

Structure and Functionality of Barley Starches

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ABSTRACT

Cereal Chem. 75(5):747-754

Amylose contents of prime starches from nonwaxy and high-amylose barley, determined by colorimetric method, were 24.6 and 48.7%, respectively, whereas waxy starch contained only a trace (0.04%) of amylose. There was little difference in isoamylase-debranched amylopectin between nonwaxy and high-amylose barley, whereas amylopectin from waxy barley had a significantly higher percentage of fraction with degree of polymerization < 15 (45%). The X-ray diffraction pattern of waxy starch differed from nonwaxy and high-amylose starches. Waxy starch had sharper peaks at 0.58, 0.51, 0.49, and 0.38 nm than nonwaxy and high-amylose starches. The *d*-spacing at 0.44 nm, characterizing the amylose-lipids complex, was most evident for high-amylose starch and was not observed in waxy starch. Differential scanning calorimetry (DSC) thermograms of prime starch from nonwaxy and high-amylose barley exhibited two prominent transition peaks: the first was >60°C and corresponded to starch gelatinization; the second was >100°C and corresponded to the amylose-lipid complex. Starch from waxy barley had only one endothermic gelatinization peak of amylopectin with an enthalpy value of 16.0 J/g. The

retrogradation of gelatinized starch of three types of barley stored at 4°C showed that amylopectin recrystallization rates of nonwaxy and high-amylose barley were comparable when recrystallization enthalpy was calculated based on the percentage of amylopectin. No amylopectin recrystallization peak was observed in waxy barley. Storage time had a strong influence on recrystallization of amylopectin. The enthalpy value for nonwaxy barley increased from 1.93 J/g after 24 hr of storage to 3.74 J/g after 120 hr. When gel was rescanned every 24 hr, a significant decrease in enthalpy was recorded. A highly statistically significant correlation ($r = 0.991$) between DSC values of retrograded starch of nonwaxy barley and gel hardness was obtained. The correlation between starch enthalpy value and gel hardness of starch concentrate indicates that gel texture is due mainly to its starch structure and functionality. The relationship between the properties of starch and starch concentrate may favor the application of barley starch concentrate without the necessity of using the wet fractionation process.

Barley is the fourth largest cereal grain crop produced worldwide, (after wheat, rice, and corn) and is the most underutilized cereal grain in terms of human consumption (Bhatti 1993). As much as 90% of the barley grown is used in alcoholic beverage production and as livestock feed.

Low levels of acceptance and consumption of barley by humans are related to culture and social status. Barley as a food source is associated with low income per capita in underdeveloped countries. On the other hand, especially in Western countries, barley is associated with natural, healthy foods (Newman and Newman 1991). As consumers become more concerned about eating food with health benefits, barley, which is a naturally healthy, easily available, and inexpensive crop, is strongly favored for increased incorporation into the human diet.

Barley for human consumption has been most extensively investigated in Korea, where barley is used as a rice substitute or as a component of wheat products, such as breads and noodles (Cheigh et al 1975, Melland et al 1984). Wheat flour replaced by barley flour at 20–30% levels provides acceptable noodle-making characteristics (Cheigh et al 1976). Ryu et al (1977) reported that a blend of hull-less barley (20%) and wheat (80%) produces acceptable instant-noodle processing characteristics and comparable quality with regard to sensory panel scores (flavor, texture, and color). Hull-less barley is preferred to hulled barley with regard to flour yield during milling and color and taste of noodles (Kim et al 1973).

Most barley studies in the United States have focused on barley product formulation, acceptance of a variety of products by consumers, and taste, color, and texture qualities. When waxy barley is incorporated into wheat-based products, product gumminess often has been reported (Newman et al 1990, 1992).

Barley is an excellent source of complex carbohydrates, which constitute ≈80% of barley grain weight (Czuchajowska et al 1992, Szczodrak et al 1992). Also, barley contains high levels of β-glucans, which are important contributors to dietary fiber, a crucial

component of the human diet (Newman and Newman 1991, Granfeldt et al 1994).

Starch is the largest single component in barley, representing up to 65% of kernel dry weight and providing a valuable source of energy. Despite the availability of barley starch, relatively little research has been done on its functional properties compared to wheat and corn starches. Part of the reason for such neglect is the fact that a high proportion of barley grain is used in animal feed without any processing. Another reason could be the difficulty of isolating starch from barley as a pure product by the wet-fractionation process, which is complex, lengthy, and requires a large amount of water. The high water-holding capacity of barley meal is due primarily to the presence of β-glucans, which absorb a lot of water and make isolation of starch by the wet fractionation process difficult.

