

Preparation and Properties of Starch Phosphates Using Waxy, Common, and High-Amylose Corn Starches. I. Oven-Heating Method

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ABSTRACT

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The structure and physicochemical properties of waxy, common, and high-amylose corn starch phosphates prepared by oven heating were studied. Starch phosphates prepared by either slurry or dry-mixing treatment before oven heating were also compared. The slurry treatment more efficiently incorporated phosphorus into starch relative to the dry-mixing treatment under the reaction conditions studied. In general, the phosphorylated starch prepared by the slurry treatment exhibited a lower gelatinization temperature, a higher peak viscosity, a lesser degree of

retrogradation, and improved freeze-thaw stability compared with those prepared by the dry-mixing treatment. Phosphorylation occurred probably in both amylose and amylopectin, and the amount and location of incorporated phosphate groups varied with starch types likely due to their different amylose and amylopectin contents. Waxy starch was more prone to phosphorylation, followed by common and high-amylose starches, respectively.

The utilization of native starch in foods is limited by its physicochemical properties. Starch granules are insoluble in cold water and heat is required to achieve dispersion. Cooked native starch has a high viscosity that is not desirable in certain applications; it normally imparts a gummy, cohesive texture to food and tends to lose its viscosity and thickening power on further heating. Furthermore, retrogradation is a common feature of cooled starch paste, characterized by losing its water binding capacity, resulting in syneresis and water separation.

Chemically modified starches show markedly altered physicochemical properties as compared with the parent starches (Rutenberg and Solarek 1984). Phosphorylation is one of the starch modification methods designed to overcome the shortcomings mentioned above. The introduction of negatively charged phosphate groups reduces interchain associations and facilitates starch hydration. Starch phosphates can be grouped into two classes: monostarch phosphate and distarch phosphates. Starch phosphate monoester is formed when one starch hydroxyl group is esterified to phosphate. Starch phosphate diester is formed when two starch hydroxy groups are esterified to the same phosphate group. During phosphorylation, pH plays a dominant role in determining the ratio of starch monoester to diester. Phosphate monoesters are produced in a pH range of 5.0–6.5 with mixtures of orthophosphates and a pH range of 5.0–9.0 with sodium tripolyphosphate (STPP) (Kerr 1947). Lim and Seib (1993) reported that phosphorylated starch prepared at pH 9.5 showed better heat and shear stability and high paste consistency using a combination of 5% STPP and 2% sodium trimetaphosphate (STMP) in the presence of 5% sodium sulfate.

Reaction with STPP and STMP at pH 10 produced starch phosphates with excessive cross-linking, indicating starch diester formation prevailed in alkali-catalyzed reactions with STPP and STMP (Kerr and Cleveland 1959). Phosphorylation of starch with different phosphate salts has also been investigated (Kerr and Cleveland 1959, 1960, 1962; Paschall 1964; Nierle 1969; Lloyd 1970; Wurzburg et al 1979, 1980; Lim and Seib 1993). Phosphorylated starches give clear pastes with high viscosity, high solubility, greater swelling power, and improved freeze-thaw stability.

Starch can be impregnated with phosphate salts using a slurry treatment before heating by suspending starch in a prepared

aqueous phosphate solution. After pH adjustment, mixing, and filtering, varying amounts of phosphate salts are retained in the starch cakes. Alternate methods include dry mixing of starch and finely powdered phosphates or spraying phosphate solutions onto dry starch or filtered cakes (Wurzburg et al 1979).

Several types of corn starch with varying amylose-to-amylopectin ratios exist naturally through genetic mutation. Waxy corn starch is composed almost exclusively of amylopectin in the endosperm, while high-amylose corn starches contain reduced amounts of amylopectin. The special properties of different corn mutant starches, such as thermal behavior during gelatinization and altered starch structures, have been described (Sanders et al 1990; Wang et al 1992). Phosphorylation of common corn starch has also been investigated but phosphorylation of other corn mutants has not been reported. It is not known if varying amylose amounts would affect the susceptibility of starch to phosphorylation reaction.

This study aimed to compare starch phosphates from waxy, common, and high-amylose corn starches using the conventional oven-heating method with respect to the reactivity of different corn starches and the efficiency of slurry and dry-mixing treatments before oven heating.

MATERIALS AND METHODS

Materials

Starch samples, including waxy corn starch ($\approx 0\%$ amylose), common corn starch (25% amylose), and two high-amylose corn starches ($\approx 50\%$ amylose [Hylon V] and $\approx 70\%$ amylose [Hylon VII]) were received from the National Starch and Chemical Company (Bridgewater, NJ).

Preparation of Starch Phosphates

Each starch was mixed with sodium tripolyphosphate (STPP) and sodium trimetaphosphate (STMP) using the slurry treatment as described by Lim and Seib (1993) as well as a dry-mixing treatment as described below before oven heating. The level of modification was chosen according to Lim and Seib (1993) to prepare starch phosphates with nearly the maximum phosphorus (0.4%) allowed by regulation (CFR 1991) using oven heating. Only one level of phosphorylation was conducted to study the effects of starch type and sample pretreatment. The reaction range was pH 9.0–9.5 to achieve high stability to heat and shear and more consistency after cooling when using a combination of STMP and STPP (Lim and Seib 1993; Muhammad et al 2000). The mixture was placed in an oven at 130°C for 2 hr to effect phosphorylation. Native starches as well as oven-treated native starches were included as references.

