 nm against a water blank in a spectrophotometer.

### Enzymatic Digestibility Using α-Amylase

The method of Zhang et al (1995) was used with slight modification. Phosphate buffer (30 mL, 0.2M, pH 6.9 ) was mixed with starch in a 50-mL test tube (1.0 g, db). The starch was heated and stirred in a water bath at 95°C for 30 min. After cooling to 25°C, 0.15 mL of *Bacillus subtilis* α-amylase (1 mg/mL, 2,135 U/mg) (Sigma Chemical Co., St. Louis, MO) was added. Tubes were placed in a shaking water bath and incubated at 30°C. After 14 hr, 5 mL of 1.0% sulfuric acid was added to inactivate the enzyme. Samples



**Fig. 1.** Representative pasting curves of waxy (W), normal (N), Gelose 50 (G), and Hi-Amylose (H) starch phosphorylated at pH 6, 8, or 10 (according to suffix) or unphosphorylated (u). Rapid Visco Analyser units (RVU).



**Fig. 2.** Differential scanning calorimetry thermograms of waxy (W), normal (N), Gelose 50 (G), and Hi-Amylose (H) starch unmodified and phosphorylated at pH 8.

were centrifuged at  $2,000 \times g$  for 20 min. The resulting pellet containing the undigested flour residue was washed with 15 mL of 80% ethanol and centrifuged again under the same conditions. The residue was dried at  $80^\circ\text{C}$  to constant weight. Starch samples were evaluated without enzymatic hydrolysis to correct for initial concentration of soluble sugar in samples. Starch digestibility was expressed as percent of weight loss after  $\alpha$ -amylase digestion.

### Starch Color

The color of dry native and phosphorylated starches was measured in triplicate using a chromameter (CR-300, Minolta Co. Ltd., Tokyo, Japan).  $L^*$ ,  $a^*$ , and  $b^*$  chromaticity values were recorded.

### Freeze-Thaw Stability

The method of Wu and Seib (1990) was used with a starch paste. A starch suspension (6% db in water) was adjusted to pH 6 and heated in boiling water bath with shaking for 1 hr. After cooling to  $25^\circ\text{C}$ , 20 g of paste was weighed into 50-mL centrifuge tubes, tightly capped, and stored at  $4^\circ\text{C}$  for 24 hr before being subjected

to freeze-thaw cycles. Holding at  $4^\circ\text{C}$  accelerates starch retrogradation by nucleating the crystallization of starch and increases the severity of the test (Slade and Levine 1987). In each cycle, the samples were frozen at  $-23^\circ\text{C}$  for 22 hr and then transferred to a water bath at  $30^\circ\text{C}$  for 2 hr. After each 24-hr cycle, the sample was centrifuged ( $1,500 \times g$ , 20 min). The percentage of separated water was the ratio of the weight of the separated water to the weight of the paste. In this study, 18 freeze-thaw cycles were conducted.

Several variables must be controlled in determining the cold-temperature stability of starch pastes: 1) exact cooking conditions of temperature, time, and agitation; 2) exact freezing conditions; and 3) reproducible methods to separate and quantitate the water of syneresis (Wu and Seib 1990).

### Scanning Electron Microscopy

Starches were sprinkled on double-backed cellophane tape attached to a scanning electron microscope stub. The stub and samples were coated with gold-palladium and examined and photographed (Steke Oscan 440, Leica Cambridge Ltd., United Kingdom).

TABLE I  
Pasting Characteristics<sup>a</sup> of Native and Phosphorylated Maize Starches