Previous studies on barley starch have been done primarily on nonwaxy types of barley cultivars in which starch contains ≈25% amylose. Starches from two other types of barley, waxy and high-amylose, have attracted interest only recently (Czuchajowska et al 1992). During food preparation, starch undergoes partial or complete gelatinization, as well as interacting with other food components (Czuchajowska and Smolinski 1997). Therefore, to promote greater use of barley in human foods, research on the thermal behavior of isolated starches and flours of different types of barley must be conducted.

Well-documented results of such research will be of great value to the food industry in selecting the right type of barley for a specific product or process. The objectives of this study were 1) to evaluate the thermal behavior of starches isolated from nonwaxy, waxy, and high-amylose barley; 2) to examine the thermal behavior of flours differing in composition as a result of abrasion; and 3) to study the retrogradation rate of amylopectin and the gel strength of three types of starch compared to the strength of gel from starch concentrate.

MATERIALS AND METHODS

Samples

Three types of hull-less barley, nonwaxy (cv. Glacier), high-amylose (cv. Glacier), and waxy (cv. Wanubet), were provided by C. W. Newman (Montana State University, Bozeman) and S. Ullrich (Washington State University, Pullman). Barley starch was iso-

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lated from three types of barley by the wet fractionation process, according to the methodology of Szczodrak and Pomeranz (1991).

Abrasion of Barley Samples

Barley was abraded with a tangential abrasive dehulling device (TADD, Venables Machine Works, Ltd., Saskatoon, Canada) to 10, 20, and 40% of kernel weight. Whole and abraded barley was ground into flour with a cyclone sample mill (Udy Corp., Fort Collins, CO) fitted with a 0.25-mm opening to determine chemical composition and thermal properties.

Chemical Analyses

Protein contents ($N \times 6.25$) of samples were determined by a nitrogen analyzer (Leco, St. Joseph, MI) equipped with a thermal conductivity detector. Moisture content was determined by oven drying for 1 hr at 130°C according to Approved Method 44-15A, ash content was determined by dry combustion for 16 hr at 580°C according to Approved Method 08-01, and free lipids content was determined by petroleum ether extraction, followed by evaporation to constant weight under vacuum according to Approved Method 30-25 (AACC 1995). Starch content was determined after enzymatic conversion to glucose by successive treatment with α -amylase, protease, and amyloglucosidase (Prosky et al 1988). The released glucose was measured colorimetrically according to Lloyd and Whelan (1969) with glucose oxidase-peroxidase reagent.

β -Glucan content was measured enzymatically, as described by Ahluwalia and Ellis (1984). β -Glucans were extracted from barley flour with 50 mM perchloric acid at 96°C for 3 min. The extract was incubated with *Penicillium funiculosum* β -glucanase, which was previously heated at 70°C for 1 hr (pH 4.0) to inactivate starch-degrading enzymes (Bamforth 1983). Glucose released by hydrolysis of β -glucans was analyzed with glucose oxidase-peroxidase reagent (Lloyd and Whelan 1969), and results were expressed on a polysaccharide basis (glucose $\times 0.9$). Appropriate substrate and enzyme blanks were included to correct for any free glucose not originating from β -glucans. All analyses were performed at least twice. Mean results of all analyses were reported on a moisture-free basis.

Characteristics of Starch

Amylose content of prime starch was determined by colorimetric method (Knutson and Grove 1994) and high-performance size-exclusion chromatography (HPSEC). The starch solubilization procedures for HPSEC used to estimate amylose content was done according to Bradbury and Bello (1993). Starch (20 mg) was solubilized in 4 mL of 90% (v/v) dimethyl sulfoxide (DMSO) at 90°C with continuous stirring for 16 hr. Solubilized starch was centrifuged at $3,000 \times g$ for 5 min and filtered through a 0.45- μ m nylon filter (Alltech Associates, Deerfield, IL) before injection. Amylose content of starch was determined by HPSEC as the ratio between total peak area and peak area corresponding to amylose (Kobayashi et al 1985).

Isoamylase-debranched amylopectin was prepared according to the method described by Yuan et al (1993) and modified by Lin and Czuchajowska (1997). Starch (10 mg) was treated with 525 units of isoamylase (EC 3.2.1.68, Sigma Chemical Co., St. Louis, MO) in 2 mL of 0.1M sodium acetate buffer (pH 3.8). The final DMSO concentration of the sample mixture was adjusted to 30% with pure DMSO before being heated in boiling water to inactivate enzymes.

The degree of polymerization (DP) of enzyme-debranched amylopectin was determined by the same HPSEC system used to determine amylose content. The DP of the linear fractions in debranched amylopectin was calculated as molecular weight (MW) divided by 162 (Bradbury and Bello 1993).

