

Extraction of Wheat Starch with Aqueous Sodium Hydroxide¹

NAOKO MATSUNAGA^{2,3} and PAUL A. SEIB^{2,4}

ABSTRACT

Cereal Chem. 74(6):851–857

Wheat starch was extracted with aqueous sodium hydroxide at 30–38% starch solids, pH 11.5–12.3, and 25–42°C for 0.17–24 hr. Stirring wheat starch at pH 12.3 and 25°C for 3 and 24 hr, then washing with water, neutralizing, and washing again, removed 70 and 90% phosphorus (P), respectively. Adding 16% sodium sulfate (dry starch basis) into the alkaline medium removed ≈80% of P at pH 12.0 and 25°C in 3 hr and >95% of P at pH 11.7 and 42°C in 3 hr. Sulfate ion was absorbed strongly by wheat starch in aqueous sodium hydroxide at pH 12.0, and sodium sulfate also increased the starch's uptake of hydroxide ion. Low-P wheat starch (>90% of P removed) retained the fatty acids in the untreated starch, but

a fatty acid-amylose complex was not detectable by differential scanning calorimetry. The enthalpy of gelatinization of the low-P wheat starch almost matched that of prime starch, as did its X-ray diffraction pattern. Those data are consistent with saponification of the lysophospholipid in the amorphous phase of the starch to form fatty acid salts and glycerol-choline or glycerol-ethanolamine phosphodiesteres that slowly diffused out of the granules. The low-P wheat starch was judged to have less "cereal" odor than the prime starch, and its pasting temperature at 9.3% starch solids was lowered by ≈10°C.

More than 90% of the lipids inside wheat starch granules are lysophospholipids (LPL). As such, they are thought to occur as an inclusion complexes with amylose (Morgan et al 1993). Practically all the phosphorus (P = 0.054%) in wheat starch is from LPL (Kasemsuwan and Jane 1996). Wheat starch lipids comprise 1% of granule weight, with surface lipids amounting to 0.05% (Eliasson et al 1981, Morrison 1988). Despite their low levels, internal lipids restrict the swelling of wheat starch (Takahashi and Seib 1988, Morrison et al 1993), and the surface lipids may oxidize and contribute to the so-called "cereal" odor of wheat starch. Furthermore, enzymolysis of wheat starch gives hydrolysis products that are difficult to filter and contain color. The latter complexities have been attributed to the LPL (Bowler et al 1985, Derez et al 1990).

Starch surface lipids are removed readily with a mixture of chloroform, methanol, and water or water-saturated butanol at room temperature (Morrison and Karkalas 1990). Removing surface protein also eliminates surface lipids (Russel et al 1987, Morrison 1988, Morrison and Karkalas 1990). The lipids inside wheat starch are removed by hot aqueous alcohol, but that method requires much solvent and time. Furthermore, the starch is damaged by hot aqueous alcohol, as shown by a decrease in the enthalpy of gelatinization (Eerlingen et al 1994).

Azudin and Morrison (1986) found that rice starch extracted from rice flour in 0.1% (w/v) sodium hydroxide contained a lower level of total lipids and LPL but an increased level of free fatty acids (FFA) as compared with proteolytically extracted rice starch. Seidel et al (1984) used aqueous alkali to remove odors and flavors from cereal starch, but they did not test for changes in lipids. Woo (1995) showed that hydroxypropylation of wheat starch done at pH 11.5 and 45°C for 24 hr removed P from the starch.

Because amylose occurs in the amorphous phase of starch and its hydroxyls begin to ionize at pH 11 (Rao and Foster 1963, Kitamura et al 1982), lipids might be removed from wheat starch without gelatinization and pasting. The objectives of this study were to: 1) determine the optimum conditions of temperature, pH,

and time to remove P from wheat starch in aqueous slurry at 31–36% starch solids; and 2) determine the fatty acid content and properties of a low-P wheat starch.

MATERIALS AND METHODS

Materials

Prime wheat starch (Midsol 50) with 0.057% nitrogen, 0.055–0.061% P, and 11.0% moisture content (mc) was obtained from Midwest Grain Products, Inc., Atchison, KS. All chemicals were reagent-grade unless otherwise specified.

Methods

Moisture content was assayed by oven-drying for 1 hr at 130°C and damaged starch was assayed by Approved Method 76-31 (AACC 1995). The P assay and sample digestion for sulfate analysis were done according to Smith and Caruso (1964). A combination electrode (model H-5510-022; Cole-Palmer, Chicago, IL) suited to high-sodium applications was used to measure pH. A pH meter/controller with attached peristaltic pump, also from Cole-Palmer, was calibrated each day with standard buffer at pH 7.0 and 11.0. Sodium ion was measured by atomic absorption (Kilmer et al 1994), and sulfate and nitrogen contents were measured by ion chromatography and Kjeldahl methods, respectively (Galbraith Laboratories Inc., Knoxville, TN). The brightness (L^* value) and yellowness (b^* value) of dry starch were measured in triplicate with a chromameter (model CR 310, Minolta Corp., Ransy, NJ). Wide-angle X-ray diffraction patterns of starches at 10% mc were recorded on a Philips X-ray diffractometer (Mahwah, NJ) with Cu-K α radiation at 35 kV and 20 mA, a θ compensator slit, and a diffraction beam monochromator.

Lipid in starch was determined as fatty acid methyl esters (FAME) by the procedure of Morrison et al (1975) without correction for loss of oleic and linoleic acids during the assay.

Total as well as individual C-16 and C-18 fatty acids in starch were determined by K. Kitahara at Kagoshima University, Kagoshima, Japan. The fatty acids were released from starch by a hot digestion with α -amylase and labeled with a fluorescent dye. The labeled acids were separated and quantitated by HPLC (Kitahara et al 1994). Total FFA were determined in a separate assay using a NEFA-C test kit (Wako Pure Chemical Industries, Osaka), which contained acyl-CoA synthetase, acyl-CoA oxidase, peroxidase, and a color-producing reagent.

Blue value of starch was measured in triplicate by a modification of the procedure of Chrastil (1987). Starch (20 mg, db) was

¹Contribution 97-332-J, Kansas Agricultural Experiment Station, Manhattan, KS.

²Graduate research assistant and professor, respectively. Department of Grain Science and Industry, Shellenberger Hall, Kansas State University, Manhattan, KS 66506.

³Present address: Kyoritsu Women's University, College of Home Economics, 2-2-1 Chiyoda-Ku, Tokyo 101, Japan.