Starch <sup>b</sup>	PV	HPV	CPV	BD	SB
W	391 ± 0.9 <sup>c</sup>	148 ± 1.1	172 ± 0.1	243 ± 1.5	24 ± 1.1
W6	386 ± 0.9	212 ± 0.9	214 ± 1.7	174 ± 0.1	2 ± 2.0
W8	455 ± 0.6	266 ± 0.9	310 ± 1.1	189 ± 1.5	44 ± 2.5
W10	443 ± 0.6	269 ± 1.1	294 ± 1.7	174 ± 1.7	25 ± 1.5
N	337 ± 0.6	142 ± 0.9	270 ± 0.9	195 ± 0.6	128 ± 2.1
N6	568 ± 0.1	182 ± 0.6	254 ± 1.7	386 ± 0.6	72 ± 1.2
N8	613 ± 1.1	225 ± 1.7	309 ± 0.7	388 ± 0.6	84 ± 2.0
N10	717 ± 0.1	228 ± 0.6	345 ± 0.1	489 ± 0.6	117 ± 0.6
G	0	103 ± 0.9	86 ± 0.9	0	0
G6	390 ± 1.5	225 ± 1.7	327 ± 0.1	165 ± 0.6	102 ± 1.7
G8	281 ± 0.9	208 ± 1.1	268 ± 0.7	73 ± 1.5	60 ± 1.5
G10	186 ± 0.6	180 ± 0.6	213 ± 1.7	6 ± 0.9	33 ± 2.1
Hi	0	17 ± 0.6	18 ± 0.6	0	1 ± 0.9
Hi6	42 ± 0.9	42 ± 0.9	44 ± 0.6	0	2 ± 1.5
Hi8	93 ± 0.6	90 ± 0.9	110 ± 0.6	3 ± 1.5	20 ± 0.6
Hi10	59 ± 0.1	59 ± 0.1	68 ± 1.7	0	9 ± 1.7

<sup>a</sup> PV = peak viscosity, HPV = hot paste viscosity, CPV = cool paste viscosity, BD = breakdown, and SB = setback measured in Rapid Visco Analyser units (RVU).

<sup>b</sup> W = waxy, N = normal, G = Gelose 50, and H = Hi-Amylose starch phosphorylated at pH 6, 8, or 10 (according to suffix) or unmodified.

<sup>c</sup> Values are means of triplicate determinations ± standard deviation.

TABLE II  
Pasting Characteristics<sup>a</sup> of Native and Phosphorylated Maize Starches in NaCl

Starch <sup>b</sup>	NaCl (M)	PV	HPV	CPV	BD	SB
W	0	391 ± 1.0 <sup>c</sup>	148 ± 0.6	172 ± 0.6	243 ± 0.6	24 ± 1.0
	0.05	400 ± 1.0	156 ± 0.6	184 ± 0.6	244 ± 1.2	28 ± 1.0
W6	0	386 ± 0.6	212 ± 1.5	214 ± 1.0	174 ± 1.0	2 ± 1.1
	0.01	370 ± 2.7	207 ± 1.0	213 ± 1.5	163 ± 3.5	6 ± 1.1
	0.05	325 ± 0.1	170 ± 0.9	198 ± 1.0	155 ± 1.5	28 ± 2.3
N	0	337 ± 0.6	142 ± 1.0	270 ± 0.6	195 ± 1.5	128 ± 0.6
	0.05	328 ± 2.6	202 ± 0.3	302 ± 0.6	126 ± 0.9	100 ± 0.6
N6	0	568 ± 0.9	182 ± 1.1	254 ± 1.7	386 ± 1.1	72 ± 0.6
	0.01	525 ± 1.6	165 ± 0.6	240 ± 1.5	360 ± 0.6	75 ± 2.1
	0.05	415 ± 1.7	143 ± 0.6	217 ± 1.7	272 ± 0.7	74 ± 2.0
G	0	0	103 ± 0.9	86 ± 1.5	0	0
	0.05	0	75 ± 0.6	52 ± 0.6	0	0
G6	0	390 ± 0.6	225 ± 1.2	327 ± 0.6	165 ± 0.6	102 ± 0.6
	0.01	293 ± 0.6	210 ± 1.7	286 ± 1.7	83 ± 1.5	76 ± 0.6
	0.05	205 ± 1.1	189 ± 0.6	207 ± 1.7	16 ± 0.1	18 ± 2.9
Hi	0	0	17 ± 0.9	18 ± 1.5	0	1 ± 1.5
	0.05	0	10 ± 0.6	10 ± 1.2	0	0
Hi6	0	42 ± 0.8	42 ± 1.5	44 ± 0.6	0	2 ± 2.0
	0.01	35 ± 1.0	35 ± 1.0	36 ± 0.9	0	1 ± 0.6
	0.05	21 ± 0.6	21 ± 0.6	21 ± 0.6	0	0

<sup>a</sup> PV = peak viscosity, HPV = hot paste viscosity, CPV = cool paste viscosity, BD = breakdown, and SB = setback measured in Rapid Visco Analyser units (RVU).