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Slurry Treatment

STPP (15 g) and STMP (6 g) were dissolved in 300 mL of deionized (DI) water containing 15 g of sodium sulfate. The solution was adjusted to pH 9.0 with 10% NaOH. Starch (300 g, db) was then dispersed in this solution. The solution was readjusted to pH 9.0 with 5% NaOH. A small amount of water was added to make a final dispersion of 45% solid. The slurry was stirred at room temperature for 1 hr and thereafter dried in an oven at 40°C to a final moisture content of 10–15% (as is).

Dry-Mixing Treatment

The moisture content of starch (300 g, db) needed to be adjusted to 15% (as is) before oven heating. The necessary additional water was used to dissolve 15 g of sodium sulfate, 15 g of STPP, and 6 g of STMP. The solution was adjusted to pH 9.0 with 5% NaOH and was added incrementally to the starch while mixing. The resulting starch was mixed in a mixer (KitchenAid, St. Joseph, MI) for an additional 30 min before proceeding to the oven-heating process.

Phosphorus Content

The phosphorus content of starch phosphates was determined according to a standard analytical method (CRA 1999). The starch phosphate was washed with methanol and water (70:30, v/v) and then oven dried at 40°C. The moisture content of the dried sample was determined using an infrared moisture analyzer. Dried sample (5 g, db) was placed in an evaporating dish and 10 mL of 10% (w/v) zinc acetate was uniformly added to the starch. The sample was allowed to evaporate to dryness in a steam bath and charred on a hot plate afterwards. The charred sample was placed in a muffle furnace at 550°C for 2 hr to destroy organic matter and convert phosphorus to inorganic phosphates. The sample was then allowed to cool at room temperature before adding 3 mL of 29% (w/v) nitric acid. The starch-acid solution was refluxed at 550°C for 30 min to ensure a complete conversion. The sample was cooled again and 10 mL of 29% nitric acid was added. DI water (15 mL) was added to the solution and it was allowed to boil on a hot plate for 10 min. The heated solution was filtered into a 250-mL volumetric flask and diluted with DI water to the mark. An aliquot of 15 mL was then put into a 100-mL volumetric flask. Nitric acid 29% (w/v) (15 mL) was added before the addition of 10 mL of 25% (w/v) ammonium vanadate and 10 mL of 5% (w/v) ammonium molybdate. The solution was diluted to the mark with DI water and allowed to stand for 15 min before spectrophotometric measurement as molybdovanadophosphoric acid complex at 460 nm.

Pasting Properties

Pasting properties of starch phosphates from waxy and common corn (7%, db), Hylon V (10%, db), and Hylon VII (15%, db) were measured using micro-viscoamylography (C.W. Brabender Instruments, South Hackensack, NJ). The slurries were first held at 50°C for 1 min, heated to 95°C at a programmed rate of 3°C/min, held at 95°C for 3 min, and cooled to 50°C at 3°C/min. Pasting temperature, peak viscosity, breakdown, and total setback were obtained from the profile. Unmodified starch and oven-treated unmodified starch, which was subjected to 130°C for 2 hr without the addition of chemicals, were also run as references.

Thermal Properties

Thermal properties of starch phosphates were assessed using differential scanning calorimetry (DSC) (Pyris-1, Perkin-Elmer Co., Norwalk, CT) to assess the effect of phosphate groups on the stability of the phosphorylated starch. The instrument was calibrated with indium and an empty pan was used as a reference. Starch (20.0 mg, db) was weighed into a standard stainless steel DSC pan. DI water (40 µL) was added using a microsyringe. The pan was sealed and allowed to stand for at least 1 hr before

thermal analysis. Thermal scanning was done from 25 to 150°C at a heating rate of 10°C/min. Onset and peak temperatures and enthalpy were automatically computed. Gelatinized samples were stored at 4°C for 14 days and rescanned to determine the enthalpy changes due to retrogradation. The degree of retrogradation was determined as the ratio of enthalpy change of retrograded starch to the enthalpy change of gelatinized starch.

Swelling Power

Swelling power was measured by suspending a 1.7% starch suspension (0.5 g of dry starch in 30 mL of DI water) into a 50-mL capped centrifuge tube. The tube was placed in a water bath of 65, 85, or 95°C for 30 min. The heated sample was rapidly cooled to room temperature in an ice-water bath and centrifuged at $7,700 \times g$ for 20 min. The swelling power was determined by measuring the sedimented paste weight divided by its initial weight (Holm et al 1985). This was done to determine the effect of phosphate groups on the swelling characteristics of the starch granules.

Freeze-Thaw Stability

The starch pastes obtained after each micro-viscoamylography measurement were used to determine the freeze-thaw stability following the method of Wu and Seib (1990). The paste was cooled to 25°C, and then exactly 20 g of the paste was weighed into a preweighed centrifuge tube and stored at 4°C for 24 hr before starting the first cycle for a total of seven cycles. In each cycle, the tubes were placed in a freezer (-23°C) for 22 hr and thawed in a 30°C water bath for 2 hr. After the thawing cycle, the tubes were centrifuged at $1,500 \times g$ for 15 min, and the amount of liquid separated from each tube was recorded.