⁴Corresponding author. Fax: 913/532-7010.

dissolved in 0.5M potassium hydroxide solution (50 mL) by stirring for 30 min at 25°C. An aliquot (1 mL) was added to 2% trichloroacetic acid solution (10 mL), which was followed by addition of 0.1 mL of aqueous iodine-potassium iodide solution (1.27 g of iodine and 30 g of potassium iodide/L). The absorbance (at a concentration of 0.004%) was measured in a 1-cm cell at 620 nm within 30 min.

Viscosity was measured by flow-through a capillary viscometer at 25°C (Ubbelohde, model No.1B, Fisher Scientific, Pittsburgh, PA) (Sorenson and Campbell 1961). Starch (150 mg, db) and 90% dimethyl sulfoxide (30 mL) were added to a capped centrifuge tube and shaken for 24 hr at room temperature. Centrifugation at 3,000 × *g* gave no sedimented gel. An aliquot (15 mL) of starch solution was added to the viscometer, and its flow time (*t* in sec) was measured as well as that of solvent (*t*₀ = 51.7 sec). Reduced specific viscosity (100 mL/g) is $[(t/t_0) - 1]/c$, where *c* is the starch concentration (g/100 mL).

Prime wheat starch and low-P wheat starch (12 g) were annealed in water (60 mL) at 45°C for 24 hr, then isolated and dried in a forced-draft oven at 30°C. The color and thermal characteristics of starches were determined.

The differential scanning calorimetry (DSC) measurements were done with a Perkin Elmer DSC-2 instrument. Starch (2 mg, db) and water (≈6 mg) were sealed together in an aluminum pan, and the pan was heated immediately from 20 to 127°C at a rate of 10°C/min and a sensitivity setting of 0.5 mcal. After cooling to 7°C, the pan was rescanned immediately to 127°C.

Pasting properties of starch were measured on a Rapid Visco-Analyser (Foss Food Technology, Eden Prairie, MN). Starch (2.6 g, db) and water (25 mL) were slurried at 50°C in the measuring cup. The slurry heating-cooling regimen was 50°C for 20 min, 50–95°C at 7.5°C/min, 95°C for 2 min, 95–50°C at 7.5°C/min, and then 50°C for 4 min. The stirring speed was 960 rpm for the first 10 sec, then 160 rpm for the rest of the test.

Swelling power and solubility were determined using the procedure of Crosbie (1991). Paste clarity was measured according to Craig et al (1989).

All analyses were done in duplicate unless stated otherwise. Statistical analysis of the data was performed using an SAS system (Release 6.09, SAS Institute Inc., Cary, NC).

Extraction of Starch with Aqueous Sodium Hydroxide: Effects of pH and Temperature

Wheat starch (78 g, wb) was mixed with water (121 mL) at 25°C; 1.0M sodium hydroxide (4, 6, 8, 13, 20, 32, and 75 mL) was added dropwise with stirring to give pH levels of 11.0, 11.3, 11.5, 11.8, 12.0, 12.2, 12.3, and 12.8, respectively. Each slurry was stirred for 2 hr at room temperature, and the starch was collected by centrifugation. Off-colored material and gelled starch on the top of the sedimented starch phase were scraped away and discarded. The ungelatinized starch was washed with water (100 mL), then recovered and resuspended in water (100 mL), and the slurry adjusted to pH 6.0 with 1M hydrochloric acid. The slurry was stirred for 30 min, centrifuged, and the starch was washed and then dried at room temperature. Treated starch was weighed and analyzed for P.

To observe the effect of temperature on the removal of P, a slurry of wheat starch (300 g, wb) and water (400 mL) was adjusted to pH 11.0 by adding 1.0M sodium hydroxide. The slurry was warmed to 50°C and stirred for 3 hr. In a similar manner, a 38% wheat starch slurry was stirred at pH 11.6 and 42°C for 3, 6, 12, and 24 hr.

Aqueous sodium hydroxide (1.0M, 250 mL) was added dropwise with stirring over a period of 30 min to a slurry of starch (600 g, wb) in water (800 mL) at 25°C to obtain pH 12.3. When the mixture (≈32% solids) reached pH 12.3, stirring was continued for 1 hr, and the slurry readjusted to pH 12.3 by adding 1.0M sodium hydroxide (1 mL). The mixture then was stirred for an additional 2, 5, 11, or 23 hr, and during that time the pH did not

change. The starch was collected by filtration and resuspended in fresh water (700 mL), and the mixture stirred for 1 hr, during which time the pH remained at 12.3. The starch was collected again by centrifugation, mixed with water (600 mL), and adjusted to pH 6 by adding 1M hydrochloric acid (120 mL). After one more stirring with water (700 mL) for 1 hr, the starch was collected and dried at room temperature. The recovery of the low-P starch that had been extracted for 24 hr was 84%. As a blank sample, starch (600 g, wb) was mixed with water (800 mL) for 24 hr and then isolated by the same procedure.

To show the need to wash the sodium hydroxide-extracted starch before neutralization to remove P, a starch slurry (starch 600 g, db) at pH 12.3 was prepared as described above and stirred for 3 hr at room temperature. The mixture was neutralized by adding 1M hydrochloric acid and filtered. The starch was isolated after washing with water (700 mL) and was assayed for P.

Extraction of Wheat Starch with Aqueous Sodium Hydroxide in the Presence of Sodium Sulfate

Starch (60 g, wb) was added to water (80 mL) at ±25°C containing 8 or 16% sodium sulfate (dsb, 4.3 or 8.6 g). Each slurry was adjusted to pH 12.0 by adding 1.0M sodium hydroxide (30 or 35 mL) under the surface of the starch slurry. The slurries containing 8 and 16% sodium sulfate (dsb) were stirred for 0.17, 0.5, 1.0, and 3 hr or for 0.17 and 3 hr. Then the slurry was centrifuged, and the recovered starch dispersed in water (80 mL) containing ≈3 mL of 1.0M hydrochloric acid. The acid prevented gelatinization of a portion of starch, apparently because water-washing had removed sodium sulfate. The mixture was stirred for 1 hr, centrifuged, and the sedimented starch was washed with water (80 mL), neutralized, washed with water again, and dried.

To prepare a low-P wheat starch, starch (180 g, wb) was added to water (240 mL) containing 16% of sodium sulfate (25.6 g). The slurry was heated to 42°C and 1.0M sodium hydroxide (35 mL) was added to the mixture to adjust the pH to 11.7 at 42°C (or 12.0 at 25°C). The mixture was stirred for 0.17, 0.5, 1, or 3 hr, and the starch recovered as before. The recovery of starch was 89% after the 3-hr extraction.

