

Acetylated and Hydroxypropylated Distarch Phosphates from Waxy Barley: Paste Properties and Freeze-Thaw Stability¹

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ABSTRACT

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Waxy barley starch was cross-linked with phosphorus oxychloride (POCl_3) at 25°C in alkali containing sodium sulfate. In a modeling study of the cross-linking reaction, the level of POCl_3 , pH, temperature, and reaction time were varied while the responses of shear stability of the hot paste and its consistency at 50°C were measured in an amylograph. The cross-linking conditions giving highest paste consistency together with highest shear stability were pH 11.1-11.5, POCl_3 0.005-0.007%, and reaction time 20-60 min. At the same pH and reaction time, the optimum level of POCl_3 to cross-link waxy maize starch was 0.010-0.015% POCl_3 . The thickening power, hot paste stability, and clarity of optimally cross-linked waxy barley starch matched those of cross-linked waxy maize

starch. Hydroxypropylation (molar substitution 0.10-0.13) of cross-linked waxy barley starch improved freeze-thaw stability more than acetylation (degrees of substitution 0.10), but the opposite was true for paste clarity. Hydroxypropylated distarch phosphate from waxy barley exhibited much better freeze-thaw stability than commercial samples of similarly modified waxy maize and tapioca starches. In fractionation-reconstitution experiments on flour in breadmaking, cross-linked waxy barley starch of low consistency was substituted for wheat starch, and the composite low-amylose flour gave bread with loaf volume and grain equivalent to those obtained with normal amylose flour.

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop, after wheat, maize, and rice (Poehlman 1985). The waxy endosperm trait in barley is conditioned by a single recessive gene (*wx*) giving opaque kernels (Eriksson 1969, Eslick 1979) that contain starch with 95-99% amylopectin (Banks et al 1970, Morrison et al 1986). Normal barley kernels appear vitreous and contain starch with 70-75% amylopectin.

In the early 1970's, waxy Compana barley was developed at Montana State University (Eslick 1981). The source of the waxy gene was waxy Oderbrucker (Goering et al 1973). Compana, which is a two-rowed spring barley, was chosen as a parent cultivar because of its large kernel size (Hockett 1981, Goering and Imsande 1960). Goering et al (1973) suggested that waxy barley with good agronomic properties could provide a new source of waxy starch. In recent years, Newman et al (1988) pointed out that the presence of β -glucan (~6%) increases the value of waxy barley, since β -glucans in the human diet appear to reduce serum cholesterol.

Waxy starches are notorious for the stringiness of their pastes; cross-linking is needed to stabilize waxy starch granules and eliminate that undesirable characteristic. At the same time, a second modification is used to improve paste clarity, consistency, and cold-temperature stability (Rutenberg and Solarek 1984, Wurzburg 1986). The second modification is usually an esterification or etherification of hydroxyls on starch molecules. The types and levels of modification to produce food-grade starches are defined in the Code of Federal Regulations (CFR 1988).

A continuing interest has prevailed at our institution to produce bread of good volume and texture that contains little amylose. Such bread may yield valuable data on the firming phenomenon. Hosney et al (1971) showed that barley and rye starch, but not corn, could be substituted for wheat starch in breadmaking. However, when waxy instead of normal barley starch was used in fractionation-substitution studies, the loaves collapsed and shrank upon cooling (Hosney et al 1978, Ghiasi et al 1984).

The objectives of this study were to model the cross-linking reaction between waxy barley starch and phosphorus oxychloride and identify conditions that gave distarch phosphate with the highest paste consistency and shear stability; to prepare food-

grade acetylated or hydroxypropylated distarch phosphates from waxy barley starch and compare their freeze-thaw properties and clarity with those of similarly modified waxy maize and tapioca starches available commercially; and to examine the breadmaking properties of cross-linked waxy barley starch.

MATERIALS AND METHODS

Materials

Wanupana, a waxy endosperm, hullless Compana barley (Fox 1981) was obtained from the Montana Agricultural Experiment Station at Bozeman, MT. It contained 51.8% starch, 15.2% protein ($N \times 6.25$), 1.8% crude fat, 1.8% ash, and 9.9% moisture. Yellow-dent waxy maize was provided by National Starch and Chemical Corp. (Bridgewater, NJ).

The wheat flour used in bread-baking tests was milled at a 70% extraction rate from Arkan wheat at Kansas State University and contained 13.1% protein ($N \times 5.7$), 0.47% ash, and 12.7% moisture. At optimum absorption (70%), mixing time (4.0 min), and oxidation (5 ppm potassium bromate), the volume of a pup loaf made from 100 g of flour (14% mb) was 1,000 cm^3 .

National Starch and Chemical Corp. and A. E. Staley Mfg. Co. (Decatur, IL) provided modified waxy maize and tapioca starch samples. The symbols assigned to the modified starches examined in this investigation are listed in Table I.

All chemicals used were reagent grade.

Analytical Methods

Protein was determined by Kjeldahl nitrogen, method 46-11; ash by dry combustion, method 08-01; and moisture by oven-drying 1 hr at 130°C, method 44-15A (AACC 1983).

Native starch lipids were estimated gravimetrically by exhaustive extraction with 75% ethanol at 85°C, followed by evaporation to constant weight under vacuum at 25°C (Morrison and Coventry 1985).

Acetyl content was determined by a titration method (Food Chemicals Codex 1981, FAO 1983) and hydroxypropyl content by a spectrophotometric method (Johnson 1969, FAO 1983). The degree of substitution (DS) and molar substitution (MS) were calculated in the usual manner (Rutenberg and Solarek 1984).