β -Amylolysis Limit

The β -amylolysis limit of starch phosphates from different treatments were determined following the procedure of Hizukuri et al (1981). DI water (4 mL) was added to a test tube containing 9 mg of starch sample. The test tube was placed in a boiling water bath for 30 min with stirring and then cooled to room temperature. Thereafter, 5 mL of 50 mM acetate buffer (pH 5.0) and 30 µL of β -amylase (potato, 150 U, Sigma Chemical) were added to the solution and the solution was incubated at 30°C for 3 hr. After incubation, 1 mL of aliquot was removed and the amounts of reducing sugar and total carbohydrates were determined following the methods of Somogyi (1952) and Nelson (1944) and the phenol-sulfuric acid method of Dubois et al (1956), respectively. A blank solution without the addition of enzyme was used as the reference. The β -amylolysis limit (%) was determined using the formula β -amylolysis limit (%) = (reducing sugar determined as maltose/total carbohydrate as maltose) \times 100.

β -Limit Dextrin

The precipitate from the β -amylolysis limit analysis (β -limits dextrin) was recovered for the structure characterization. Starch (20 mg) was added to a test tube containing 10 mL of DI water.

TABLE I
Phosphorus Contents of Starch Phosphates from Four Different Starch Types and Two Sample Treatments Using the Oven-Heating Method^a

Sample	Treatment	Phosphorus Content (% db)
Waxy	Slurry	0.570a
	Dry-mixing	0.513b
Common	Slurry	0.393c
	Dry-mixing	0.326e
Hylon V	Slurry	0.307f
	Dry-mixing	0.282g
Hylon VII	Slurry	0.340d
	Dry-mixing	0.310f

^a Mean values of two replicates followed by a common letter are not significantly different based on the Tukey test ($P < 0.05$).

Then the solution was allowed to stand in a boiling water bath for 30 min before the addition of 12 mL of 50 mM acetate buffer and 80 μ L of β -amylase. The mixture was incubated at 30°C for 3 hr, followed by centrifugation. The collected precipitate was washed with 90% methanol five times and DI water two times. The washed sample was redissolved in 5 mL of DI water before the addition of 0.7 mL of 0.1M acetate and 30 μ L of isoamylase (*Pseudomonas amylodeamosa*, 1,250,00 U, Sigma Chemical) and then incubated at 40°C for 48 hr. After incubation, the solution was heated in boiling water for 30 min. A mixed-bed exchange resin (IONAC NM-60, J.T. Baker) was added to the debranched solution for 1 min to eliminate the interference from the buffer before chromatography analysis. Then the solution was filtered through a 0.45- μ m membrane and analyzed by high-performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD) (Dionex DX500, Sunnyvale, CA) (Kasemsuwan et al 1995). The system consisted of an LC20-1 chromatography organizer, GP50 gradient pump, ED40 electrochemical detector, 4 \times 50-mm CarboPac PA1 guard column, 4 \times 250-mm CarboPac PA1 analytical column, and AS40 automated sampler.

Statistical Analyses

All analyses were done in duplicate. Experimental data were analyzed by using ANOVA (SAS Institute, Cary, NC). Significant differences among treatments and starch types were identified using the Tukey test at the 95% confidence level.

RESULTS AND DISCUSSION

Phosphorus Content

The phosphorus content of starch phosphates from four different starch types and two sample treatments using the oven-heating method are shown in Table I. The slurry treatment was more effective when comparing the phosphorylation efficiency of the two sample treatments. The suspension in the slurry treatment was maintained at pH 9.0 for 1 hr with mixing and dried to remove excessive moisture before oven heating. The pH of the starch mixture using the dry-mixing treatment was likely lower

than pH 9.0 due to the acidic nature of starch and the phosphorylation reaction, although the same amount of phosphate salts was added. Nevertheless, the decrease in pH would not have contributed to the significant differences observed in phosphorylation efficiency between the dry-mixing and slurry treatments using STPP and STMP because Lim and Seib (1993) and Muhammad et al (2000) demonstrated that the total phosphorus level was higher in starch phosphates prepared at pH < 9 than those prepared at pH \geq 9 under the same reaction conditions. Therefore, it is suspected that the slurry treatment using a suspension of starch, phosphate salts, and water permitted better mixing before oven phosphorylation, thus resulting in higher phosphorylation efficiency. The much larger amount of water (55%) in the slurry treatment was sufficient to hydrate and distribute the phosphate salts to most starch granules to effect phosphorylation. In contrast, the small amount of water (15%) in the dry-mixing treatment possibly allowed limited starch hydration. More phosphate salts were present in areas such as amorphous regions where fewer starch molecules were present with the dry-mixing treatment, whereas more phosphate salts were distributed to semicrystalline regions where most starch molecules were present with the slurry treatment. Therefore, lowered phosphorylation efficiency occurred in samples prepared by the dry-mixing treatment relative to samples prepared by the slurry treatment.

Among different types of starches, waxy starch had the largest amount of incorporated phosphorus, followed by common starch. Two high-amylose starches had lower phosphorus contents. These results implied that phosphorylation occurred in both amylose and amylopectin. It has been reported that the linear segments in amylopectin were as reactive as amylose (Morrison et al 1984; Batres and White 1986; Eliasson and Ljunger 1988). Schierbaum et al (1986) found that the linear segments of amylopectin, amylose, or dextrans of a certain critical length can interact or react with other chemicals in a solution. It is hypothesized that amylopectin might be more reactive than amylose in a dry reaction such as phosphorylation because of its branching structure that helped retain phosphate salts within the starch molecules (i.e., semicrystalline regions), thus allowing for enhanced phosphorylation. Otherwise, the introduced phosphate salts might be distributed more in the amorphous regions where mostly water is present. However, Sitohy et al (2000) reported that corn amylose bound a larger amount of phosphate than corn amylopectin. It should be noted that purified amylose instead of high-amylose corn starches was used in their study. Other possibilities that might cause the observed discrepancy included different sample preparations where they filtered the salt solution out after mixing, use of a vacuum oven to effect phosphorylation, and use of different phosphate salts ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and Na_2HPO_4) in their study.