HPSEC

Solubilized starch (100 μ L) or isoamylase-debranched starch solution was injected into a two-column HPSEC system (Bio-Sil SEC 125, 300 \times 7.8 mm, Bio-Rad Laboratories, Richmond, CA),

using 30% DMSO as the mobile phase at a flow rate of 0.5 mL/min. The two-column system was preceded by a guard column (80 \times 7.8 mm) and a 2- μ m precolumn filter (A315, Upchurch Scientific, Oak Harbor, WA). The HPSEC system consisted of an autosampler (model 1050, Hewlett-Packard, Wilmington, DE), a solvent-delivering system (model 2350 HPLC pump and model 2360 gradient programmer, ISCO, Lincoln, NE), a differential refractometer (R401, Waters Associates, Milford, MA), and a computer equipped with HPLC 3D ChemStation Software (Hewlett-Packard). The columns and detector (sensitivity at 32 \times) were maintained at a constant temperature (35°C). The HPSEC system was calibrated using four pullulan standards (Polymer Laboratories, Amherst, MA) at MW 112,000, 22,800, 5,900 and 738, respectively. A linear relationship ($r^2 = 0.992$) between log MW and retention time was obtained.

Wide-Angle X-ray Diffraction of Barley Starch

X-ray diffraction was performed on barley starch hydrated to 16% by storing it in a chamber maintained at 4°C and 95% rh for ≈ 20 hr. Starches were densely packed in an aluminum frame. X-ray diffraction patterns of specimens were recorded on a diffractometer (D 500, Siemens, Madison, WI) operating at 35 kV and 30 mA. Diffractograms were obtained from 4° 2 θ to 30° 2 θ with a step of 0.05° 2 θ , counting 4 sec on each step.

X-ray diffraction data was reported by interplanar d -spacing values (nm). The crystallinity of starch samples was evaluated with respect to the integrated normalized intensities of diffraction peaks and sharpness of pattern. Integrated normalized intensities were calculated on the basis of the number of counts recorded by the scintillation counter (Czuchajowska et al 1991, Sievert et al 1991).

Differential Scanning Calorimetry

Thermal behavior of three types of barley starches and flours was followed by differential scanning calorimetry (DSC), as described by Czuchajowska and Pomeranz (1989), on a Perkin-Elmer (Norwalk, CT) DSC-2 instrument using large-volume stainless-steel capsules. Also, the rate of starch retrogradation after storage of gel at 4°C for up to 120 hr was measured by DSC.

Experiment 1. In the first experiment, 30 subsamples of dry starch (10 mg) isolated from nonwaxy and waxy barley were weighed in large pans, water was added (20 μ L), and the pans were sealed and scanned from 20 to 180°C at a heating rate of 10°C/min. The 30 pans were divided into six sets of five pans each and stored at 4°C. Each set was rescanned once after 24, 48, 72, 96, and 120 hr.

Experiment 2. In the second experiment, six replicates of nonwaxy and waxy prime starches were scanned every 24 hr for up to 120 hr. After each scan, the pans were stored at 4°C to evaluate the effect of repeated heating on the intensity of retrogradation of nonwaxy and waxy barley starches.

Pasting Properties of Abraded Barley

The pasting properties of abraded barley were measured with a Brabender Viscoamylograph according to Shuey and Tipples (1980), with 10% flour in 450 mL of water. The slurry was heated at 1.5°C/min from 30°C to the temperature at which a peak in the pasting curve appeared. Peak viscosity was determined from the pasting curve.

Gel Texture from Prime Starch and Starch Concentrate

The texture of gel, representing 60% of the inner part of the barley kernel (starch concentrate), was measured with a texture analyzer (TX.XT2, Stable Micro System, Haslemere, England). The gel was prepared in a Brabender Viscoamylograph. The slurry of 10% starch or 10% flour was heated to 90°C and held at 90°C for 20 min. The hot paste was poured into molds (30 mm diameter, 35 mm high), covered to avoid evaporation of water, and stored at 4°C for up to 120 hr. Gel texture was measured by two attachments: a Plexiglas plunger (12 mm diameter) and a disk (60 mm diameter).

Gel prepared from waxy barley was too weak to stand by itself. Therefore, to have comparable data, the texture of gels prepared from nonwaxy, high-amylose, and waxy barley was measured with the Plexiglas plunger. The gels were penetrated once by the plunger to 30% of gel height, and the force of penetration was recorded. Nonwaxy and high-amylose barley gels were compressed by disk to 30% of the gel height, and gel hardness was recorded. The texture of gels was measured during a 120-hr period at 24-hr intervals.