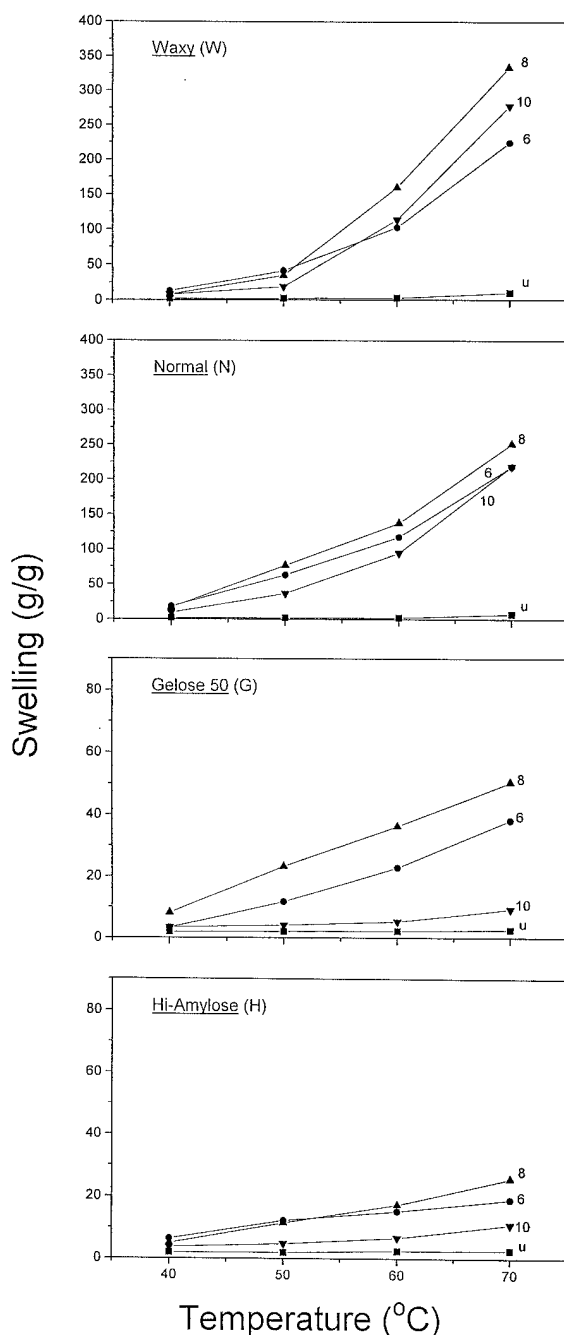
<sup>b</sup> W = waxy, N = normal, G = Gelose 50, and H = Hi-Amylose starch phosphorylated at pH 6 or unmodified.

<sup>c</sup> Values are means of triplicate determinations ± standard deviation.

## RESULTS AND DISCUSSION

The pasting properties of native and phosphorylated starches prepared at pH 6, 8, and 10 are shown in Fig. 1 and Table I. Phosphorylation had a much greater effect on  $T_p$  and PV of *ae* and normal starches than on *wx* starch. Because *wx* starch contained little amylose, it did not show appreciable changes in pasting properties after phosphorylation. The inclusion of negatively charged phosphate groups can cause interchain repulsion that prevents the close association necessary for interchain hydrogen bonding. In the normal and *ae* starches, the reduction of amylose chain bonding enabled more extensive hydration of the starch granule, resulting in greater swelling and giving a higher peak viscosity at a lower gelatinization temperature.