Absorption of Ions by Wheat Starch at pH 12.0 and 25°C in the Presence and Absence of 4% Sodium Sulfate

Wheat starch (30 g, wb) was mixed with water (40 mL) containing 0 or 4% sodium sulfate (1.20 g, dsb). The pH of each mixture was adjusted to 12.0 by adding 1.0M sodium hydroxide (5.0 and 9.5 mL, respectively). After 1 hr, the slurry was centrifuged at 3,500 × for 10 min. The supernatant was decanted, and water (35 mL) was added to the sedimented starch. An aliquot (35 mL) of the supernatant and the entire slurry of sedimented starch were titrated to pH 7.0 with standard 1.0M hydrochloric acid using a pH meter.

In a second sample, an aliquot (25 mL) of the supernatant was diluted 30,000-fold with 0.1M hydrochloric acid, and sodium ion was assayed by atomic absorption (Kilmer et al 1994). The sodium content in the sedimented starch was calculated by difference. A third aliquot (5 mL) of the supernatant was neutralized with 1M hydrochloric acid, and its sulfate ion content was determined. The neutralized sedimented starch was dried in an air oven at 30°C, and a portion (5 g) of the dried starch and salt mixture was ashed in a muffle furnace. The ash was dissolved in 29% nitric acid (Smith and Caruso 1964), and the solution was assayed for sulfate and sodium ions.

RESULTS AND DISCUSSION

Phosphorus Removal from Wheat Starch by Alkali

Because wheat starch molecules are devoid of hexose phosphates (Morrison 1988, Lim et al 1994, Kasemsuwan and Jane 1996) and the lipids in wheat starch are LPL (Hargin and Morri-

son 1980, Morrison 1988), a P assay on wheat starch can be used to monitor the extraction of lipids. But when wheat starch is treated with alkali, besides the fatty acid salts, LPL undergoing saponification releases mostly a 6–8:1 mixture of glycerol-choline (I) and glycerol-ethanolamine (II) phosphodiester (Fig. 1), plus low levels of two other phosphodiester and several polyols (Morrison 1988).

Seidel et al (1984) emphasized the need to thoroughly wash alkali-treated starches to remove odorous components before neutralization. The same was true for removal of P. For example, 70% of P in wheat starch was removed by extraction with aqueous sodium hydroxide at pH 12.3 for 3 hr at 25°C and then washing once with an equal weight of water. In contrast, only 10% of P was removed when the alkaline starch slurry was neutralized before the washing step. That phenomenon, together with the initially rapid consumption of alkali by starch, suggest that saponification of the wheat starch lipids occurred quickly at pH > 11.5 and 25°C, and that the P in the form of phosphodiester I and II slowly diffused through the granules in the rate-limiting step. In this study, we did not attempt to wash alkali-extracted wheat starch with acidified water.

Adjusting the pH of a Wheat Starch Slurry

Morrison (1988) calculated the %lipids in wheat starch using the relationship %lipid = 16.4 × %P. From that expression, one can calculate a formula weight of 507 Da (16.4 g of lipid/g of P × 30.9 g of P/mol) for the average molecular weight of wheat starch lipid. If wheat starch contains 1% lipid by weight, then 100 g of dry starch contains ≈ 2 mmol of lipid, in agreement with Kitahara et al (1997). Because the lipid molecules are predominantly mono-acyl lipids, and because phosphodiester linkages are stable at alkaline pH (Plimmer and Burch 1928), saponification of wheat starch lipid would consume ≈ 2 meq of alkali (80 mg of sodium hydroxide) per 100 g of starch and release 2 meq of FFA.

Solid-state ¹³C-NMR and glucoamylase digestion studies have indicated that the lysophospholipid in wheat starch is complexed with amylose (Morgan et al 1993, Kitahara et al 1997). The fatty acid chain is located inside the helical cavity of amylose, and its bulky polar end is likely exposed to the bulk medium (Godet et al 1993). Initially, the amount of sodium hydroxide added to wheat starch to achieve pH 12.3 was 46.8 meq (1.87 g) per 100 g of dry starch. To achieve pH 12.0, 29.3 meq (1.17 g)/100 g of dry starch was added. Those amounts of alkali represent a 10- to 20-fold excess over the amount needed to hydrolyze all the fatty acids from the LPL.

A wheat starch slurry (≈38% solids) at 25°C, adjusted to pH 12.3 by adding 46.8 meq of 1.0M sodium hydroxide per 100 g of starch and stirred for 70 min, required 0.17 meq of additional alkali per 100 g of starch to restore pH to 12.3, which brought the total to 48.0 meq per 100 g of starch in 145 mL of water. When

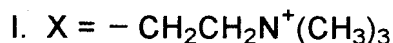
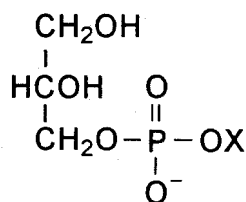


Fig. 1. Structure of major phosphodiester formed by saponification of lysophospholipids in wheat starch: glycerol-choline phosphodiester (I) and glycerol-ethanolamine phosphodiester (II).

145 mL of water alone was adjusted to pH 12.3, the alkali needed was 5.8 meq. If the lipids in the starch (100 g) required 2 meq of alkali for saponification, then that accounted for 7.8 meq out of the total of 48.0 meq of hydroxide. Much of the remaining 40.2 meq of hydroxide was reacted with or was adsorbed by the 100 g of starch.

Before gelatinization, the absorption of alkali by starch is expressed by the Freundlich equation ($X = aC^b$), where X is the alkali absorbed in meq per grams of dry starch, C is the equilibrium concentration (molarity) of alkali in the aqueous phase, and a and b are constants (Leach et al 1961). Lancaster and Conway (1968) reported that the absorption of sodium hydroxide by corn starch was independent of starch solids in a slurry (9–30%) when the initial alkali concentration was between 0.1–0.4 M.

The propensity of starch to absorb or react with sodium hydroxide in aqueous solution causes experimental limitations in preparing mixtures of starch in an alkaline medium. For example, if one attempts to add one part wheat starch to two parts 0.24M alkali at 25°C to obtain a slurry with pH 12.3, the starch must be added all at once to avoid partial gelatinization. However, adding the alkali slowly to an agitated slurry of starch is preferred. Leach et al (1961) reported that at ≈5% starch solids, absorption of >40 meq and >32 meq of sodium hydroxide per 100 g caused gelatinization of corn and potato starches, respectively. Alkali absorption of wheat starch varied somewhat between lots of commercial wheat starch. For example, when adjusted to pH 12.0 in aqueous slurry, the two lots used here absorbed amounts of sodium hydroxide that differed by 0.5 meq/100 g.