Isolation of Starch

Isolation and purification of waxy barley starch were done based on the methods described by Goering et al (1970) and Goering and Imsande (1960). Pearled barley (500 g) was steeped with 0.2% sodium metabisulfite (1,000 ml) at room temperature. After 24 hr, the steeping water was decanted, the barley kernels

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were ground in a Waring Blendor, and the resulting slurry was screened through a 10XX (132 μm) nylon bolting cloth. The residue was reground in the blender with water, the mixture was screened, and the process was repeated several more times. The combined starch slurry was centrifuged in 250-ml bottles at $1,000 \times g$ for 5 min; the liquid was decanted, and the pigmented fraction (tailings) atop the starch was carefully scraped away. The crude starch was then suspended in cold 0.15% NaOH and centrifuged 5 min at $1,000 \times g$; the supernatant and tailings were discarded. The alkali-washing step was repeated three times, at which point, the tailings appeared negligible. The starch was washed with H_2O (2 L) to remove excess alkali, screened through 16XX (73 μm) bolting cloth, neutralized with 1M HCl, washed with distilled water, and dried in a convection oven at 40°C to give approximately 200 g of starch. The waxy barley starch contained 0.2% protein ($N \times 6.25$), 0.1% ash, 0.7% lipid, and 9% moisture.

Waxy maize starch was isolated using the procedure described by Watson (1964). The waxy maize starch contained 0.3% protein ($N \times 6.25$), 0.05% ash, 0.6% lipid, and 8% moisture.

Experimental Design for Cross-Linking Reaction

Cross-linking of starch was done with phosphorus oxychloride (POCl_3) in alkali containing sodium sulfate (Na_2SO_4 , 2% based on dry weight of starch) as described by Felton and Schopmeyer (1943) and Wetzstein and Lyon (1956). The concentration of the starch in a slurry was approximately 35%.

The experimental design for response surface analysis was a central composite, rotatable, second-order design (Cochran and Cox 1957). Following preliminary trials, four independent variables were selected: pH of the reaction mixture (pH 10.0–11.5), amount of POCl_3 (0.005–0.020% based on dry weight of starch), reaction temperature (15– 35°C), and reaction time (20–100 min). The design had 31 treatments with seven replications at the center point. Five dependent variables (responses) were monitored: paste stability, which was measured by the stability of a paste's consistency while stirring at 95°C in the amylograph; consistency of paste when cooled to 50°C in the amylograph; swelling power of starch; solubility of starch; and clarity of a starch paste.

The data obtained from the study were treated by multiple regression analysis for a second-order response-surface equation that contained linear, quadratic, and interaction terms for the four independent variables. The best equation for each dependent variable was found using the stepwise regression procedure described by Draper and Smith (1981). To determine the effects of independent variables on starch paste properties, contour plots for each quality parameter were generated as a function of two variables, while the other variables were held constant at their center points. The optimum reaction conditions were determined by superimposing contour plots.

Double Modification of Starch

The percentages of reagents used to modify starch were all based on the dry weight of starch. Acetylated distarch phosphate was prepared by first reacting starch with POCl_3 . Then, the starch was esterified with acetic anhydride, according to the procedure of Wurzburg (1964). Dual modification of starch was also done by first reacting starch with propylene oxide followed by POCl_3 (Kesler and Hjermstad 1950, Tuschhoff et al 1969). The etherification reaction was done at 45°C for 24 hr using 7.5–10% propylene oxide in a 35% starch slurry containing 1% sodium hydroxide and 15% sodium sulfate. Following etherification, the modified starch was cross-linked at pH 11.6 for 1 hr using 0.005–0.015% POCl_3 .

Starch Paste Properties

The pasting behavior of starch samples was examined in a Brabender Viscograph-E (C. W. Brabender Instruments, Inc., Hackensack, NJ), at 700 cm-g and 75 rpm. The standard procedure described in *The Amylograph Handbook* (Tipples 1980) was followed, using a 6.25% starch suspension for all starch samples. The following measurements were taken from an amylograph

curve: the pasting temperature, the peak consistency during the heating stage, the consistency after being held for 30 min at 95°C , and the consistency after cooling to 50°C (Tipples 1980, Mazurs et al 1957). The shear stability of a paste was determined by the difference in Brabender units (BU) between the pasting peak and the consistency after the paste was stirred for 30 min at 95°C (Dengate 1984).

Swelling power and solubility of granular starches at 85°C were determined as described by Schoch (1964). The clarity (% T_{650}) of a 1% starch paste, which had been heated in a boiling water bath for 30 min and cooled to 25°C , was evaluated using percent transmittance at 650 nm against a water blank in a Spectronic 21 spectrophotometer (Craig et al 1987).

Differential Scanning Calorimetry

All samples were prepared by sealing in aluminum pans 2.5 mg of starch or modified starch (db) and 7.5 mg of water. Samples were heated at $10^\circ\text{C}/\text{min}$ from 7 to 127°C at a sensitivity of 0.5 mcal/sec (Zeleznaek and Hosney 1987) in a Perkin-Elmer DSC-2 instrument equipped with a FTS Systems Flexicooler and temperature controller (Perkin-Elmer Corp., Norwalk, CT). The thermograms were recorded and analyzed with a thermal analysis data system (TADS).

Starch gelatinization was characterized by onset temperature of gelatinization (T_o) and gelatinization enthalpy (ΔH).

Breadmaking

Wheat flour was fractionated into gluten, starch, and water-solubles, and the fractions were dried by lyophilization (Hosney et al 1971, Shogren et al 1969). The 10-g microloaf baking procedure of Shogren and Finney (1984) was followed. All fractions were reconstituted at their original ratios: gluten (1.8 g), starch (6.3 g), and water-solubles (0.5 g), all on a dry-weight basis. In test loaves, the wheat starch was totally exchanged with unmodified or cross-linked waxy barley starch. Loaf volume was measured by rapeseed displacement, and crumb grain and texture were evaluated by sensory methods.

Freeze-Thaw Stability and Freezer Storage Tests

Two types of starch pastes were tested for freeze-thaw and freezer storage stability (Schoch 1968). The first was prepared by suspending 6.25% starch in distilled water at pH 6.0, heating in the amylograph to 95°C , holding at 95°C for 30 min, and cooling from 95 to 50°C with continued stirring. In the other method, starch (25 g) was suspended in a solution of sucrose (125 g) and water (350 g). To the suspension (500 g) was added 0.5–1.0 ml of 10% malic acid to bring the pH to 3.5, and the suspension was heated in the amylograph and held at 95°C for 5 min.