Pasting Properties

The pasting profiles of native and phosphorylated starches from each starch type prepared using the slurry and dry-mixing treatments are shown in Figs. 1 and 2. Phosphorylated starches exhibited much higher peak, final, and total setback viscosities and swelled earlier compared with their parent starches (Solarek 1986; Wu and Seib 1990; Lim and Seib 1993; Liu et al 1999; Sitohy et al 2000; Becker et al 2001). The introduced negatively charged phosphate groups are known to cause interchain repulsion and reduce hydrogen bond formation among adjacent molecules. This enables extensive water penetration and starch granule hydration, resulting in a lower gelatinization temperature, greater swelling, and higher peak viscosity (Liu et al 1999). The extent of decrease in the pasting temperature from introduced phosphorus decreased when starch amylose content increased for amylose-containing starches, suggesting amylose inhibited starch swelling (Tester and Morrison 1990).

Starch phosphates prepared using the slurry treatment showed higher peak and final viscosities compared with those prepared

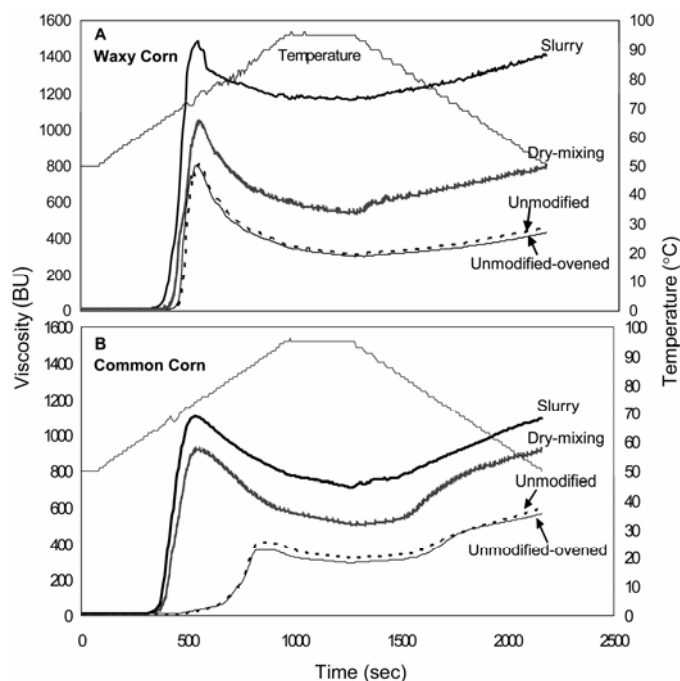


Fig. 1. Representative pasting curves of phosphorylated and unmodified (A) waxy (7%) and (B) common (7%) corn starches using slurry and dry-mixing treatments.

using the dry-mixing treatment. This was attributed to the higher efficiency of the slurry treatment in incorporating more phosphate groups into the starch by better mixing. The location where the phosphorus was introduced might also be different for starch phosphates prepared using two different treatments. The viscosity of the oven-treated unmodified starch was slightly lower than that of native unmodified starch, presumably because of the heat-moisture treatment that slightly increased its crystalline structure. Less breakdown was observed for the slurry-reacted waxy starch compared with that of the native starch, which indicates the occurrence of cross-linking; however, cross-linking was not observed in other phosphorylated starches.

Thermal Properties

The thermal properties of unmodified and phosphorylated starches are summarized in Table II. A significant decrease in onset and peak temperatures was observed in all phosphorylated starches as compared with their parent starches. Significant differences were also observed in enthalpy and degree of retrogradation.

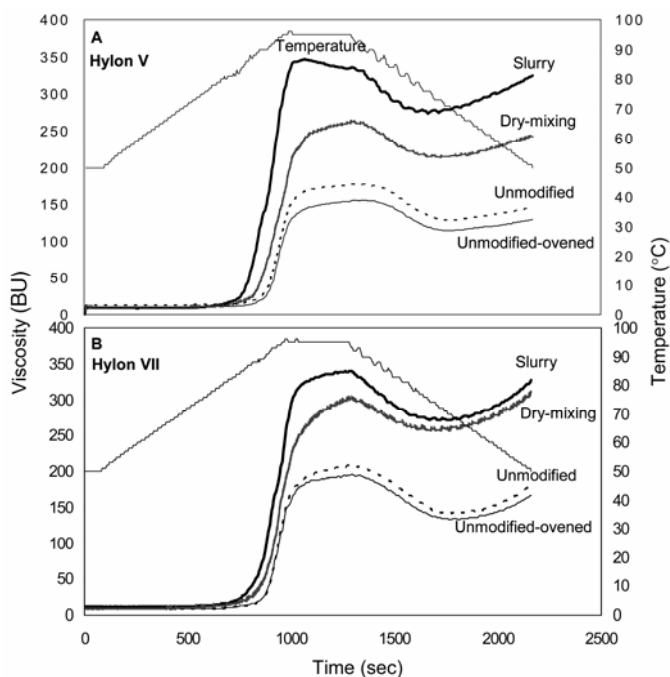


Fig. 2. Representative pasting curves of phosphorylated and unmodified (A) Hylon V (10%) and (B) Hylon VII (15%) corn starches using slurry and dry-mixing treatments.