Effect of pH on Removal of Phosphorus from Wheat Starch

Stirring wheat starch in approximately one part water at pH 6 and 25°C for 24 hr did not remove P, but the loss of P from starch accelerated at pH >11.5 (Fig. 2). Seidel et al (1984) also found that a pH >11.5 but <12.4 (pasting) was best to improve the flavor of wheat starch. In the present study, we noted that odor began to emanate from an aqueous slurry of wheat starch at pH ≥11 at 25°C. Presumably, the odor signaled the dissociation of helical complexes between amylose and volatile organic compounds or their enhanced diffusion from the granule.

Slurrying starch in aqueous sodium hydroxide could cause swelling and damage to the starch granules. However, damaged starch remained low (1.5–2.0%) for all samples (Fig. 2) compared

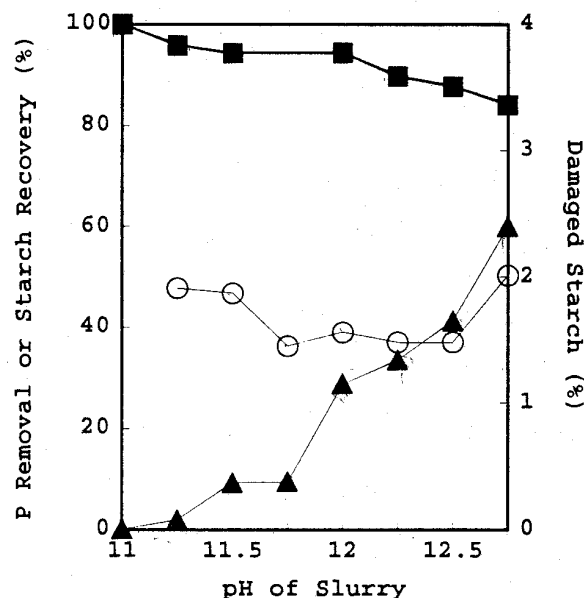


Fig. 2. Phosphorus removal (▲), recovery of starch (■), and degree of damaged starch (○) in wheat starch (30–35%) after stirring in aqueous sodium hydroxide 2 hr at 25°C. Degree of damaged starch in prime wheat starch = 2.9%.

to the 2.9% damage for the prime starch. The treated starches showed reduced damage because highly swollen granules were removed during washing and centrifuging steps. The recovery of alkali-treated starches was 84–96% (Fig. 2).

When wheat starch was slurried in water with increasing levels of sodium hydroxide, a corresponding increase in saponification of the acyl groups might be expected with a concomitant increase in loss of glycerol-aminoalcohol phosphodiester I and II from the starch. Indeed, Fig. 2 shows that at pH >11.5, P loss appeared to accelerate. Kitamura et al (1982) found that amylose started to change conformation in aqueous solution at pH 11.5 and attributed this to the beginning of ionization of amylose hydroxyl groups. That ionization somehow increased the loss of P.

Effects of Time and Temperature on Removal of P from Wheat Starch in Aqueous NaOH at pH 12.3 and 25°C

More than 70% of P was removed from wheat starch during the first 3 hr of extraction in aqueous sodium hydroxide at pH 12.3 and 25°C. However, the remaining P was removed more slowly up to 95% (Fig. 3). The slow phase of P removal was not caused by slow uptake of alkali by wheat starch. We found that the amount of sodium hydroxide (29.3 meq/100 g of starch) absorbed by wheat starch at pH 12.0 and 25°C was constant after stirring for 10 min to 3 hr (data not shown), which agrees with the reported rapid uptake (<15 min at 30°C) of sodium hydroxide by corn and potato starches (Leach et al 1961).

The slow removal of the last 20–30% of P may be explained by structural differences among the phosphodiester of glycerol-choline compared to those of glycerol-ethanolamine, inositol, or serine. The phosphodiester of glycerol-choline (structure I, Fig. 1), which accounts for about two-thirds of the phospholipids in wheat starch (Morrison 1988), has a net neutral charge at alkaline pH. The remaining 20–30% phosphodiester are negatively charged, as illustrated by glycerol-ethanolamine phosphodiester (structure II, Fig. 1). The phosphodiester with a net negative charge could be attracted to the amorphous phase of starch by the same force that causes absorption of sulfate ion. We should mention that the removal of P from wheat starch, which amounts to ≈1.9 mmol/100 g, was accompanied by a change in Kjeldahl nitrogen from 0.06 to 0.05%, which is a loss of nitrogen of ≈0.7 mmol/100 g. In theory, the nitrogen level in wheat starch freed of the phosphodiester I and II should have decreased to 0.03%. Those results remain unexplained.

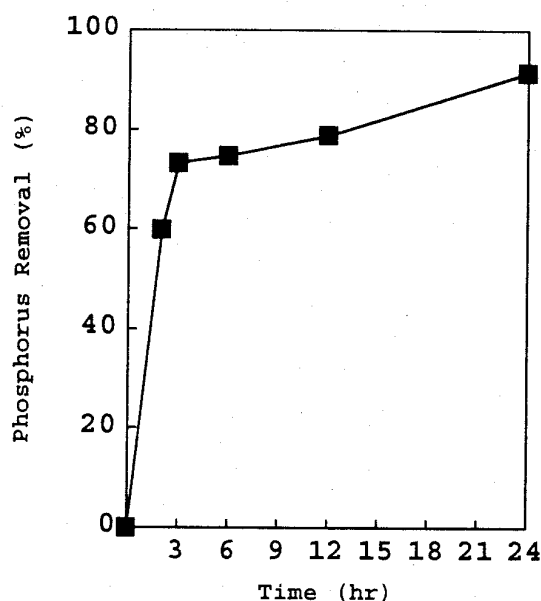


Fig. 3. Phosphorus removal (%) from wheat starch (≈32%) slurried over time in aqueous sodium hydroxide at pH 12.3 and 25°C.

Effect of Sodium Sulfate on Removal of Phosphorus

By adding sodium sulfate to a slurry of wheat starch before adding alkali, the amount of hydroxide ion absorbed by or reacted with starch as pH was adjusted to 12.0 was increased, even though the volume of the aqueous phase increased. Table I shows that the hydroxide ions added to a wheat starch slurry to give pH 12.0 were increased by 68, 88, and 119% with 4, 8, and 16% sodium sulfate, respectively.