Statistical Analysis

All tests were run at least twice. Least significant difference, analysis of variance, and correlation analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Composition of Whole Barley

The composition of three barley types is summarized in Table I. Protein content ranged from 12.5% in high-amylose barley to 15.5% in waxy barley. Protein content varied in barley based on growing conditions, with an average value of 13%, which is almost equal to wheat and higher than in other cereal grains (Newman and Newman 1991). Ash content in high-amylose barley (3.01%) was significantly higher than in waxy and nonwaxy barley (2.61 and 2.11%, respectively). Free lipids contents in nonwaxy and waxy barley were comparable, whereas high-amylose barley had significantly lower free lipids content.

More than 65% of the barley content was starch. Similar values were recently reported by Baik and Czuchajowska (1997) and Czuchajowska et al (1992). The wide variation in starch content in barley, ranging from 48 to 72%, may be due to the determination method or to both genetic and environmental effects (Åman et al 1985, Morrison et al 1986, Bach Knudsen et al 1987, Henry 1987b). β -Glucan content was highest in waxy barley (6.6%) and lowest in high-amylose barley (5.6%). Czuchajowska et al (1992) also reported that waxy barley had the highest β -glucan content.

Effect of Abrasion on Composition of Barley

Changes in barley composition due to abrasion at 10, 20, and 40% are summarized in Table II. Compared to whole kernels, protein content significantly decreased when the percentage of abrasion increased. A similar pattern was observed for all three types of

barley. The largest change in protein content, a decrease of 4.2%, took place in high-amylose barley at 40% abrasion compared to whole grain, whereas nonwaxy and waxy barley decreased in protein content by 3.4 and 3.6%, respectively, compared to whole grain. The ash and free lipids contents decreased more than two times due to the removal of 40% of the outer layer of the kernels. Free lipids content of <1% in abraded barley at the 40% abrasion level was equal to the lipids content in wheat flour milled to a 60% extraction level (Czuchajowska and Pomeranz 1993). This low level of free lipids in barley flour obtained from abraded barley would extend its shelf life.

In contrast to protein, ash, and free lipids, starch content increased with abrasion. In the 40%-abraded kernels, starch content increased by 12.4% in waxy barley and by >15% in nonwaxy and high-amylose barley. Similar changes in barley composition due to abrasion were reported by Baik and Czuchajowska (1997). In all three types of barley, β -glucan content was highest in the inner part when abraded at the 40% level. This result agrees with a study by Henry (1987b), who reported that most β -glucans are present in the endosperm. The relationship between β -glucan content and cell wall thickness was suggested by Aastrup (1983). In contrast, low β -glucan levels were reported in the outer part of barley kernels by Henry (1987a).

Composition of Isolated Barley Starches

Isolating starch from barley is time-consuming and laborious (McDonald and Stark 1988), requiring several washing and centrifugation steps. The composition of purified prime starch is presented in Table III. As indicated by average protein and ash contents of 0.5 and 0.2%, respectively, all three types of barley starches had high purity. β -Glucans were not detected in these starches. Similar barley starch compositions were reported by Czuchajowska et al in 1992.

Amylose content of prime starch differed statistically between the three types of barley, as determined by both HPSEC and colorimetric method. Amylose content of prime starch from nonwaxy and waxy types of barley, measured by HPSEC, was higher than that measured by colorimetric method. A similar pattern was reported by Kobayashi et al (1985) and more recently by Lin and Czuchajowska (1997) for wheat starch. These authors reported that a very

TABLE I
Composition (%) of Whole Barley Kernels

Barley	Protein	Ash	Free Lipids	Starch	Total β -Glucans
Nonwaxy	13.6b ^a	2.11c	2.60	67.6a	6.21ab
High-amylose	12.5c	3.01a	2.16b	65.2b	5.56c
Waxy	15.5a	2.61b	2.65a	65.6b	6.60a

^a Values in a column followed by different letters are significantly different at $P = 0.05$.

TABLE III
Composition (%) of Prime Starch

Barley	Protein	Ash	Starch	Amylose	
				HPSEC ^a	Colorimetric
Nonwaxy	0.56b ^b	0.21b	97.4b	32.7b	24.6b
High-amylose	0.61a	0.18c	98.4a	39.7a	48.7a
Waxy	0.35c	0.23a	97.8b	7.4c	0.04c

^a High-performance size-exclusion chromatography.

^b Values in a column followed by different letters are significantly different at $P = 0.05$.