Liu et al (1999) proposed that phosphorylation reduced the stability of starch structure by inhibiting the formation of double helices through hydrogen bonding, thus reducing the energy required for the structural transition in gelatinization. The present results agreed with previous reports (Lim and Seib 1993; Lin and Czuzhajowska 1998) that the introduction of phosphorus decreases the gelatinization temperatures of starches. Using X-ray and NMR spectroscopy, Cooke and Gidley (1990) also demonstrated that gelatinization enthalpy was proportional to granule stability, thus supporting the role of the phosphate groups in destabilizing starch granules.

In general, slurry-reacted starch phosphates showed a lower onset temperature, enthalpy, and degree of retrogradation compared with those prepared by the dry-mixing treatment; however their peak gelatinization temperatures were similar. The higher phosphorus content and possibly different locations of the introduced phosphates by the slurry treatment contributed to observed differences in gelatinization properties. The steric hindrance and repulsive force from negatively charged phosphate groups destabilized the starch structure, thus phosphorylated starches have lower gelatinization

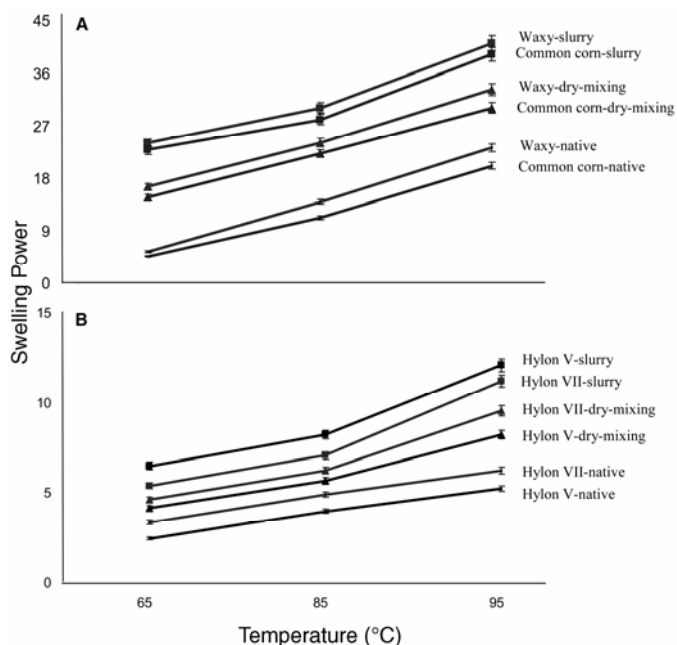


Fig. 3. Swelling power of native and phosphorylated starches from four different starch types and two sample treatments using the oven-heating method. A, Waxy and common corn starches. B, Hylon V and Hylon VII corn starches.

TABLE II
Thermal Properties of Unmodified and Phosphorylated Starches from Four Different Starch Types and Two Sample Treatments Using the Oven-Heating Method^a

Samples	Treatment	Gelatinization			Degree of Retrogradation (%) 14-days
		Onset Temp (°C)	Peak Temp (°C)	Enthalpy (J/g)	
Waxy	Unmodified	70.3c	76.1d	12.7a	35.2d
	Slurry	58.9i	64.4h	10.1c	12.5i
	Dry-mixing	59.9h	64.7h	11.8ab	13.4h
Common	Unmodified	71.0bc	74.5e	11.0b	37.9c
	Slurry	61.9g	66.1g	7.3f	13.7h
	Dry-mixing	63.0f	66.8g	8.2ef	14.5g
Hylon V	Unmodified	71.9b	80.1b	10.2c	47.9b
	Slurry	65.5e	72.6f	8.6de	16.7f
	Dry-mixing	65.9e	73.3ef	9.9c	17.2f
Hylon VII	Unmodified	74.5a	82.9a	11.7ab	53.7a
	Slurry	66.0e	77.9c	8.1ef	26.5e
	Dry-mixing	68.1d	77.4cd	9.6cd	26.9e

^a Mean values of two replicates in a column followed by a common letter are not significantly different based on the Tukey test ($P < 0.05$).

temperature range and are more resistant to chain association upon retrogradation. Because amylopectin is arranged in a semi-crystalline structure within starch granules and waxy starch is composed almost exclusively of amylopectin, waxy starch required more energy to melt even after phosphorylation when compared with common and high-amylose starches, which are composed of ≈25–75% amylopectin (Eliasson and Gudmundsson 1996).