When 1.0M aqueous sodium hydroxide was added to a wheat starch slurry (38% solids) in the absence of sodium sulfate to obtain pH 12, 80% of sodium ions and 80% of hydroxide or alkoxide ions were present in the starch phase (Table II) as determined independently by atomic absorption spectroscopy and by titration. Assuming that one hydroxyl group ionizes per anhydroglucose unit and that the entire 14.7 meq of sodium ion in starch (Table II) was associated with ionized starch hydroxyls, the maximum degree of dissociation of the starch hydroxyl was 14.7 meq/620 meq, or 2.4%, which gives $pK_a = 13.6$ ($-\log_{10}$ ionization constant). Using a pK_a of 13.3 for wheat starch at 25°C (Saric and Schofield 1946, Lammers et al 1993), the predicted ionization of the starch hydroxyl at pH 12.0 is 5.0%, which is in fair agreement considering the possible contaminants in wheat starch.

When 4% sodium sulfate was present in the pH 12.0 medium, hydroxide or alkoxide ion in the starch doubled, and 93% of sulfate ion was present in the starch phase as determined by sulfate assay (Table II). In addition, the starch granules assumed a net negative charge (44 meq of excess negative charge/100 g of dry starch) and the continuous phase became positively charged

TABLE I
Effect of Na₂SO₄ on the Reaction and Absorption of NaOH Ion by Wheat Starch (38% starch solids) at 25°C and pH 12.0 and on Removal of Phosphorus from Starch

% Na ₂ SO ₄ ^a	Meq Added/100 g of Starch		P Removal (%)	
	Na ₂ SO ₄ Added	pH 12.0 ^b	0.17 hr	3 hr
0	0	29.3	29	54
4	56.3	49.3	45	71
8	112.3	55.0	54	75
16	225.4	64.0	55	82

^a Based on dry weight of starch.

^b Slurry adjusted to pH 12.0 by adding 1.0M NaOH per 100 g of dry starch.

TABLE II
Components in the Discontinuous (Starch) and Continuous (Aqueous) Phases in a Wheat Starch Slurry (≈35% starch solids) at pH 12.0 With and Without 4% Na₂SO₄^a

		Component	Amount
Without Na ₂ SO ₄			
Discontinuous phase			
	Starch		100 g
	Na ⁺ ion ^b		14.7 meq
	OH ⁻ and starch O ⁻ ions		14.7 meq
Continuous phase			
	Water		169 g
	Na ⁺ ion		3.7 meq
	OH ⁻ ion		3.7 meq
With Na ₂ SO ₄			
Discontinuous phase			
	Starch		100 g
	Na ⁺ ion ^b		35.9 meq
	OH ⁻ and starch O ⁻ ions		30.6 meq
	SO ₄ ⁻² ion		49.8 meq
Continuous phase			
	Water		186 g
	Na ⁺ ion		55.7 meq
	OH ⁻ ion		4.7 meq
	SO ₄ ⁻² ion		6.5 meq

^a Based on dry weight of starch. Equivalent weight of sulfate ion is 48 g/eq.

^b Determined by difference between total added and amount determined in the continuous phase.

(excess sodium ions at 44.5 meq/100 g of dry starch). The surprisingly high absorption of sulfate ion inside the negatively charged starch granule was confirmed by a separate sulfate assay on the supernatant, which showed 11.5% of that ion added was found in the continuous phase compared to 7.0% calculated by difference (Table II). And, the deficiency of sodium ion in the starch phase was confirmed by a separate assay of that phase after drying (0.8% sodium, db) in fair agreement with that calculated (0.78% sodium) by difference. If all the sodium added as hydroxide and sulfate had remained in starch phase, the starch phase would have contained $\approx 2.0\%$ sodium.

Other workers (Leach et al 1961, De Willigen and De Groot 1971) had shown previously that sodium sulfate increased the absorption of alkali by starch, but we are unaware of data on the high absorption of sulfate ions by starch to generate a net negative charge on the granules in aqueous sodium hydroxide. Oosten (1982) suggested that starch absorbs sodium ion from sodium chloride solution to form starch alcoholate ions due to the Donnan potential between the granules and the bulk water phase. Furthermore, he suggested that adding sodium hydroxide to an aqueous slurry of starch containing sodium chloride would cause extra sodium ion to enter the granule with the exclusion of chloride ion. However, at the concentrations of reagents used in our work, more of the negative ion (sulfate) from the salt rather than positive ion (sodium) was absorbed at equilibrium by wheat starch in a mixture of aqueous sodium hydroxide and sulfate.

In the presence of 8 and 16% of sodium sulfate at pH 12.0, after 10 min of extraction, $>50\%$ of P was removed from wheat starch.

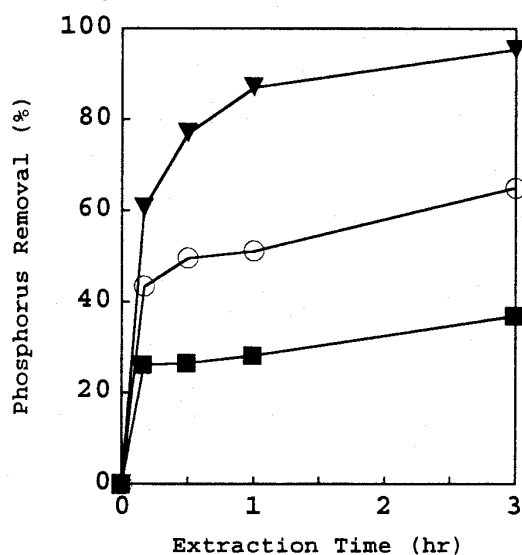


Fig. 4. Phosphorus removal (%) from wheat starch at pH 12.0 and 25°C with (○) or without (■) 8% sodium sulfate (dsb) and at pH 11.7 and 42°C with 16% sodium sulfate (dsb) (▲).

After 3 hr of extraction, 71–82% of P was removed at pH 12.0 in the presence of 4–16% sodium sulfate, compared to 54% in the blank (Table I). Phosphorus removal from wheat starch at pH 12.0 with 8% sodium sulfate at 25°C still occurred in two stages, the same as without sodium sulfate (Fig. 4).

Extracting Wheat Starch with Alkali at Increased Temperature

When the extraction temperature was increased from 25 to 42°C, the pH of the alkaline medium containing no sodium sulfate had to be decreased from pH 12.3 to 11.6 to avoid pasting of starch. Figure 5 shows that at 25°C and pH 11.6, 24 hr of extraction time gave 80% removal of P from wheat starch. Increasing the extraction temperature to 50°C while lowering alkalinity to pH 11.0 removed 62% P after 3 hr of stirring (data not shown) compared to 54% removed at pH 11.6 and 42°C. However, damage to the starch was difficult to control at 50°C.