In normal starch, increased reaction pH led to a corresponding increase in PV, indicating that an increased level of phosphory-

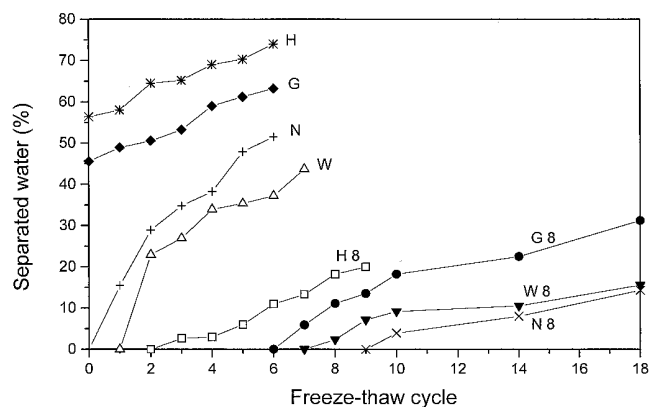


**Fig. 3.** Swelling (g/g) of waxy (W), normal (N), Gelose 50 (G), and Hi-Amylose (H) starch phosphorylated at pH 6, 8, or 10 (according to suffix) or unmodified (u).

lation had further reduced interchain bonding, facilitating granule swelling. With the Gelose 50 starch, however, the opposite was observed: an increase in pH leading to a decrease in PV. This could be due to the formation of distarch phosphates that are favored at high pH levels and act as cross-links that would restrict the swelling and hydration of the starch granule. With the highest amylose starch, Hi-Maize, the highest PV was observed at pH 8 and a reduction only occurred at pH 10. This may be due to the high amylose level of the starch, requiring a greater number of cross-links before the level of cross-linking is sufficient to influence the overall pasting properties or the formation of a reduced number of cross-links in the starch.

DSC thermograms of the native and phosphorylated maize starch prepared at pH 8 are shown in Fig. 2. After phosphorylation, the gelatinization enthalpy (data not shown) of all the starches greatly decreased, especially for *ae* and normal starches. By inhibiting double helix formation through hydrogen bonding, phosphorylation greatly reduces stability of the starch structure and, consequently, reduces energy required for the structural transitions in gelatinization.

The presence of NaCl with phosphorylated *ae*, *wx*, and normal starches caused a dramatic decrease in paste viscosity which was dependent on the NaCl concentration (Table II). The increase in ionic strength will cause shielding of the negative charges of phosphate groups and swelling of phosphorylated starches will generally



**Fig. 4.** Freeze-thaw stability (% separated water) of native waxy (W), normal (N), Gelose 50 (G), and Hi-Amylose (H) starch unmodified and phosphorylated at pH 8.

**TABLE III**  
Gel Texture of Native and Phosphorylated Maize Starches

Probe Size and Sample <sup>a</sup>	Hardness (g)	Adhesiveness (g/sec)	Springiness <sup>b</sup>	Cohesiveness <sup>b</sup>
5 mm				
N	66 ± 0.6 <sup>c</sup>	66 ± 0.9	0.94	0.48
N6	31 ± 0.6	0	0.97	0.66
N8	30 ± 0.8	0	1.00	0.71
N10	33 ± 0.5	15 ± 0.5	0.95	0.59
G	45 ± 0.8	45 ± 0.8	0.86	0.35
G6	62 ± 0.8	119 ± 0.9	0.96	0.58
G8	51 ± 1.0	108 ± 0.8	0.95	0.58
G10	49 ± 0.7	81 ± 1.0	0.97	0.45
Hi	15 ± 0.7	19 ± 0.7	0.52	0.32
Hi6	10 ± 0.9	32 ± 0.7	0.91	0.54
Hi8	24 ± 1.2	68 ± 0.6	0.93	0.56
Hi10	15 ± 1.1	33 ± 1.0	0.93	0.58
20 mm				
W	23 ± 1.1	5 ± 0.5	1.00	0.85
W6	28 ± 0.9	10 ± 0.1	0.97	0.85
W8	29 ± 1.0	22 ± 0.7	0.96	0.85
W10	29 ± 0.6	15 ± 0.6	0.96	0.86

<sup>a</sup> W = waxy, N = normal, G = Gelose 50, and H = Hi-Amylose starch phosphorylated at pH 6, 8, or 10 (according to suffix) or unmodified.

<sup>b</sup> Measurements from a texture analyzer. Standard deviation was ± 0.02 or less.

<sup>c</sup> Values are means of triplicate determinations ± standard deviation.

be reduced. For normal and *ae* starches, even in the presence of NaCl, paste viscosities of the phosphorylated starches all remained higher than the corresponding native starches. The anomalous behavior may be a reflection of higher levels of substitution in normal and *ae* starch. This will be the subject of further investigation.