Phosphorylation effectively reduced the tendency of starch retrogradation during storage. The decrease in the degree of retrogradation ranged from 21.8% for waxy starch from the dry-mixing treatment to 31.2% for Hylon V starch from the slurry treatment. Among the phosphorylated starches, Hylon VII exhibited the highest degree of retrogradation. The greater proportion of longer chains (degree of polymerization, DP > 30), which tended to quickly reorganize to form double helices after heating, was ascribed to the observed rapid retrogradation in high-amylose starches (Thompson and Blanshard 1995; Fisher and Thompson 1997; Klucinec and Thompson 1999). Varavinit et al (2003) rationalized the positive correlation of amylose and the degree of retrogradation. They also stated that the inability of waxy and common starches to quickly reassociate might be due to a large proportion of short chains (DP < 30) and the branch nature of amylopectin that limited the interaction of neighboring chains. Although phosphorylation effectively impeded starch reassociation, the inherent differences in terms of retrogradation tendency among the four starch types was still evident. Hylon VII had a more retrogradation than the other starches.

Swelling Power

Figure 3 depicts the swelling behavior of all starches. There was an increase in swelling power for all the starches after

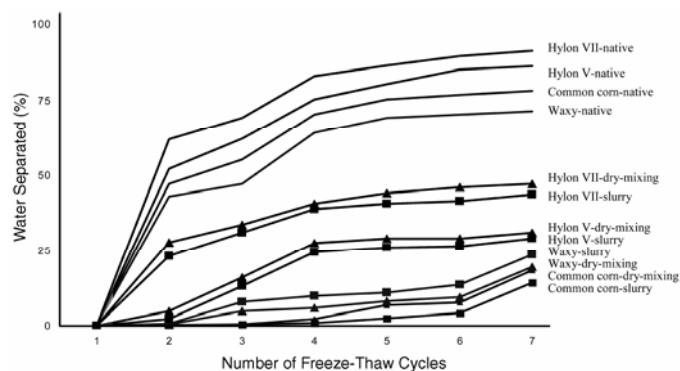


Fig. 4. Freeze-thaw stability of native and phosphorylated starches from four different starch types and two sample treatments using the oven-heating method.

phosphorylation, as anticipated. Because swelling behavior of cereal starches was primarily related to the amylopectin fraction (Tester and Morrison 1990), and the rigidity of the granule was inversely proportional to the swelling power (Lii et al 1996), the high swelling power suggested less rigid structure of phosphorylated starches. Phosphate groups in starch clearly improved water hydration and retention, thus increasing starch swelling. Starch phosphates prepared by the slurry treatment gave a higher swelling power relative to those prepared by the dry-mixing treatment, agreeing with previous pasting results (Figs. 1 and 2).

Freeze-Thaw Stability

The freeze-thaw stability of native and phosphorylated starches is displayed in Fig. 4. Several variables were carefully controlled when determining the cold temperature stability of starch (Wu and Seib 1990). At the initial stage of the experiment, the samples were stored at 4°C for 24 hr to initiate nucleation and to manifest a severe freeze-thaw condition (Slade and Levine 1987).

Little syneresis was observed in the first freeze-thaw cycle; however the rate of water loss increased afterwards. This phenomenon was due to the reassociation of starch chains, which begins with nucleation and then is followed by propagation until crystal perfection (Slade and Levine 1987). Phosphorylated starch retained more water after each cycle than did its unmodified counterpart. The repulsive effect of introduced phosphate groups within the starch molecules prevented reassociation and promoted water binding. Among the phosphorylated starches, common and waxy starch phosphates experienced less syneresis than did high-amylose starch phosphates. The introduced phosphate groups were not sufficient to block strong reassociation of starch molecules, mainly from amylose, in the two high-amylose corn starch phosphates. Through the cycles, the texture of all starch pastes changed from a smooth gel to a rough-textured porous gel with a spongy structure.

Structural Characteristics

Table III summarizes the structural characteristics of different starch phosphates. It was apparent that phosphorylated starches had lower β-amylolysis limits as compared with their unmodified counterparts. β-Amylolytic limit represents the percent release of reducing sugar from β-amylase hydrolysis. β-Amylase hydrolyzed starch polymers exowise at the second α-(1→4) linkages from the nonreducing terminal residue until near the branch linkages, thus both β-maltose and β-limit dextrans were produced. The decrease in β-amylolysis limits among phosphorylated starches indicated the inhibitory effect of introduced phosphate groups on β-amylase action. Many theories regarding the blocking mechanism of phosphate groups have been proposed. Feller et al (1996) and D'Amico et al (2000) proposed that the phosphate group competed for

TABLE III
Structural Characteristics of Unmodified and Phosphorylated Starches from Four Different Starch Types and Two Sample Treatments Using the Oven-Heating Method^a

Samples	Treatment	β-Amylolytic Limit (%)	β-Limit Dextrin, % Area			
			DP 1	DP 2	DP 3	DP 4
Waxy	Unmodified	54.1b	6.4d	85.8a	3.0j	4.7i
	Slurry	43.3 f	2.2g	10.8h	80.0b	7.0g
	Dry-mixing	48.7d	2.3g	12.0g	63.8f	21.7b
Common	Unmodified	61.6a	1.5h	16.9e	72.4 d	9.3f
	Slurry	40.1g	2.8g	3.2i	90.5a	3.5j
	Dry-mixing	44.4e	5.6e	9.2i	69.1e	16.1d
Hylon V	Unmodified	50.1c	2.8g	6.0j	76.3c	14.7e
	Slurry	37.6h	6.1d	13.2f	72.9d	7.8g
	Dry-mixing	40.6g	17.7a	73.5b	8.5i	0.3k
Hylon VII	Unmodified	43.5f	3.2f	5.2k	72.9d	18.7c
	Slurry	31.0j	11.3b	54.9c	28.4g	5.5h
	Dry-mixing	35.7i	10.4c	20.8d	15.9h	53.0a

^a Mean values of two replicates in a column followed by a common letter are not significantly different based on the Tukey test ($P < 0.05$).

metal ions that are essential to enzyme activity. Evers et al (1997) discovered that the degradation by β -amylase was incomplete when using 2-deoxy-maltooligosaccharides as the substrate, and the substitution of an hydroxyl group by phosphate may have affected β -amylase activity.