After cooling to 25°C , either paste was weighed (exactly 20 g each) into approximately 20 polypropylene centrifuge tubes (50 ml capacity), and the tubes were tightly capped. The tubes containing starch pastes in water were stored at 4°C for 24 hr,

TABLE I
Abbreviations for Native and Modified Starches

Starch Source	Cross-Linking Conditions	Substitution	%	Symbol
Waxy barley	none	WB
	pH 11.0, 0.010% POCl_3	WB-X
	pH 11.0, 0.010% POCl_3	acetyl	2.5	WB-XA25
	pH 11.6, 0.005% POCl_3	hydroxypropyl	4.0	WB-XH40
	pH 11.6, 0.007% POCl_3	hydroxypropyl	4.6	WB-XH46
Waxy maize	none	WM
	pH 11.0, 0.020% POCl_3	WM-X
	pH 11.0, 0.020% POCl_3	acetyl	2.5	WM-XA25
	pH 11.6, 0.015% POCl_3	hydroxypropyl	4.2	WM-XH42
	unknown	hydroxypropyl	4.7	WM-XH47
Tapioca	unknown	hydroxypropyl	4.4	T-XH44

whereas those with starch pastes in sugar/acid solution were stored for three weeks before being subjected to freeze-thaw cycles. In each cycle, the tubes were put into a freezer at -23°C (-10°F) for 22 hr and thawed at 30°C (86°F) in a waterbath for 2 hr. After each cycle, triplicate tubes were centrifuged for 15 min at $1,500 \times g$, and the amount of liquid separated was determined by weight.

For freezer storage stability, the freshly cooked pastes in the tubes were placed immediately in a freezer at -23°C (-10°F). Each month, three tubes were removed, thawed in a 30°C (86°F) waterbath for 2 hr, and the amount of separated liquid after centrifugation was determined by weight.

RESULTS AND DISCUSSION

Isolation of Waxy Barley Starch

The yield of waxy barley starch (0.2% protein) was about 40% of the kernel weight on a dry basis, which accounted for 70% of the total starch in the grain. The yield of starch from waxy maize (0.3% protein) was approximately 70% of its total starch. Recovery of starch could have been increased by additional processing of tailings.

Aqueous sodium hydroxide (0.15%, pH 12.5) was needed to purify the crude waxy barley starch (Goering and Imsande 1960) as opposed to water for waxy maize. The alkali dispersed barley protein and β -glucans, which facilitated their separation from the starch by centrifugation. Meuser et al (1987) reported high-pressure disintegration when water alone was used to isolate 58% starch (1.2% protein) from normal barley.

Pasting of Waxy Barley and Cross-Linked Waxy Barley Starches

Amylograms of waxy barley and waxy maize starches are shown in Figure 1. The high peak consistency and rapid breakdown during the heating cycle followed by low set-back during cooling are typical of waxy starches. The waxy maize starch reached its

peak consistency at a lower temperature than waxy barley (Fig. 1). The amylose content of waxy barley starch was $\sim 5\%$ compared with $\sim 2\%$ for waxy maize (Morrison et al 1984). Perhaps the amylose inhibited swelling of waxy barley starch.

To check if α -amylase activity might account for the somewhat lower consistency of waxy barley versus waxy maize starch, mercuric chloride was added to the starch suspensions heated in the amylograph (Goering and Eslick 1976). The starches with or without HgCl_2 gave identical pasting curves, indicating the absence of α -amylase. The cross-linked waxy barley starch gave a paste with increased consistency and improved shear stability (Fig. 1). Furthermore, as expected, the cooked paste showed reduced "stringiness" as evidenced visually during spooning of the paste.

Effect of Salt on Cross-Linking Reaction

When Na_2SO_4 was omitted in one trial reaction with waxy barley (WB) starch (pH 11.0, 0.02% POCl_3 , 25°C , 60 min), little cross-linking occurred, as evidenced by an unaltered pasting curve compared to native WB. It appears that Na_2SO_4 or NaCl must be included in the reaction mixture to ensure cross-linking with 0.02% or less POCl_3 . The amount of salt may vary from 0.1 to 10% based on starch by weight, with 2% being the median level (Wetzstein and Lyon 1956).

The mechanism whereby sodium sulfate or sodium chloride increases the efficiency of the cross-linking reaction is not known. Perhaps sulfate or chloride forms an ion pair with a phosphonium reaction intermediate, or builds the structure of water. Either mechanism could reduce the side reaction of POCl_3 with water. Alternatively, those salts may cause close juxtaposition of starch molecules and frequent cross-linking because of retarded swelling of starch granules in the alkaline medium (Rutemberg and Solarek 1984).

Effect of Reaction Conditions on Cross-Linking

The extent of cross-linking of waxy starches was judged by the loss of paste consistency between the pasting peak and the final consistency at 95°C . Generally, that loss is thought to be due, at least in part, to breakdown of swollen granules, which is counteracted by increasing cross-linking. A second criterion for the extent of cross-linking is paste consistency at 50°C , which passes through an optimum value as cross-linking increases (Schoch 1969).