When 16% of sodium sulfate was added to the aqueous medium at 42°C and pH 11.7, a 0.17-hr (10-min) extraction period removed 60% of P from wheat starch, and the removal was more than 95% complete within 3 hr to give a 89% yield of low-P starch (Fig. 4). In contrast, an extraction time of 24 hr was required to achieve 95% removal of P at 25°C and pH 12.3 to give 84% low-P starch (Fig. 3).

Properties of Wheat Starch Extracted with Sodium Hydroxide

Two low-P wheat starches ($\approx 95\%$ of P removed) and prime wheat starch were pasted at 9.3% starch solids in a pasting instrument (Fig. 6). The general shape of the pasting curves were similar, but the low-P starches pasted 5–10°C below the prime wheat starch, indicating more rapid swelling of low-P wheat

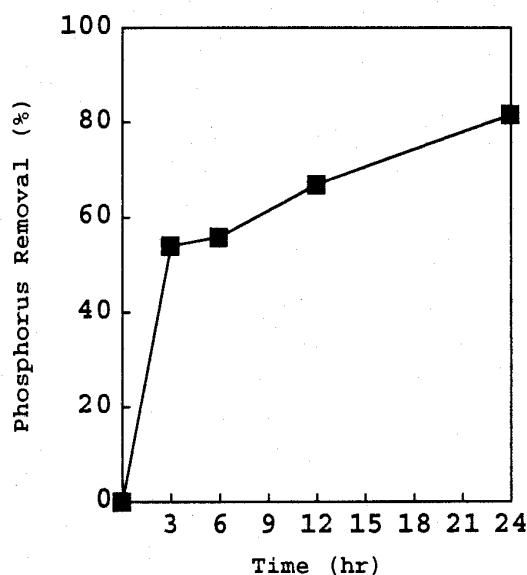


Fig. 5. Phosphorus removal (%) from wheat starch ($\approx 35\%$) slurried over time at 42°C in aqueous sodium hydroxide at pH 11.6.

TABLE III
Physical Properties and Lipids in Prime and Low-P Wheat Starches

Wheat Starch	FAME ^a	Blue Value ^b	Paste Clarity ^c	Solubility ^d	Swelling Power ^e	Reduced Specific Viscosity (dl/g)
Prime	350a ^f	0.626a	11.6a	16.4a	16.1a	2.92a
Extracted at pH 12.3, 25°C, 24 hr	377a	0.632b	26.2b	15.2a	16.5a	2.97b
Extracted at pH 11.7, 16% Na ₂ SO ₄ , 42°C, 3 hr	352a	0.625a	25.4b	17.3a	18.7b	3.11c

^a Fatty acid methyl esters (mg/100 g of starch, db) determined by acid-hydrolysis method (Morrison et al 1975). Not corrected for losses of oleic and linoleic acid during assay.

^b A at 0.004% and 1 cm. Blue value = 0.652 for starch after extraction with 70% *n*-propanol (Morrison and Coventry 1985).

^c %T. Paste clarity of 1% potato starch paste in water = 87%T.

^d Solubility (%) at 93°C = amount of soluble carbohydrate/dry weight of starch.

^e Swelling power at 93°C (g) = gel weight/dry weight of the gel phase.

^f Values followed by the same letter are not significantly different ($P = 0.05$).

starches. The granules in low-P starches showed almost the same size distribution in dilute saline at 25°C on a Coulter instrument as did the blank starch (data not given).

An assay of low-P starches showed that they contained 0.35 or 0.38 wt% FAME compared to 0.35% FAME for the prime wheat starch (Table III). FAME assays of starch entailed losses for which we did not correct. Total FFA analysis at Kagoshima University showed that treated starches contained 1.91 or 1.99 meq/100 g compared to 0.28 meq/100 g in the blank wheat starch (Table IV), which is equivalent to 0.47 or 0.49 wt% FFA, compared to 0.07 wt% FFA, respectively, calculated as palmitic acid. The total FFA assay shows that practically all fatty acids originally present in wheat starch (≈ 2 meq/100 g) were saponified by the alkali and retained in starch granule. The individual C-16 and C-18 fatty acids captured inside the two low-P wheat starches were almost the same (Table IV).

The blue values of low-P wheat starches were only slightly elevated as compared to prime starch, but they were much lower than the 0.652 found for wheat starch that had been extracted with hot 70% aqueous propanol (Table III). The blue value data was consistent with the fatty acids remaining in the low-P wheat starch.

The low-P starch showed a gelatinization endotherm with an initiation temperature (T_i) of 57.5 or 58.3°C, which was ≈ 0.2 – 0.8 °C higher than that of prime starch (Fig. 7). The enthalpy of gelatinization of the low-P starch was 9–11% more than that (2.3 cal/g) of the prime starch, but the X-ray diffraction patterns were identical with strong reflections at 2θ of 15, 18, and 23° (data not shown). The DSC and X-ray data indicate no damage to the crystalline phase by the alkali treatment, which is consistent with damaged starch data (Fig. 2). Surprisingly, no melting endotherm for an amylose-

lipid complex was observed for the low-P starch, whereas the prime wheat showed the endotherm at 100–105°C (Fig. 7). Rescanning of both low-P samples or converting the sodium salts of the fatty acids in the low-P starches to their free-acid form did not result in the appearance of an amylose-lipid peak (curve not shown).

When the low-P wheat starch was annealed at 45°C, it showed a new broad endotherm over the 85–100°C range (Fig. 7). Apparently, upon annealing, the fatty acids in the low-P wheat starch formed weak complexes with amylose, or possibly amylopectin. This weak complex condition was not due to depolymerization of starch molecules during treatment with sodium hydroxide. The reduced specific viscosity of a dimethyl sulfoxide solution of the low-P wheat starch (0.5%) exceeded that of the prime starch (Table III). The elevated viscosity of low-P starch was probably caused by the 11–16% loss of damaged prime starch during isolation. The weak complexing of FFA with amylose may explain why the low-P starch forms a paste some 10°C below prime wheat starch. The lack of DSC evidence for amylose-fatty acid complexing in the low-P wheat starch is difficult to explain if the LPL occur as a helical complex in wheat starch. Complexing of fatty