TABLE II
Composition (%) of Abraded Barley Kernels

Barley	Abrasion Level	Protein	Ash	Free Lipids	Starch	Total β -Glucans
Nonwaxy	Whole kernel	13.6a ^a	2.11a	2.60a	67.6d	6.2ab
	10%	12.3b	1.68b	1.88b	73.7c	6.6a
	20%	11.6c	1.45c	1.47c	77.2b	6.8a
	40%	10.2d	1.00d	0.95d	82.9a	6.8a
High-amylose	Whole kernel	12.5a	2.05a	2.16a	65.2d	5.6c
	10%	10.3b	1.62b	1.62b	71.0c	6.4b
	20%	9.9c	1.36c	1.18c	77.8b	6.5b
	40%	8.3d	0.92d	0.80d	81.0a	7.1a
Waxy	Whole kernel	15.5a	2.61a	2.65a	65.6d	6.60cd
	10%	14.6b	1.90b	1.76b	70.5c	6.87c
	20%	13.5c	1.46c	1.23c	74.6b	7.31b
	40%	11.9d	0.96d	0.86d	78.0a	8.00a

^a Values in a column within each type of barley followed by different letters are significantly different at $P = 0.05$.

accurate measurement by HPSEC of amylose content <30% is difficult to achieve due to overlapping between amylose and amylopectin. In the current study, the overlapping peaks of amylose and amylopectin measured by HPSEC are shown in Fig. 1.

Independent of applied methodology, however, the highest amylose content was found in starch from high-amylose barley, followed by starch from nonwaxy and waxy barley. Amylose content, determined by colorimetric method, from these three types of starch agreed with previous work by Czuchajowska et al (1992). Waxy starch contained 0.04% amylose, whereas amylose content in high-amylose barley reached ≈50%. Prime starch from nonwaxy barley had an amylose content of ≈25%, which is close to most wheat starches.

Fine Structure of Amylopectin

Isoamylase-debranched amylopectin from the three types of barley had a trimodal pattern for the nonwaxy type (Fig. 2). The area and peak were related to a high MW (HMW) with DP > 35, intermediate MW (IMW) with DP < 35 but > 15, and low MW (LMW) with DP < 15. These results are consistent with those reported by Hanashiro et al (1996) and MacGregor and Morgan (1984).

The distribution of the average MW of the branch chains of all three evaluated starches is summarized in Table IV. The average branch chain distribution of amylopectin showed little difference

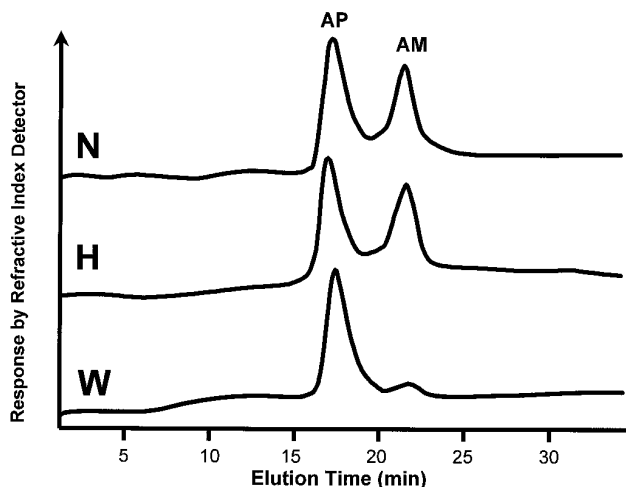


Fig. 1. Amylopectin (AP) and amylose (AM) peaks of solubilized barley starch eluted with two high-performance size-exclusion columns and 30% (v/v) dimethyl sulfoxide as an eluent at a flow rate of 0.5 mL/min. N, H, and W = nonwaxy, high-amylose, and waxy starches, respectively.

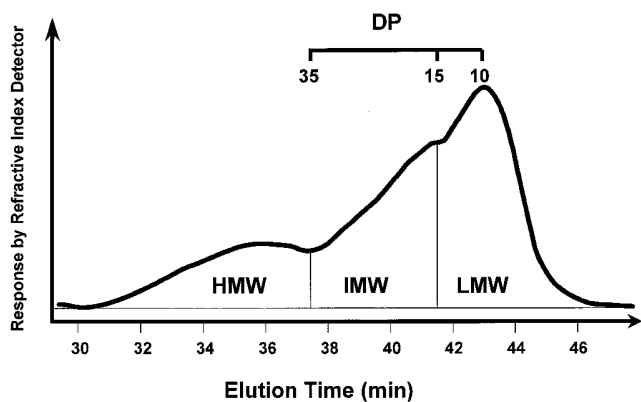


Fig. 2. Elution profile of isoamylase-debranched barley amylopectin eluted with two high-performance size-exclusion columns and 30% (v/v) dimethyl sulfoxide as an eluent at a flow rate of 0.5 mL/min. HMW (high molecular weight) degree of polymerization (DP) > 35; IMW (intermediate molecular weight) DP < 35 but > 15; LMW (low molecular weight) DP < 15.

between nonwaxy and high-amylose barley. Waxy barley contained significantly more HMW and LMW but fewer IMW fractions of debranched amylopectin than did nonwaxy and high-amylose barley. Actual data agree with that of MacGregor and Morgan (1984), in that the HMW fraction of debranched amylopectin from waxy barley represented ≈20% of the total relative peak area.