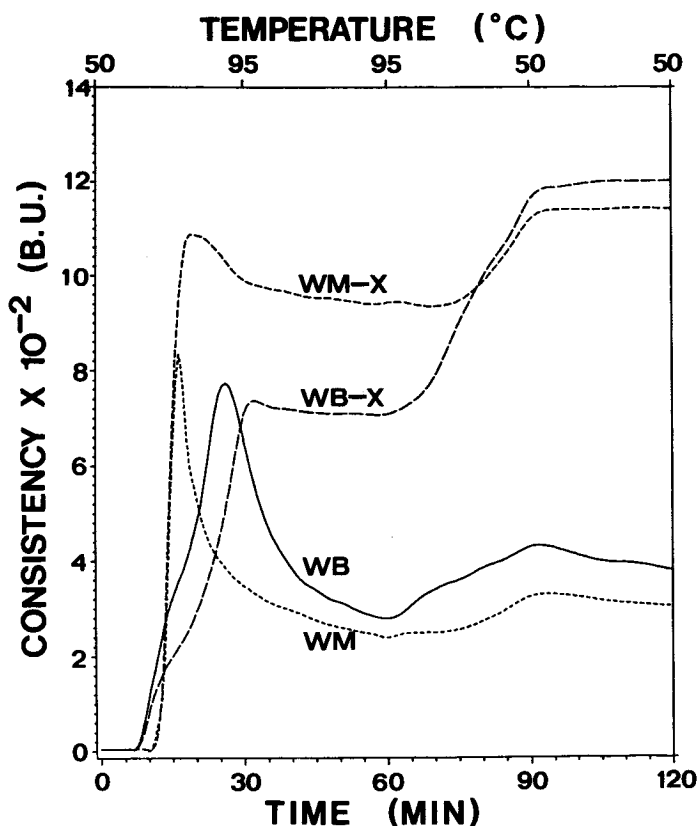


Fig. 1. Pasting curves (6.25% starch solids) of unmodified (WB) and cross-linked waxy barley (WB-X) and waxy maize starches (WM and WM-X). Cross-linking conditions: waxy barley starch, pH 11.0, 0.01% POCl_3 , 25°C , 60 min; waxy maize starch, pH 11.0, 0.02% POCl_3 , 25°C , 60 min.

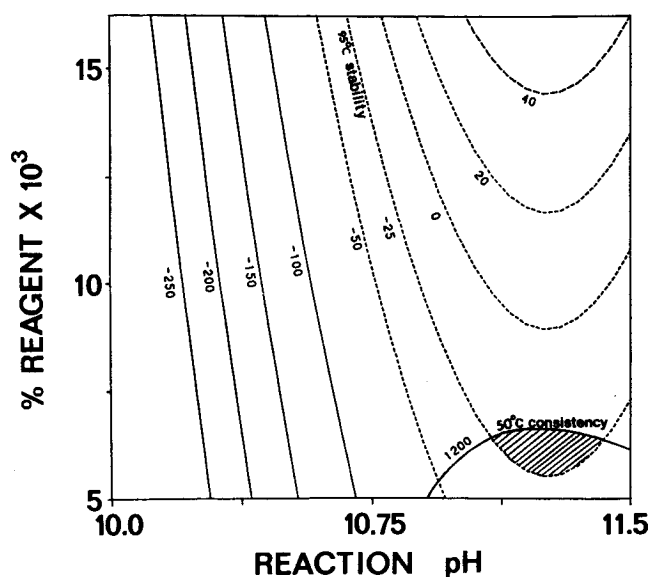


Fig. 2. Contour plot of paste stability (Brabender units) at 95°C of cross-linked waxy barley starch as a function of reaction pH and POCl_3 level, with reaction temperature and time constant at 25°C and 60 min. Shaded area indicates levels of POCl_3 and reaction pH giving the highest consistency at 50°C and shear stability of the paste.

Reaction pH showed a decisive effect upon cross-linking of WB starch with phosphorus oxychloride. Cross-linking with POCl₃ was reported to be efficient between pH 8-12 (Rutenberg and Solarek 1984). However, at pH 10.0 or below, with reaction temperature 15-35°C and 0.02% POCl₃ or less, very little cross-linking was observed (amylograms not shown). As the pH increased from 10 to 12, the efficiency of cross-linking also increased (Fig. 2).

The effect of reaction temperature on cross-linking WB starch was noticeable at pH 10.0-10.75, but not at pH 11.5 or higher. For example, the amylograms of WB starches that were cross-linked at pH 11.5 with 0.02% POCl₃ at 15 and 35°C were almost identical. At pH 10.0-10.75, cross-linking appeared to be more efficient when reaction temperature increased (amylograms not shown).

Reaction times between 20 and 100 min showed little effect on the cross-linking reaction, as judged by paste viscosity and stability. Thus, the cross-linking reaction with POCl₃ was complete in less than 20 min, and the cross-links were stable for up to 240 min at 25°C and pH 11.5.

The regression equations relating reaction variables to shear stability at 95°C, consistency at 50°C, clarity, swelling power, and solubility are given in Table II. As expected, swelling power, solubility, and paste clarity all decreased with increased cross-linking (contour plots not shown), whereas shear stability increased (Fig. 2).

High paste consistency and shear stability may be considered the most important characteristics of cross-linked starches used as thickeners without pressure cooking. Those two features were

TABLE II
Best Prediction Equations Selected for Paste Properties of Waxy Barley Starches Cross-Linked Under Various Reaction Conditions

Paste Property	Equation ^a	R ²
Shear stability at 95°C (BU)	$-32,019 + 5,491X_1 + 84.62X_4 - 235.6X_1^2 - 7.53X_1X_4 + 287.3X_2X_4$	0.93
Consistency at 50°C (BU)	$-51,748 + 8,698X_1 + 405,277X_2 + 31.62X_3 + 158.3X_4 - 351.1X_1^2 - 39,352X_1X_2 - 3.02X_1X_3 - 14.37X_1X_4$	0.67
Clarity (% T ₆₅₀)	$1,612 - 276.1X_1 + 12.09X_1^2 - 3.77X_2X_3 - 15.21X_2X_4$	0.92
Swelling power (g/g starch)	$254.1 - 37.16X_1 - 0.0084X_3 - 1.24X_4 + 1.44X_1^2 - 18.09X_1X_2 + 0.11X_1X_4$	0.87
Solubility (%)	$107.4 - 16.71X_1 - 0.14X_3 + 0.66X_1^2 - 4.66X_1X_2 + 0.012X_1X_3$	0.74

^a Equations were derived by stepwise regression. Variables: X₁ = reaction pH, X₂ = POCl₃ (%), X₃ = reaction time (min), and X₄ = reaction temperature (°C). Limits: pH 10.0-11.5, POCl₃ 0.005-0.020%, 20-100 min, and 15-35°C.