The difference in β -amylolysis limit among starch phosphates might be attributed to a combined effect of the amylose content and the amount and location of incorporated phosphates. Both unmodified high-amylose starches had a lower β -amylolysis limit compared with common and waxy starches, presumably because of their low solubility that hindered β -amylase action (Klucinec and Thompson 1998). The slurry-reacted starch phosphates had a lower β -amylolysis limit than those prepared by the dry-mixing treatment, which was ascribed to the higher phosphorus content and possibly different locations of introduced phosphates in slurry-reacted starch phosphates.

When the β -limit dextrins of unmodified and phosphorylated starches were debranched with isoamylase and analyzed by HPAEC-PAD, the location of phosphate groups within the starch can be hypothesized (Table III). A large percentage of DP 1–2 suggested that the phosphate groups were bound closer to the branch points, whereas a large percentage of DP 3–4 implied the phosphate groups were located near the nonreducing ends. Waxy and common corn starch phosphates prepared using both treatments contained a larger proportion of DP 3–4 compared with their parent starches, agreeing with their β -amylolysis limit results. A higher percentage of DP 4 was noted in starch phosphates prepared by dry mixing than in those prepared by slurry, indicating that more phosphate groups were bound closer to the branching points in slurry-reacted starch phosphates as compared with starch phosphates prepared by dry mixing. However, this trend was not observed in Hylon V and VII starch phosphates according to their β -limit dextrin structure analysis by HPAEC-PAD, although their β -amylolysis limit results supported this trend. Amylose is mostly linear in nature in contrast to the highly branched structure of amylopectin. Phosphorylation was proposed to occur in both amylose and amylopectin as previously discussed, but the location of phosphorylation in amylose might be random, whereas phosphorylation in amylopectin might occur mostly in the amorphous lamellae that primarily constitute the branching structure of amylopectin, which might contribute to the observed discrepancy in structural characteristics of the β -limit dextrins among starches with different amylose-to-amylopectin ratios.

CONCLUSIONS

This study demonstrated that the degree and location of phosphorylation varied with starch composition and type of treatment when using oven heating to effect phosphorylation. The slurry treatment was more effective in incorporating phosphate groups into starch than the dry-mixing treatment. The improved phosphorylation of the slurry treatment was attributed to its use of a large amount of water that was able to distribute phosphate salts to more starch molecules relative to the limited amount of water in the dry-mixing treatment. The phosphorylated starch prepared by the slurry treatment exhibited a higher peak viscosity, a lower gelatinization temperature, a lesser degree of retrogradation, and improved freeze-thaw stability compared with those prepared by dry mixing. Phosphorylation probably occurred in both amylose and amylopectin, and amylopectin was proposed to be more prone to phosphorylation.

LITERATURE CITED

Batres, L., and White, P. J. 1986. Interaction of amylopectin with mono-glycerides in model system. *J. Am. Oil Chem. Soc.* 63:1537-1542.
Becker, A., Sandra, E. H., and Mitchell, J. R. 2001. Relevance of amylose-lipid complexes to the behavior of thermally processed starches. *Starch* 53:121-130