X-ray Diffraction Pattern of Barley Starches

All three barley starches exhibited the A-type X-ray diffraction pattern (Fig. 3). A higher percent relative intensity indicates a higher degree of starch crystallinity. Major peaks for barley starches were observed around *d*-spacings of 0.58, 0.51, 0.49, 0.44, and 0.38 nm. Zobel (1988) reported that X-ray *d*-spacings of 0.58, 0.52, and 0.38 nm are characteristic of an A-type starch crystal that is common in most cereal starches. Waxy barley starch differed from nonwaxy and high-amylose barley, with sharper peaks at 0.58, 0.51, 0.49, and 0.38 nm. The *d*-spacing at 0.44 nm is characteristic of the amylose-lipid complex. No peak was observed in waxy starch at 0.44 nm; high-amylose barley had the most evident peak at 0.44 nm. The data agree with the findings of Vasanthan and Bhatti (1996), who reported that waxy barley had no peak at 0.44 nm and that there were sharper peaks at 0.44 nm in high-amylose than in nonwaxy barley.

Viscosity of Abraded Barley

The large changes in composition of barley kernels due to abrasion (Table II) caused significant changes in the thermal behavior of abraded barley. The increase in starch content due to abrasion resulted in increased viscosity for all three types of barley. In nonwaxy barley, viscosity increased from 680 BU in whole meal to 970 BU in flour from 40%-abraded kernels (Table V). This increase in viscosity was due mainly to an increase in starch content from 67.6 to 82.9%, because total β-glucans showed no significant changes due to abrasion.

TABLE IV
Branch Chain Distribution (%) of Isoamylase-Debranched Amylopectin

Barley	HMW ^a	IMW ^b	LMW ^c
Nonwaxy	15.5b ^d	47.5a	37.0b
High-amylose	16.3b	46.4a	37.4b
Waxy	19.7a	35.4b	45.0a

^a High molecular weight: degree of polymerization (DP) > 35.

^b Intermediate molecular weight: DP < 35 but > 15.

^c Low molecular weight: DP < 15.

^d Values in a column followed by different letters are significantly different at *P* = 0.05.

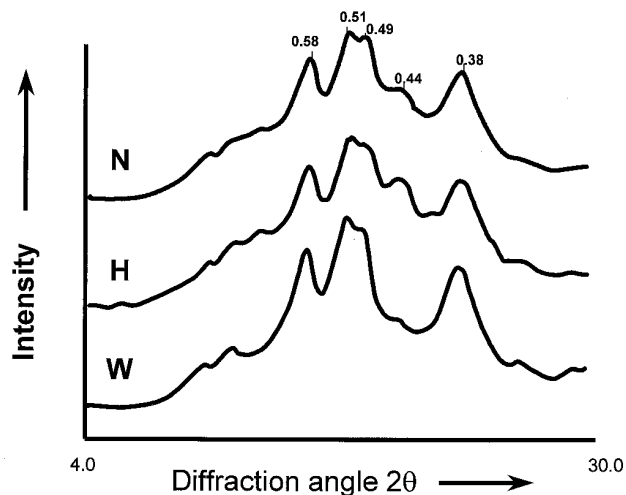


Fig. 3. X-ray diffraction patterns of barley starches. N, H, and W = nonwaxy, high-amylose, and waxy starches, respectively. Numbers indicate starch crystal *d*-spacings (2θ) of the major diffraction peaks.

For high-amylose barley, viscosities were comparable to non-waxy barley. The largest changes in viscosity due to abrasion took place in waxy barley. The viscosity of 40%-abraded barley was three times higher than that of whole meal. It is interesting that this large increase in peak viscosity in all three types of barley was not accompanied by increases in peak temperature. Therefore, independent of the level of abrasion for each type of barley, temperature was almost constant. For nonwaxy barley, the amylograph peak temperature ranged between 85 and 86°C; for high-amylose barley, the temperature was 83°C; and for waxy barley, maximum peak viscosity occurred between 64 and 65°C, ≈20°C lower than for nonwaxy and high-amylose barley. Peak viscosity temperature of prime starch from high-amylose, waxy, and non-waxy barley corresponded to peak viscosity temperature of starch concentrates for the same type of barley. For example, waxy prime starch peak viscosity was at 68°C, whereas peak viscosity of starch concentrate was at 65°C. The peak viscosity temperature of high-amylose prime starch was at 93°C, whereas peak viscosity of starch concentrate was at 87°C. However, large differences in viscosities were recorded between prime starch and starch concentrate. Waxy prime starch increased in peak viscosity to 2,060 BU compared to 1,580 BU of starch concentrate at the same 10% concentration. Prime starch from high-amylose barley decreased in peak viscosity compared to starch concentrate, from 940 to 360 BU. Similarly, prime starch from nonwaxy barley decreased in peak viscosity, from 970 to 630 BU.