TABLE III
Paste Properties of Modified Waxy Barley and Waxy Maize Starches

Starch	Temperature (°C)		Stability ^a at 95°C (BU)	Consistency ^b at 50°C (BU)	Swelling Power at 85°C	Solubility at 85°C (%)	Clarity ^c (% T ₆₅₀)
	Pasting	Peak					
WB	61	90	-490	430	22.3	4.3	65.2
WB-X	61	95	-20	1,200	16.1	3.0	32.0
WB-XA25	52	88	-120	1,430	20.5	7.1	39.7
WB-XH40	51	80	-190	1,480	21.8	5.6	36.0
WM	67	75	-600	320	28.1	42.9	65.0
WM-X	67	83	-60	1,050	18.6	1.6	26.5
WM-XA25	60	73	-210	1,420	22.5	4.4	38.5
WM-XH42	58	70	-200	1,440	19.5	1.8	28.0
WM-XH47 ^d	60	72	-90	1,300	15.4	1.6	24.5

^a Stability = (consistency after 30 min at 95°C) - (peak consistency).

^b Consistency when paste cooled to 50°C.

^c Clarity (% T₆₅₀) of 1% starch suspension after cooking at 100°C for 30 min.

^d Commercial sample.

used as criteria to determine optimum cross-linking conditions. By superimposing the contour plots of consistency at 50°C and stability at 95°C, the optimum cross-linking conditions were indicated by the shaded area in Figure 2. The optimum conditions to cross-link WB starch at 25°C were pH 11.1-11.5, 0.005-0.007% POCl₃ (based on starch), and 20-60 min. For waxy maize (WM) starch, the optimum level of cross-linking agent increased to 0.010-0.015% POCl₃ when the other variables were kept the same. The paste properties of the optimally cross-linked waxy barley (WB-X) and waxy maize (WM-X) starches are given in Table III.

Breadmaking with WB-X Starch

Unmodified WB and two WB-X starches were interchanged with wheat starch in reconstituted flours obtained by fractionation. One was cross-linked to give the highest paste consistency and shear stability, whereas the other was more highly cross-

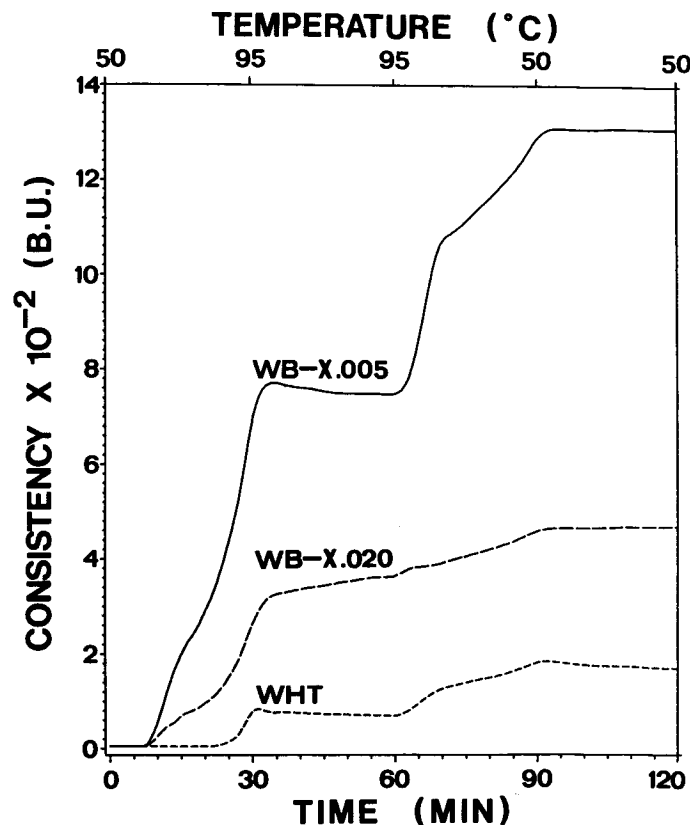


Fig. 3. Pasting curves of two cross-linked waxy barley starches and native wheat starch (6.25% suspension) used in breadmaking. Cross-linking conditions: pH 11.5, 25°C, 60 min, 0.005% POCl₃ and 0.020% POCl₃ for high and low consistency, respectively.

linked and gave a pasting curve whose consistency was lower but somewhat above that of wheat starch (Fig. 3).

The breadmaking results are summarized in Table IV. Unmodified WB starch gave microloaves with soft and somewhat sticky crumb, very open grain, and 20% less volume than the control loaves (with wheat starch). Moreover, the WB loaves shrank excessively after cooling. The WB-X starch with high paste consistency gave loaves with 10% increase in volume over WB loaves, but the crumb grain remained open, and the texture was soft. Loaves baked with the WB-X starch of low consistency were comparable to the control loaves in loaf volume and crumb grain. Thus, modified WB starch in flour can give a low-amylose bread otherwise equivalent to that from flour with normal wheat starch.

Doubly-Modified WB Starch

Acetylation of cross-linked WB starch with 8% acetic anhydride at pH 8.0–8.4 gave doubly modified WB starch with acetyl 2.5% or DS 0.10. The reaction efficiency was 70% based on acetic anhydride. Cross-linked WM reacted almost identically. WB-XA25 and WM-XA25 gave increased swelling power, solubility, consistency, and clarity (Table III) compared with their cross-linked starches (WB-X and WM-X). Moreover, the pasting temperature of WB-XA25 was 90°C lower than that of WB-X.

WB starch, when slurried at 35% solids in a mixture of 1% sodium hydroxide and 15% sodium sulfate (both based on starch), reacted with 7.5% propylene oxide (based on starch) at 45°C to give 3.5–4.0% hydroxypropyl groups (MS 0.10–0.12). Using 10% propylene oxide, 4.6–4.8% hydroxypropyl groups were substituted on the WB starch (MS 0.13–0.14).

Hydroxypropylation (MS 0.10–0.13) was equally as effective as acetylation (DS 0.10) in increasing paste consistency, swelling power, and solubility (Table III). However, acetylation gave better clarity but poorer freeze-thaw stability. WM-XH starch exhibited similar properties to those of WB-XH.

TABLE IV
Microloaves Baked from Reconstituted Flours
Containing Different Starches

Starch in Reconstituted Flour	Loaf ^a Volume	Crumb Grain	Firmness of Freshly Baked Crumb
Wheat (control)	90 ± 2	very good	++
WB ^b (unmodified)	72 ± 2	very open, excessive shrinking	+
WB-X, high paste consistency ^c	79 ± 2	open grain	+
WB-X, low paste consistency ^d	90 ± 2	good, somewhat open	++

^a Quadruplicate loaves each made from 10 g flour.