All the starches showed increased swelling power after phosphorylation (Fig. 3), as anticipated from the pasting properties. The effects of increased cross-linking at high pH in *ae* starches

were apparent; swelling power was clearly reduced for the starches prepared at pH 10.

The gel texture of native starches and phosphorylated starches prepared at different pH levels is shown in Table III. After phosphorylation, the adhesiveness, springiness, and cohesiveness of all the starch gels was increased, except for the adhesiveness of normal starch gels and springiness of *wx* starch gels, which showed a decrease, and cohesiveness of *wx* starch gels, which did not show a change.

After phosphorylation, the hardness of normal starch gels decreased and that of *wx* starch gels increased; both of these effects were relatively independent of pH level. In the *ae* starches the highest value for gel hardness was observed at pH 6 in Gelose 50 and pH 8 with Hi-Maize. Gel properties in the *ae* starches may be influenced by variation in the extent of amylose leaching during gelatinization.

The clarity of phosphorylated starches prepared at different pH levels is shown in Table IV. After phosphorylation, the clarity of all the phosphorylated starches was greatly increased when compared with the corresponding unmodified starches. The highest clarity was observed with the *ae* starch and the lowest with *wx* starch. Phosphorylated starches, which are readily hydrated, can form pastes with high light transmittance. At high pH levels, however, there is a drop in clarity that reflects the increase in diester cross-linking of starch that will reduce the transmittance of the starch (Kerr and Cleveland 1959). The biggest loss of clarity at high pH levels was found with the *ae* starch Gelose 50, indicating that cross-linking will occur more readily in this starch.

The digestibility of native and phosphorylated starches prepared at different pH levels is given in Table IV. The digestibility of *ae* starches was greatly increased after phosphorylation, which suggested easier penetration by enzymes into the starch. However, the digestibility of *wx* and normal starches did not show any change after phosphorylation.

The color of native and phosphorylated starches prepared at different pH levels was shown in Table IV. After phosphorylation, the brightness ( $L^*$ ) of all starches decreased and redness ( $a^*$ ) increased with increasing pH level. The yellowness ( $b^*$ ) of all the phosphorylated starches was minimum at intermediate pH levels, with the highest value at pH 10. Alkaline conditions are expected to increase the yellow color of starches and this effect would appear to be unrelated to phosphorylation. Overall, the changes in color did not appreciably affect the usefulness of the modified starches.

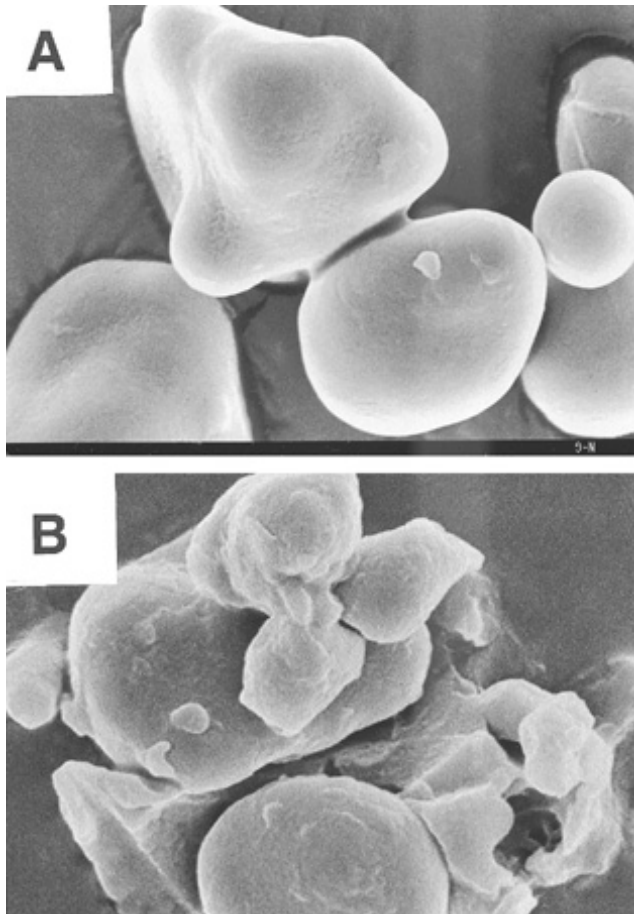


Fig. 5. Scanning electron micrographs of Gelose 50 starch phosphorylated at pH 8 (A) and unmodified (B).