DSC Thermograms of Flour and Starch

DSC is an excellent technique for studying the thermal behavior of starch in the presence of other components, such as proteins, fiber, or lipids (Erdogdu et al 1995, Czuchajowska and Smolinski

1997). DSC thermograms of whole-meal barley, 20% of outer kernels, 80% of inner kernels, and pure starch of nonwaxy barley are presented in Fig. 4. Both size and shape of enthalpy peak and gelatinization temperature depended on the complexity of the material. For example, the enthalpy value of 80% of inner endosperm was much lower compared to pure starch than enthalpy calculated from factors of dilution for the other components. Instead of an enthalpy value of 7.9 J/g, 80% of inner endosperm had a value of 5.3 J/g. We concluded that this result was due to the interaction of starch with other flour components (protein, lipids, or fiber).

Peak gelatinization temperature was highest for whole meal and the outer layer of the kernel, lower for 80% inner kernel, and lowest for prime starch. Nonwaxy prime starch showed a sharp, narrow, well-defined peak, whereas complex materials of whole-meal barley and the outer or inner part of kernels produced broader, flatter peaks. DSC thermograms for high-amylose and waxy barley (data not shown) had a pattern similar to nonwaxy barley.

DSC thermograms of starch gelatinization and recrystallization for the three types of barley during storage are presented in Fig. 5. Both nonwaxy and high-amylose barley exhibited two prominent transitions over similar temperature ranges. The first transition temperature, >60°C, corresponded to endotherms of starch gelatinization. The second transition, >100°C, corresponded to the amylose-lipids complex. The starch from waxy barley had only one large gelatinization peak of amylopectin at 16 J/g (Fig. 5A). High-amylose prime starch had a higher amylose-lipids complex than prime starch from nonwaxy barley, which may have been due to higher amylose content determined by HPSEC and colorimetric method (Table III). Also, the higher amylose-lipids complex of high-amylose starch than those of waxy and nonwaxy starches

TABLE V
Amylograph Parameters of Abraded Barley

Barley	Abrasion Level	Peak Temp. (°C)	Peak Viscosity (BU)
Nonwaxy	Whole kernels	85	680
	10%	85	860
	20%	85	930
	40%	85	970
High-amylose	Whole kernels	83	...
	10%	83	...
	20%	83	930
	40%	83	940
Waxy	Whole kernels	64	510
	10%	64	980
	20%	64	1,380
	40%	65	1,580

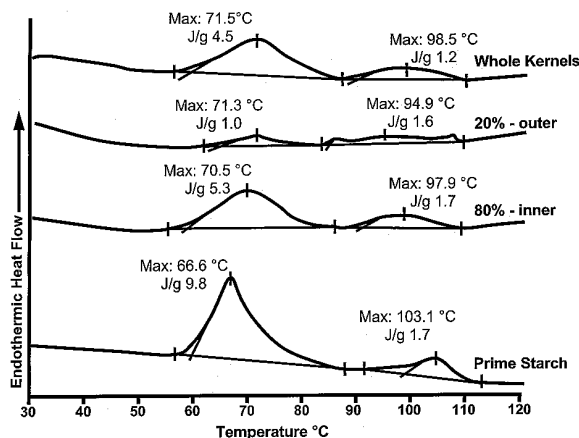


Fig. 4. Differential scanning calorimetry thermograms of whole kernels, 20% outer part and 80% inner part of barley kernels, and prime starch of non-waxy barley.

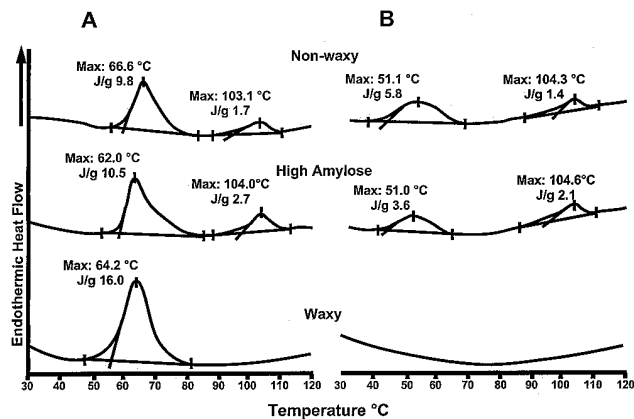


Fig. 5. Differential scanning calorimetry thermograms of prime starch from nonwaxy, high-amylose, and waxy barley for first scan without storage. (A) and rescan after two weeks of storage at 4°C (B).