^b Waxy barley.

^c Cross-linking conditions: pH 11.5, 0.005% POCl₃.

^d Cross-linking conditions: pH 11.5, 0.020% POCl₃.

TABLE V
Differential Scanning Calorimetry Measurements
on Modified Starches at 75% Moisture (wb)

Starch	T _o (°C)	T _p (°C)	ΔH (cal/g)	DS (Acetyl) or MS (Hydroxypropyl) ^a
WB	57.4 ^b	61.7 ^b	3.28 ^c	...
WB-X	56.5	60.7	3.04	...
WB-XA25	49.7	54.4	2.77	0.10
WB-XH46	48.7	53.1	2.37	0.13
WM	63.3	68.3	3.71	...
WM-X	63.2	68.3	3.62	...
WM-XA25	56.1	61.9	3.32	0.10
WM-XH42	55.6	60.4	2.83	0.12

^a DS = degree of substitution; MS = molar substitution.

^b Standard deviation = 0.1°C.

^c Standard deviation = 0.03 cal/g.

Acetylation and hydroxypropylation reduced the pasting temperature in amylograms of the starches by 7–10°C (Table III), as well as the temperature and enthalpy of the gelatinization (Table V). Gelatinization temperatures for WB and modified WB were approximately 6°C below those for WM.

Freeze-Thaw Stability of Modified Starches

Several variables must be controlled in determining the cold-temperature stability of starch pastes, including 1) exact cooking conditions of temperature, time, and stirring; 2) exact storage conditions; and 3) reproducible methods to separate and quantify the water of syneresis.

To increase the severity of the freeze-thaw testing of starches, sealed tubes containing cooked pastes (6.25% starch in water) were held first at 4°C for 24 hr and then subjected to freeze-thaw cycles (–23°C/30°C). Holding at 4°C accelerates starch retrogradation by nucleating the crystallization of starch (Slade and Levine 1987).

In freeze-thaw tests, little or no syneresis was observed in the early cycles. Then suddenly, syneresis was induced, and the rate of water lost from a starch paste or gel increased rapidly. In this study, the number of freeze-thaw cycles that caused 10% water loss was arbitrarily used as an index of freeze-thaw stability.

Native WB and WM starches both showed poor freeze-thaw stability (<1–2 cycles). Cross-linking decreased, but acetylation improved the freeze-thaw stability of WB or WM starch (Fig. 4). Hydroxypropylation was more effective than acetylation in improving freeze-thaw stability. With 2.4% hydroxypropyl groups (MS 0.07) WB-XH24 could withstand nine freeze-thaw cycles, whereas WB-XA25 (acetyl DS 0.10) could withstand only two cycles (Fig. 4).

Unmodified WM starch paste lost approximately 3% water after one freeze-thaw cycle, whereas WM-X starch paste lost 25% and was much more opaque. After four freeze-thaw cycles, pastes of native or cross-linked WM starch were transformed into spongelike structures from which water was easily squeezed out. Pastes of unmodified or modified WB starch were less opaque

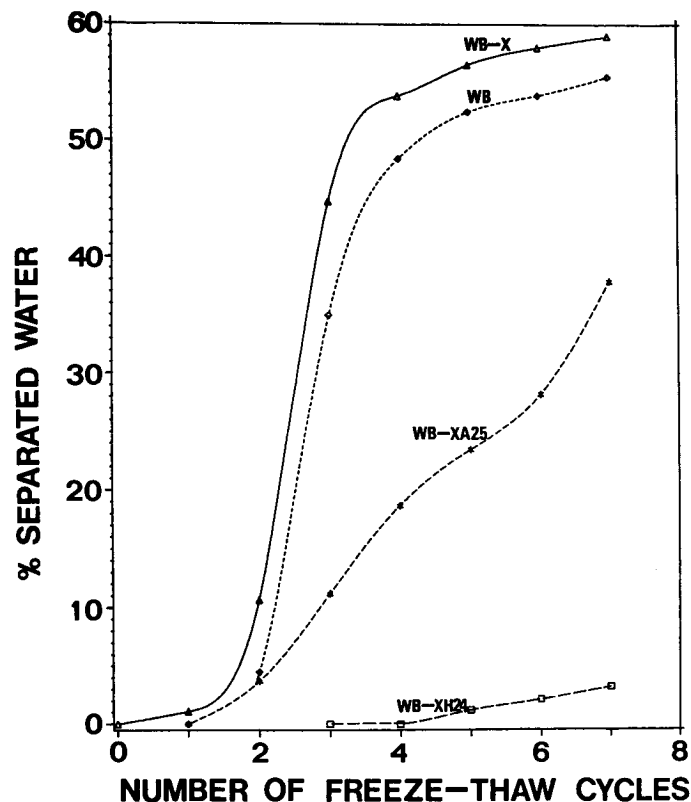


Fig. 4. Freeze-thaw stability of pastes of modified waxy barley starches (6.25% starch in water). Cross-linking conditions: WB-X and WB-XA25, pH 11.0, 0.01% POCl₃; WB-XH24, pH 11.6, 0.005% POCl₃.

than those from WM and remained as swollen gels even when syneresis finally occurred.

Hydroxypropylated tapioca (T-XH44) was the most freeze-thaw stable (12 cycles) of all the commercial starches tested in this work (Fig. 5). One of the doubly modified WB starches, WB-XH46, was much more freeze-thaw stable (>23 cycles) than all the commercial modified starches, including WM and tapioca (Fig. 5). The thickening power of WB-XH46 was also comparable to or better than that of similarly modified commercial starches when cooked in the amylograph.