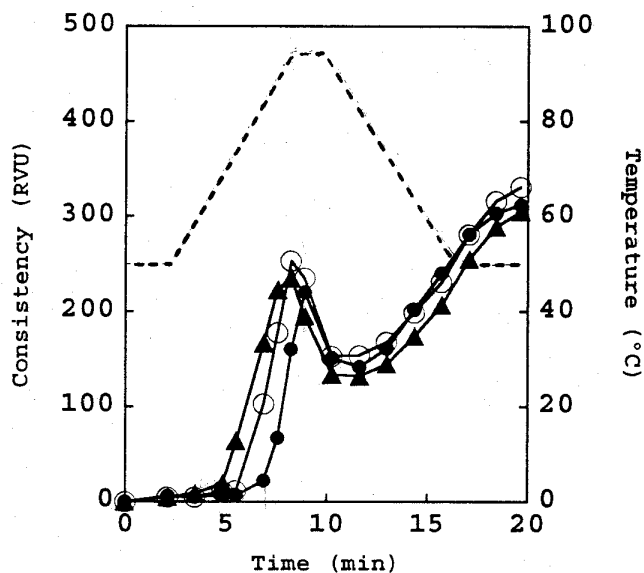


Fig. 6. Rapid ViscoAnalyser curves of native (●), and low-P wheat starches prepared either at pH 12.3, 25°C with 24 hr stirring (○) or at pH 11.7, 16% sodium sulfate (dsb), 42°C with 3 hr stirring (▲). Pasting of starch solids (9.3%, w/w) in water with a heating rate of 7.5°C/min. Dotted line indicates temperature of the paste. RVU = Rapid ViscoAnalyser units.

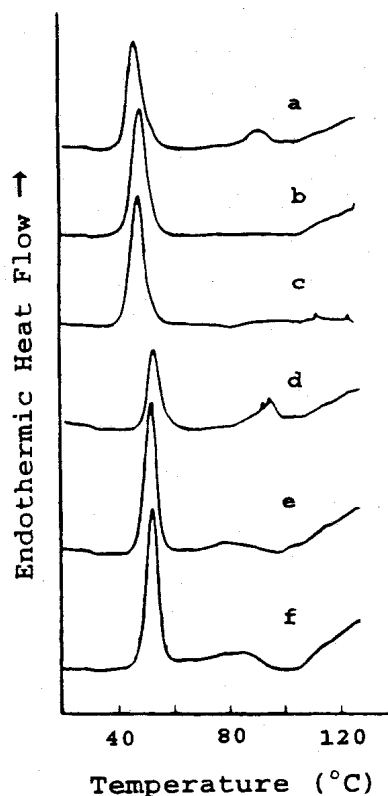


Fig. 7. Thermograms of wheat starch and water (1:3, w/w) measured using differential scanning calorimetry at a heating rate of 10°C/min. Prime (a); low-P starch prepared at pH 12.3, 25°C for 24 hr (b); low-P starch prepared at pH 11.7, 16% sodium sulfate (dsb), 42°C for 3 hr (c); prime starch annealed in five parts water at 45°C for 24 hr (d); low-P starch prepared at pH 12.3, 25°C for 24 hr, annealed (e); and low-P starch prepared at pH 11.7, 16% sodium sulfate (dsb), 42°C for 3 hr, annealed (f).

TABLE IV
Total and Individual C-16 and C-18 Fatty Acids in Wheat Starches^a

Wheat Starch	Free Fatty Acids ^b (μmol/g of starch)					Total ^c
	16:0	18:0	18:1	18:2	18:3	
Prime	0.81	0.11	0.30	0.42	0.08	1.72 (2.78)
Extracted at pH 12.3, 25°C, 24 hr	7.41	0.41	1.75	7.81	0.41	17.8 (19.9)
Extracted at pH 11.7, 42°C, 3 hr	7.39	0.37	1.68	8.01	0.43	17.9 (19.1)

^a Determined at Kagoshima University (Kitahara et al 1994).

^b Determined by the fluorescence labeling-HPLC method.

^c Values in parentheses determined colorimetrically using NEFA-C test kit.

acid salts with amylose is known to occur at pH 12 and 20°C (Karkalas and Raphaelides 1986), so why are those complexes not readily observed by DSC in the low-P wheat starch? One possibility is that the alkalinity inside the starch granule was greater than pH 12, which is the alkalinity of the continuous phases.

The low-P wheat starch showed improved microbiological stability, which was noted during annealing. The low-P wheat starch ($L^* = 97.7$) gave the same lightness as prime starch ($L^* = 97.5$), with a slight reduction in yellowness ($b^* = 2.04$ and 2.37 , respectively). But the prime starch appeared to undergo fermentation upon annealing at 45°C, with loss of lightness ($L^* = 96.6$) and formation of yellow color ($b^* = 2.56$).

The low-P starches had somewhat increased paste clarity, and the low-P starch produced at pH 11.7 and 42°C for 3 hr in the presence of 16% sodium sulfate had somewhat increased solubility and swelling power (Table III).

CONCLUSIONS

Stirring wheat starch (30–35%, w/w) in aqueous sodium hydroxide at pH 11.5–12.3 and 25–42°C releases odorous substances and apparently saponifies the LPL in the starch but causes no damage to the crystalline phase and no depolymerization of the starch molecules. The fatty acid salts remain in the granules, whereas the phosphodiester of alcohol amines appear to diffuse into the medium. The diffusion occurs in two stages, with a rapid loss of ≈70% P and then a slow loss of the rest. The diffusion of the phosphodiester is accelerated by sodium sulfate, apparently because sulfate ions are absorbed rapidly and highly by starch in aqueous sodium hydroxide and thereby increase the negative charge in the granules. Saponification of the LPL decreases the pasting temperature of the low-P starch, and may be helpful when converting wheat starch to starch-hydrolysis products.

ACKNOWLEDGEMENT

We thank Indira Reddy for FAME analyses, Kanefumi Kitahara for total and individual free fatty acid analyses, and C. C. Maningat for measuring granule size distribution.