CFR. 1991. Code of Federal Regulation. Food Starch-Modified. Title 21, Chapter 1, Part 172, Sect. 172.892 in: *Food Additives Permitted in Food for Human Consumption*. GPO: Washington, DC.
Cooke, D., and Gidley, M. J. 1990. Loss of crystalline and molecular order during starch gelatinization: Origin of enthalpic transition. *Carbohydr. Res.* 20:103-112.
CRA. 1999. *Standard Analytical Methods of the Member Companies of Corn Refiners Association*. 6th Ed. The Association: Washington DC.
D'Amico, S., Gerday, C., and Feller, G. 2000. Structural similarities and evolutionary relationships in chloride-dependent α -amylase. *Gene* 253:95-105.
Dubois, M., Gillies, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Phosphorylating starch. *Anal. Chem.* 8:350-354.
Eliasson, A. C., and Gudmundsson, M. 1996. Starch: Physicochemical and functional aspects. Pages 431-503 in: *Carbohydrates in Foods*. A. C. Eliasson, ed. Marcel Dekker: New York.
Eliasson, A. C., and Ljunger, G. 1988. Interactions between amylopectin and lipid additives during retrogradation in a model system. *J. Sci. Food Agric.* 44:353-358.
Evers, B., Petricek, M., and Thiem, J. 1997. Specificity of amylases and cyclodextrins-glucanotransferase in reactions with 2-deoxymaltoligosaccharides. *Carbohydr. Res.* 300:153-159.
Feller, G., Le Bussy, O., Houssier, C., and Gerday, C. 1996. Structural and functional aspects of chlorine binding to alteromonas haloplanetis α -amylase. *J. Biol. Chem.* 271:23836-23841.
Fisher, D. K., and Thompson, D. B. 1997. Retrogradation of maize starch after thermal treatment within and above the gelatinization temperature range. *Cereal Chem.* 74:344-351.
Hizukuri, Y., Takeda, Y., and Yasuda, M. 1981. Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* 94:205-213.
Holm, J., Björck, L., Asp, N., Sjöberg, L. B., and Lundquist, I. 1985. Starch availability in vitro and in vivo after flaking steam cooking and popping of wheat. *J. Cereal Sci.* 3:193-206.
Kasemsuwan, T., Jane, J.-L., Schnable, P., Stinard, P., and Robertson, D. 1995. Characterization of the dominant mutant amylose-extender (*ael*-5180) maize starch. *Cereal Chem.* 72:457-464.
Kerr, R. W. 1947. Orthophosphate ester of starch. U.S. patent 2,884,413.
Kerr, R. W., and Cleveland, Jr., F. C. 1959. Orthophosphate esters of starch. U.S. patent 2,884,413.
Kerr, R. W., and Cleveland, Jr., F. C. 1960. Process for preparing inorganic starch ester. U.S. patent 2,961,440.
Kerr, R. W., and Cleveland, Jr., F. C. 1962. Thickening agent and method of making the same. U.S. patent 3,021,222.
Klucinec, J. D., and Thompson, D. B. 1998. Fractionation of high-amylose maize starches by differential alcohol precipitation and chromatography of the fractions. *Cereal Chem.* 75:887-896.
Klucinec, J. D., and Thompson, D. B. 1999. Amylose and amylopectin interact in retrogradation of dispersed high-amylose starches. *Cereal Chem.* 76:282-291.
Lii, C.-Y., Tsai, M.-L., and Tseng, K. H. 1996. Effects of amylose content on the rheological properties of rice starch. *Cereal Chem.* 73:415-420.
Lim, S., and Seib, P. A. 1993. Preparation and pasting properties of wheat and corn starch phosphates. *Cereal Chem.* 57:137-144.
Lin, P. Y., and Czuzhajowska, Z. 1998. Role of phosphorus in viscosity, gelatinization and retrogradation of starch. *Cereal Chem.* 75:705-709.
Liu, H., Ramsden, L., and Corke, H. 1999. Physical properties and enzymatic digestibility of phosphorylated ae, wx, and normal maize starch prepared at different pH levels. *Cereal Chem.* 76:943-983.
Lloyd, N. E. 1970. Starch esters. U.S. patent 3,539,551.
Morrison, W. R., Milligan, T. P., and Azudin, M. N. 1984. A relationship between the amylose and lipid contents of starches from diploid cereals. *J. Cereal Sci.* 2:257-261.
Muhammad, K., Hussin, F., Man, Y. C., Ghazali, H. M., and Kennedy, J. F. 2000. Effect of pH on phosphorylation of sago starch. *Carbohydr. Polym.* 42:85-90.
Nelson, N. 1944. A photometric adoption of the Somogyi method for the determination of glucose. *J. Biol. Cereal Chem.* 70:385-391.
Nierle, V. W. 1969. The influence of manufacturing conditions on the properties of phosphated corn starched and their application. *Starch* 21:13-18.
Paschall, E. F. 1964. Phosphation with inorganic phosphate salts. Pages 294-298 in: *Methods in Carbohydrate Chemistry*. Vol. IV. R. L. Whistler, ed. Academic Press: New York.
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.
- Schierbaum, F., Vorweg, W., Kettlitz, B., and Reuther, F. 1986. Interaction of linear and branched polysaccharides in starch gelling. *Nahrung* 30:1047-1052.
- Sitohy, M. Z., El-Saadany, S. S., Labib, S. M., Ramadan, M. F., and Mohamed, Z. 2000. Physicochemical properties of different types of starch phosphate monoester. *Starch* 52:101-105.
- Slade, L., and Levine, H. 1987. Recent advances in starch retrogradation. Pages 387-430 in: *Recent Development in Industrial Polysaccharides*. S. S. Stivala, V. Crescenzi and I. C. M. Dea, eds. Gordon and Breach Publishers: New York.
- Solarek, D. B. 1986. Phosphorylated starches and miscellaneous inorganic esters. Page 56 in: *Modified Starches: Properties and Uses*. O. B. Wurzburg, ed. CRC Press: Boca Raton, FL.
- Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* 195:19-23.
- Tester, R. F., and Morrison, W. R. 1990. Swelling and gelatinization of cereal starch. I. Effects of amylopectin, amylose, and lipids. *Cereal Chem.* 67:551-557.
- Thompson, D. B., and Blanshard, J. M. V. 1995. Retrogradation of selected *wx*-containing maize starches. *Cereal Foods World* 40:670-675.
- Varavinit, S., Shobsngob, S., Varayanond, W., Chinachoti, P., and Naivikul O. 2003. Effect of amylose content on gelatinization, retrogradation and pasting properties of flours from different cultivars of Thai rice. *Starch* 55:410-415.
- Wang, Y.-J., White, P. J., and Pollak, L. 1992. Thermal and gelling properties of maize mutants from OH43 inbred line. *Cereal Chem.* 69:293-304.
- 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.
- Wurzburg, O. B., Jarowenko, W., and Rubens, R. W. 1979. Process for phosphorylating starch in alkaline metal triphosphate salts. U.S. patent 4,166,173.
- Wurzburg, O. B., Jarowenko, W., and Rubens, R. W., and Patel, J. K. 1980. Continuous process for phosphorylating salt. U.S. patent 4,216,310.

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