The good freeze-thaw stability of modified WB starch could result from the short chain-length of its amylopectin. The average chain-lengths of A-chains of amylopectin in waxy rice, tapioca, and maize have been reported to be 13, 12, and 15, respectively (Hizukuri 1988). It is well known that glycogen with the same linkages as amylopectin, but an average chain-length of 8-9, does not crystallize (Gidley and Bulpin 1987). The predominant chain-length in WB starch was 12, as reported by MacGregor and Morgan (1984). Since amylopectin molecules retrograde (re-associate) by crystallization of their unit chains (Ring et al 1987), WB and tapioca starches may be more freeze-thaw stable than WM starch because their branch chains of \overline{DP} 12-13 are less prone to recrystallize than those of WM with \overline{DP} approximately 15.

A model paste system was devised to simulate an acidic fruit filling for a frozen pie or pastry. In the freeze-thaw tests with pastes made from 5% starch in 25% sucrose containing 0.01-0.02% malic acid (pH 3.5), none of the hydroxypropylated starches showed syneresis after 40 cycles ($-23^{\circ}\text{C}/30^{\circ}\text{C}$) by our centrifugation method. This same observation was made in freezer storage tests with pastes made from 5% starch in sucrose/acid solutions, in which no syneresis occurred for any of the hydroxypropylated starches after six months at -23°C . Because the presence of sugar hinders association of the starch molecules (Schoch 1968), more freeze-thaw cycles and a longer storage time are needed to differentiate the best cold-stable starch for frozen pie fillings.

CONCLUSIONS

A hydroxypropylated distarch phosphate from waxy barley (WB-XH46), which was modified by food-grade standards, exhibited extraordinary freeze-thaw stability and also displayed the thickening power, clarity, and paste stability expected of a modified waxy starch. Good quality bread could be made with

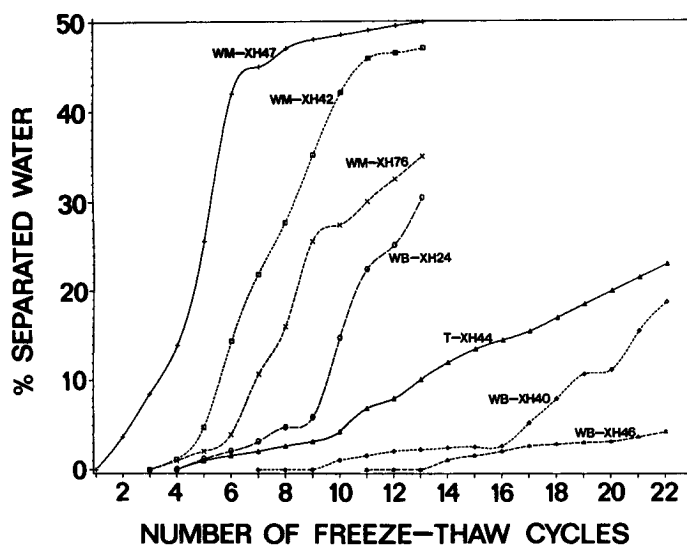


Fig. 5. Freeze-thaw stability of pastes of hydroxypropylated distarch phosphates (6.25% starch in water). WM-XH47, WM-XH76, and T-XH44 were commercial starches. Cross-linking conditions: WM-XH42, pH 11.6, 0.015% POCl_3 ; WB-XH24 and WB-XH40, pH 11.6, 0.005% POCl_3 ; WB-XH46, pH 11.6, 0.007% POCl_3 .

only traces of amylose in its starch. The substitution of modified waxy barley for wheat starch in baked foods may permit texture variations and improvement in their cold-temperature stability.

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LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Methods 08-01 and 44-15A, revised October 1981; Method 46-11, revised October 1982. The Association: St. Paul, MN.
- BANKS, W., GREENWOOD, C. T., and WALKER, J. T. 1970. Studies on the starches of barley genotypes: The waxy starch. *Starch/Staerke* 22:149.
- COCHRAN, W. G., and COX, G. M. 1957. Some methods for the study of response surfaces. Pages 335-375 in: *Experimental Designs*, 2nd ed. John Wiley & Sons: New York.
- CODE OF FEDERAL REGULATIONS. 1988. Title 21, Part 172.892. Food starch-modified. U.S. Government Printing Office: Washington, DC.
- CRAIG, S. A. S., MANINGAT, C. C., SEIB, P. A., and HOSENEY, R. C. 1987. Starch paste clarity. *Cereal Foods World* 32:679.
- DENGATE, H. N. 1984. Swelling, pasting, and gelling of wheat starch. *Adv. Cereal Sci. Technol.* 6:49-82.
- DRAPER, N., and SMITH, H. 1981. *Applied Regression Analysis*, 2nd ed. John Wiley & Sons: New York. 709 pp.
- ERIKSSON, G. 1969. The waxy character. *Hereditas* 63:180.
- ESLICK, R. F. 1979. Barley breeding for quality at Montana State University. Pages 2-25 in: *Proc. Joint Barley Utilization Seminar*. Korean Science and Engineering Foundation and U.S. National Science Foundation, Suwon, Korea.
- ESLICK, R. F. 1981. Mutation and characterization of unusual genes associated with the seed. Pages 864-867 in: *Proc. Int. Barley Genet. Symp.* 4th, Edinburgh.
- FAO. 1983. Modified starches. Pages 76-92 in: *Specifications for Identity and Purity*. Joint FAO/WHO Expert Committee on Food Additives. FAO Food and Nutrition Paper 28. Food and Agriculture Organization of the United Nations: Rome, Italy.
- FELTON, G. E., and SCHOPMEYER, H. H. 1943. Thick-bodied starch and method of making. U.S. patent 2,328,537; *Chem. Abstr.* 38:889, 1944.
- FOOD CHEMICALS CODEX. 1981. Acetyl groups. Page 543 in: 3rd ed. National Academy of Sciences, National Academy Press: Washington, DC.
- FOX, G. J. 1981. The effect of the waxy endosperm, short awn, and hullless seed genes upon biochemical and physiological seed characteristics important in barley (*Hordeum vulgare* L.) utilization. Ph.D. dissertation. Montana State Univ.: Bozeman, MT.
- GHIASI, K., HOSENEY, R. C., ZELEZNAK, K., and ROGERS, D. E. 1984. Effect of waxy barley starch and reheating on firmness of bread crumb. *Cereal Chem.* 61:281.
- GIDLEY, M. J., and BULPIN, P. V. 1987. Crystallization of malto-oligosaccharides as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.* 161:291.
- GOERING, K. J., and ESLICK, R. F. 1976. Barley starch. VI. A self-liquefying waxy barley starch. *Cereal Chem.* 53:174.
- GOERING, K. J., and IMSANDE, J. D. 1960. Barley flour composition and use for starch production. *J. Agric. Food Chem.* 8:368.
- GOERING, K. J., ESLICK, R., and DEHAAS, B. 1970. Barley starch. IV. A study of the cooking viscosity curves of twelve barley genotypes. *Cereal Chem.* 47:592.
- GOERING, K. J., ESLICK, R., and DENS, B. W. 1973. Barley starch. V. A comparison of the properties of waxy Compana barley starch with the starches of its parents. *Cereal Chem.* 50:322.
- HIZUKURI, S. 1988. Recent advances in molecular structure of starch. *J. Jpn. Soc. Starch Sci.* 35(3):185 (in Japanese).
- HOCKETT, E. A. 1981. Registration of hullless and hullless short-awned spring barley germplasm. *Crop Sci.* 21:146.
- HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. 1971. Functional (breadmaking) and biochemical properties of wheat flour components. VIII. Starch. *Cereal Chem.* 48:191.
- HOSENEY, R. C., LINEBACK, D. R., and SEIB, P. A. 1978. Role