TABLE IV  
Clarity, Digestibility, and Color of Native and Phosphorylated Maize Starches

Sample <sup>a</sup>	Clarity <sup>b</sup> (T%)	Digestibility (%)	Color		
			$L^*$	$a^*$ <sup>c</sup>	$b^*$ <sup>c</sup>
W	45.2 ± 0.3 <sup>d</sup>	98.9 ± 0.4	97.3 ± 0.3	-0.06	1.59
W6	83.9 ± 0.4	99.0 ± 0.4	97.2 ± 0.2	0.18	0.96
W8	85.5 ± 0.2	99.1 ± 0.1	95.4 ± 0.2	0.03	1.29
W10	83.9 ± 0.3	99.0 ± 0.2	94.5 ± 0.1	0.19	1.94
N	16.6 ± 0.3	90.3 ± 0.5	96.5 ± 0.3	-0.81	4.00
N6	80.0 ± 0.2	91.5 ± 0.6	96.2 ± 0.2	-0.52	3.01
N8	76.5 ± 0.2	90.9 ± 0.3	95.2 ± 0.2	-0.34	2.99
N10	71.4 ± 0.4	90.5 ± 0.4	95.9 ± 0.2	-0.25	3.07
G	2.7 ± 0.3	55.7 ± 0.5	96.2 ± 0.2	-0.80	5.47
G6	51.5 ± 0.6	77.2 ± 0.3	94.8 ± 0.4	-0.28	4.10
G8	30.3 ± 0.2	73.2 ± 0.3	94.0 ± 0.1	0.04	3.81
G10	12.3 ± 0.3	68.4 ± 0.3	91.9 ± 0.3	0.18	5.20
Hi	1.6 ± 0.1	40.1 ± 0.5	96.0 ± 0.3	-0.50	2.99
Hi6	5.0 ± 0.2	58.4 ± 0.4	94.6 ± 0.1	-0.01	2.98
Hi8	8.9 ± 0.2	59.4 ± 0.5	93.9 ± 0.1	0.24	3.40
Hi10	5.7 ± 0.1	54.4 ± 0.4	91.5 ± 0.5	0.63	5.17

<sup>a</sup> W = waxy, N = normal, G = Gelose 50, and H = Hi-Amylose starch phosphorylated at pH 6, 8, or 10 (according to suffix) or unmodified.

<sup>b</sup> Evaluated using % transmittance at 650 nm using a spectrophotometer.

<sup>c</sup> Standard deviation for all samples was ± 0.03 or less.

<sup>d</sup> Values are means of triplicate determinations ± standard deviation.

## LITERATURE CITED

The freeze-thaw stability of native and phosphorylated starches prepared at pH 8 is shown in Fig. 4. After phosphorylation, the freeze-thaw stability of all starches was greatly increased when compared with unmodified starches. Phosphorylated normal amylose starch showed an exceptionally high stability with no water loss occurring until after nine freeze-thaw cycles.

Figure 5 shows scanning electron microscopy of native and phosphorylated starch granules prepared at pH 8. The shape and size of granules was not affected by the phosphorylation reaction. The main visible effect was the presence of surface erosion after phosphorylation which could assist water penetration into the starch granule.

## CONCLUSIONS

Phosphorylation of starch shows considerable differences in its effects depending on the amylose content of the starch being modified. Waxy starch was relatively little affected by phosphorylation but this modification was of value for increasing freeze-thaw stability of the starch pastes. For normal and *ae* starches, there were significant changes in the pasting properties, freeze-thaw stability, and clarity that are all potentially beneficial in a large number of food applications. The *ae* starches can benefit from an increase in pasting viscosity that approaches the pasting viscosity observed in normal starch and extends the use of these starches. The potential for phosphate cross-linking of these starches also renders it possible to achieve high levels of freeze-thaw stability.