LITERATURE CITED

- American Association of Cereal Chemists. 1995. Approved Methods of the AACCC, 9th ed. Method 46-13, approved October 1976, revised October 1986, reviewed October 1994; Method 76-31, approved January 1995. The Association: St. Paul, MN.
- Azudin, M. N., and Morrison, W. R. 1986. Non-starch lipids and starch lipid in milled rice. *J. Cereal Sci.* 4:23-31.
- Bowler, P., Towersey, P. J., Waight, S. G., and Galliard, T. 1985. Minor components of wheat starch and their technical significance. Pages: 71-79 in: *New Approaches to Research on Cereal Carbohydrates*. R. D. Hill and L. Munck, eds. Elsevier: New York.
- Craig, S. A. S., Maningat, C. C., Seib, P. A., and Hosney, R. C. 1989. Starch paste clarity. *Cereal Chem.* 66:173-182.
- Crosbie, G. B. 1991. The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. *J. Cereal Sci.* 13:145-150.
- Chrastil, J. 1987. Improved colorimetric determination of amylose in starch or flours. *Carbohydr. Res.* 159:154-158.
- De Willigen, A. H., and De Groot, P. W. 1971. Adsorption of electrolytes by potato starch. Heat development, swelling, and contraction. *Stärke* 23:37-42.
- Derez, F. G. H., Desadeleer, J. W. G. C., and Reeve, A. L. 1990. Carbohydrate refining process and novel enzyme compositions suitable for use therein. U. S. patent 4,916,064.
- Erlingen, R. C., Cillen, G., and Delcour, J. A. 1994. Enzyme-resistant starch. IV. Effect of endogenous lipids and added sodium dodecyl sulfate on formation of resistant starch. *Cereal Chem.* 71:170-177.
- Eliasson, A.-C., Carlson, T. L.-G., Larsson, K., and Mieziš, Y. 1981. Some effect of starch lipids on the thermal and rheological properties of wheat starch. *Starch/Stärke* 33:130-134.
- Godet, M. C., Buleon, A., Tran, V., and Colonna, P. 1993. Structural features of fatty acid-amylose complexes. *Carbohydr. Polym.* 21:91-95.
- Hargin, K. D., and Morrison, W. R. 1980. The distribution of acyl lipids in the germ aleurone, starch and non-starch endotherm of four wheat varieties. *J. Sci. Food Agric.* 31:877-878.
- Karkalas, J., and Raphaelides, S. 1986. Quantitative aspects of amylose-lipid interactions. *Carbohydr. Res.* 157:215-234.
- Kasemsuwan, T., and Jane, J.-L. 1996. Quantitative method to survey starch phosphate derivative and starch phospholipid by ^{31}P -nmr spectroscopy. *Cereal Chem.* 73:702-707.
- Kilmer, O. L., Seib, P. A., and Hosney, R. C. 1994. Effects of minerals and apparent phytase activity in the development of the hard-to-cook state of beans. *Cereal Chem.* 71:476-482.
- Kitahara, K., Sukanuma, T., and Nagahama, T. 1994. Bound free fatty acids in glucoamylase-digested starches of corn and sweet potato. *Cereal Chem.* 71:439-443.
- Kitahara, K., Tanaka, T., Sukanuma, T., and Nagahama, T. 1997. Release of bound lipids in cereal starches upon hydrolysis by glucoamylase. *Cereal Chem.* 74:1-6.
- Kitamura, S., Yunokawa, H., Mitsue, S., and Kuge, T. 1982. Study on polysaccharide by the fluorescence method. II. Micro-Brownian motion and conformational change of amylose in aqueous solution. *Polym. J.* 14:93-99.
- Lammers, G., Stamhuis, E. J., and Beenackers, A. A. C. M. 1993. Kinetics of the hydroxypropylation of potato starch in aqueous solution. *Ind. Eng. Chem. Res.* 32:835-842.
- Lancaster, E. B., and Conway, F. 1968. Alkali sorption and swelling of starch. *Cereal Foods World* 13:248-250.
- Leach, V. H. W., Schoch, T. J., and Chessman, E. F. 1961. Adsorption von alkalien das stärkekor. *Starch/Stärke* 6:200-203.
- Lim, S. T., Kasemsuwan, T., and Jane, J.-L. 1994. Characterization of phosphorus in starch by ^{31}P -nuclear magnetic resonance spectroscopy. *Cereal Chem.* 71:488-493.
- Morgan, K. R., Furmeaux, R. H., and Larsen, N. G. 1993. Solid-state NMR studies on the structure of starch granules. *Cereal Chem.* 70:385-391.
- Morrison, W. R., Mann, D. L., Soon, W., and Coventry, A. M. 1975. Selective extraction and quantitative analysis of non-starch and starch lipids from wheat flour. *J. Sci. Food Agric.* 26:507-521.
- Morrison, W. R., and Coventry, A. M. 1985. Extraction of lipids from cereal starches with hot aqueous alcohol. *Starch/Stärke* 37:83-87.
- Morrison, W. R. 1988. Lipids in cereal starches: A review. *J. Cereal Sci.* 8:1-15.
- Morrison, W. R., and Karkalas, J. 1990. Starch. Pages 323-352 in: *Methods in Plant Biochemistry*, Vol. 2. P. M. Dey and J. B. Harbone, eds. Academic Press: San Diego.
- Morrison, W. R., Tester, R. F., Gidley, M. J., and Karkalas, J. 1993. Resistance to acid hydrolysis of lipid-complexed amylose and lipid-free amylose in lintnerised waxy and non-waxy barley starches. *Carbohydr. Res.* 245:289-302.
- Oosten, B. J. 1982. Tentative hypothesis to explain how electrolytes affect the gelatinization temperature of starches in water. *Starch/Stärke* 34:233-239.
- Plimmer, R. H. A., and Burch, J. N. 1929. Esters of phosphoric acid. I. Phosphates of cetyl alcohol, cholesterol chloroethyl alcohol, and ethylene glycol. *J. Chem. Soc.* 279-291.
- Rao, V. S., and Foster, J. F. 1963. Studies of the confirmation of amylose in solution. *Biopolymers* 1:527-544.
- Russel, P. L., Gough, B. M., Greenwell, P., Fowler, A., and Munro, H. S. 1987. A study by ESCA of the surface of native and chlorine-treated wheat starch granule: the effect of various surface treatments. *J. Cereal Sci.* 5:83-100.
- Saric, S. P., and Schofield, R. K. 1946. The dissociation constants of the carboxyl and hydroxyl groups in some insoluble and sol-forming polysaccharides. *Proc. R. Soc. A* 185:431-447.
- Seidel, W. C., Orozouich, G. E., Medcalf, D. G. 1984. Method of preparing a clear flavor cereal starch. U. S. patent 4,477,480.
- Smith, R. J., and Caruso, J. 1964. Determination of phosphorus. *Methods Carbohydr. Chem.* 4:42-46.
- Sorenson, W. R., and Campbell, T. W. 1961. Characterization of polymers. Pg. 33 in: *Preparative Methods of Polymer Chemistry*. Interscience: New York.
- Takahashi, S., and Seib, P. A. 1988. Paste and gel properties of prime corn starch and wheat starches with and without native lipids. *Cereal Chem.* 65:474-483.
- Woo, K. 1995. Cross-linking of wheat starch and hydropropylated starch in alkaline slurry with sodium trimetaphosphate. MS thesis. Kansas State University: Manhattan, KS.