- of starch in baked foods. *Baker's Dig.* 52(4):11.
- JOHNSON, D. P. 1969. Spectrophotometric determination of the hydroxypropyl group in starch ethers. *Anal. Chem.* 41:859.
- KESLER, C. C., and HJERMSTAD, E. T. 1950. Preparation of starch ethers in original granule form. U.S. patent 2,516,633; *Chem. Abstr.* 44:11142 (1950).
- MacGREGOR, A. W., and MORGAN, J. E. 1984. Structure of amylopectins isolated from large and small starch granules of normal and waxy barley. *Cereal Chem.* 61:222.
- MAZURS, E. G., SCHOCH, T. J., and KITE, F. E. 1957. Graphical analysis of the Brabender viscosity curves of various starches. *Cereal Chem.* 34:141.
- MEUSER, F., WITTIG, J., HUSTER, H., and HOLLEY, W. 1987. Recent developments in the extraction of starch from various raw materials. Pages 285-299 in: *Cereals in a European Context*. I. D. Morton, ed. Ellis Horwood: Chichester, U.K.; VCH Publishers: New York.
- MORRISON, W. R., and COVENTRY, A. M. 1985. Extraction of lipids from cereal starches with hot aqueous alcohols. *Starch/Staerke* 37:83.
- 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.
- MORRISON, W. R., SCOTT, D. C., and KARKALAS, J. 1986. Variation in the composition and physical properties of barley starches. *Starch/Staerke* 38:374.
- NEWMAN, R. K., NEWMAN, C. W., GRAHAM, H., MCGUIRE, C. F., and GRAY, D. G. 1988. Nutritional functions of barley β -glucans. *Cereal Foods World* 33:678.
- POEHLMAN, J. M. 1985. Adaptation and distribution. Pages 1-17 in: *Barley*. D. C. Rasmusson, ed. *Agron. Monogr.* 26. Am. Soc. Agronomy: Madison, WI.
- RING, S. G., COLONNA, P., I'ANSON, K. J., KALICHEVSKY, M. T., MILES, M. J., MORRIS, V. J., and ORFORD, P. D. 1987. The gelation and crystallization of amylopectin. *Carbohydr. Res.* 162:277.
- RUTENBERG, M. W., and SOLAREK, D. 1984. Starch derivatives: Production and uses. Pages 311-388 in: *Starch: Chemistry and Technology*, 2nd ed. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.
- SCHOCH, T. J. 1964. Swelling power and solubility of granular starches. Pages 106-108 in: *Methods of Carbohydrate Chemistry*, Vol. 4. R. L. Whistler, ed. Academic Press: New York.
- SCHOCH, T. J. 1968. Effects of freezing and cold storage on pasted starches. Page 44-56 in: *The Freezing Preservation of Foods*. Vol. 4., 4th ed. D. K. Tressler, W. B. van Arsdell, and M. J. Copley, eds. Avi Publishing Co.: Westport, CT.
- SCHOCH, T. J. 1969. Starch in foods. Pages 395-420 in: *Symposium on Foods: Carbohydrates and Their Roles*. H. W. Schultz, R. F. Cain, and R. W. Wrolstad, eds. Avi Publishing Co.: Westport, CT.
- SHOGREN, M. D., and FINNEY, K. F. 1984. Bread-making test for 10 grams of flour. *Cereal Chem.* 61:418.
- SHOGREN, M. D., FINNEY, K. F., and HOSENEY, R. C. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. I. Solubilizing gluten and flour protein. *Cereal Chem.* 46:93.
- 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.
- TIPPLES, K. H. 1980. Uses and applications. Pages 12-24 in: *The Amylograph Handbook*. W. C. Shuey and K. H. Tipples, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- TUSCHHOFF, J. V., KESSINGER, G. L., and HANSON, C. E. 1969. Phosphorus oxyhalide cross-linked hydroxypropyl starch derivative. U.S. patent 3,422,088; *Chem. Abstr.* 67:63097 (1967).
- WATSON, S. A. 1964. Corn starch isolation. Pages 3-5 in: *Methods in Carbohydrate Chemistry*. Vol. 4. R. L. Whistler, ed. Academic Press: New York.
- WETZSTEIN, H. W., and LYON, P. 1956. Manufacture of modified starches. U.S. patent 2,754,232; *Chem. Abstr.* 50:13490 (1956).
- WURZBURG, O. B. 1964. Acetylation. Pages 286-288, in: *Methods in Carbohydrate Chemistry*. Vol. 4. R. L. Whistler, ed. Academic Press: New York.
- WURZBURG, O. B. 1986. Forty years of industrial starch research. *Cereal Foods World* 31:897.
- ZELEZNAK, K. J., and HOSENEY, R. C. 1987. The glass transition in starch. *Cereal Chem.* 64:121.

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