The greatly improved freeze-thaw stability after phosphorylation of all starches appears to be one of the most useful consequences of phosphorylation. However, these benefits are also apparent when phosphorylation is performed at lower pH levels where cross-linking will be reduced. Potentially, the improved water-holding capacity of phosphorylated starches is equally important to any increase in physical stability derived from cross-linking.

The differences reported here in starch properties after phosphorylation for starches of different amylose content may arise from 1) different levels of phosphorylation, 2) differences in the distribution of phosphate between amylose and amylopectin, 3) different types of phosphorylation, as either monophosphate or diphosphate cross-links.  $^{31}\text{P}$  nuclear magnetic resonance spectroscopy can distinguish among the various consequences of phosphorylation (Kasemsuwan and Jane 1996). Further work with this technique will examine the basis for the variation in phosphorylated starch properties.

## ACKNOWLEDGMENTS

Financial support was received from the Hong Kong Research Grants Council. We thank Di Miskelly and Starch Australasia Ltd., Lane Cove, Australia, for generous supply of starch samples.

- Hizukuri, S., Tabata, S., and Nikuni, Z. 1970. Studies on starch phosphates. *Starch/Staerke* 22:338-240.
- Jane, J., Xu, A., Radosavljevic, M., and Seib, P. A. 1992. Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chem.* 69:405-409.
- Kasemsuwan, T., and Jane, J. L. 1996. Quantitative method for the survey of starch phosphate derivatives and starch phosphate by  $^{31}\text{P}$  nuclear magnetic resonance spectroscopy. *Cereal Chem.* 73:702-707.
- Lim, S., and Seib, P. A. 1993. Preparation and pasting properties of wheat and corn starch phosphates. *Cereal Chem.* 70:137-144.
- Morrison, W. R. 1978. Cereal lipids. In: *Advances in Cereal Science and Technology*, Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Nierle, W. 1969. The influence of the manufacturing conditions on the properties of phosphorylated corn starches and their application. *Starch/Staerke* 21:13-18.
- Paschall, E. F. 1964. Phosphorylation with inorganic phosphate salts. *Methods Carbohydr. Chem.* 4:294-296.
- Posternak, T. 1951. On the phosphorous of potato starch. *J. Biol. Chem.* 188:317-325.
- Rutenberg, M. W., and Solarek, D. 1984. Starch derivatives: Production and uses. Pages 312-388 in: *Starch: Chemistry and Technology*, 2nd ed. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: London.
- Sanders, E. B., Thompson, D. B., and Boyer, C. D. 1990. Thermal behavior during gelatinization and amylopectin fine structure for selected maize genotypes as expressed in four inbred lines. *Cereal Chem.* 67:594-602.
- Slade, L., and Levine, H. 1987. Recent advances in starch retrogradation. Pages 387-430 in: *Recent Developments in Industrial Polysaccharides*. S. S. Stivala, V. Crescenzi, and I. C. M. Dea, eds. Gordon and Breach Science: New York.
- Solarek, D. B. 1986. Phosphorylated starches and miscellaneous inorganic esters. In: *Modified starches: Properties and Uses*. O. B. Wurzburg, ed. CRC Press: Boca Raton, FL.
- Subramanian, V., Hoseney, R. C., and Bramel-Cox, P. 1994. Shear thinning properties of sorghum and corn starches. *Cereal Chem* 71:272-275.
- Tabata, S., Nagata, K., and Hizukuri, S. 1975. Studies on starch phosphates. 3. On the esterified phosphates in some cereal starches. *Starch/Staerke* 27:333-335.
- Wang, Y. J., White, P., and Pollak, L. 1992. Thermal and gelling properties of maize mutants from the Oh43 inbred line. *Cereal Chem.* 69:328-334.
- Wu, Y., and Seib, P. A. 1990. Acetylated and hydroxylated distarch phosphate from waxy barley: Paste properties and freeze-thaw stability. *Cereal Chem.* 67:202-208.
- Zhang, D., Collins, W. W., and Andrade, M. 1995. Estimation of genetic variance of starch digestibility in sweetpotato. *Hort. Science* 30:348-349.

[Received January 19, 1999. Accepted August 30, 1999.]