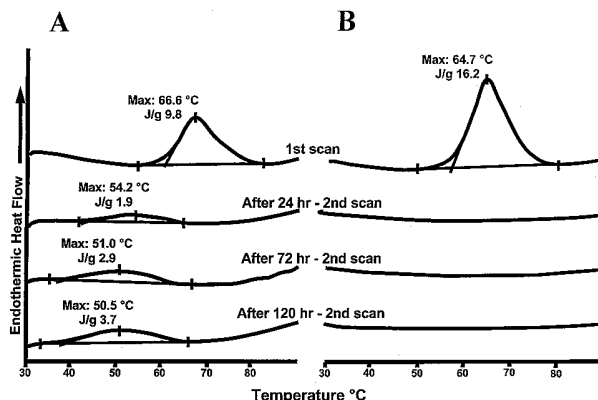


Fig. 6. Differential scanning calorimetry thermograms of starches from nonwaxy (A) and waxy (B) barley. Starches were scanned (first) and rescanned (second) after up to 120 hr at 4°C.

was confirmed by X-ray crystallography (Fig. 3). The intensity of the peak at 0.44 nm represents the amylose-lipids complex (Czuchajowska et al 1991).

The retrogradation of gelatinized starch of three types of barley stored at 4°C for two weeks is presented in Fig. 5B. The enthalpy values of retrograded amylopectin of nonwaxy and high-amylose barley were comparable when the recrystallization was calculated based on the percentage of amylopectin. No recrystallization peak of amylopectin was found in waxy barley. This result may be explained by the presence of a high percentage of low DP of branches of waxy amylopectin determined by HPSEC of isoamylase-debranched amylopectin (Table IV).

The highly diverse composition and thermal behavior of barley starches directed this research toward a more in-depth study of the rate of retrogradation of gelatinized starch and the changes in gel texture when stored. Retrogradation of amylopectin was determined by DSC; the gel texture was determined with a texture analyzer.

Retrogradation of Amylopectin: DSC Study

Two experiments were done on nonwaxy and waxy prime starches. In the first experiment, after the first scan (five sets of samples, six replicates each), gels of DSC pans containing both nonwaxy and waxy starch were stored at 4°C. Each set was rescanned one time after a 24-hr storage interval. In the second experiment, all samples were rescanned five times at 24-hr intervals.

The retrogradation rate of prime starch from nonwaxy barley is presented in Fig. 6A. As indicated by the size of the enthalpy peak, the retrogradation of amylopectin increased with storage time. After 120 hr, an enthalpy value of 3.74 J/g was recorded. The onset temperature of recrystallized amylopectin after 120 hr of storage was 38°C, ≈10°C lower than the onset of gelatinized starch. The lower temperature and enthalpy peak of recrystallized amylopectin

indicates it has less perfectly ordered structure. In waxy barley, again, the retrogradation of amylopectin did not occur during storage of the gel under the same conditions (Fig. 6B). Because waxy starch did not show recrystallization enthalpy during storage in the first or second experiment, changes in recrystallization due to rescanning of each gel five times are shown only for starch of nonwaxy barley (Fig. 7A).

Storage time had a strong influence on recrystallization of amylopectin (Fig. 7A). The enthalpy value increased from 1.93 J/g after 24 hr to 3.74 J/g after 120 hr. However, a significant decrease in enthalpy was recorded when gels were rescanned five times at 24-hr intervals (Fig. 7B). These results indicate that not only is a certain amount of time needed to recrystallize amylopectin but also that frequent melting can change the inner structure of amylopectin and delay retrogradation. This observation could be important to the food industry, because it may enable processors to affect the texture of products by delaying recrystallization.

Gel Texture

The effect of storage on texture of gels from prime starch and starch concentrate of three types of barley measured by a Plexiglas plunger is presented in Fig. 8. A plunger was used to penetrate the gel because it was impossible to remove the gel of waxy barley from molds. The hardness of waxy starch gel was <0.7 N and did not change during storage. Gels from nonwaxy and high-amylose starches showed distinctly higher hardness than waxy starch, ranging from 5.8 to 11.1 N (Fig. 8A). The gel texture from starch concentrate, also measured by a plunger, showed a similar pattern but slightly lower values (Fig. 8B). Hardness of gel from waxy starch concentrate was <0.6 N, whereas hardness of gels from nonwaxy and high-amylose starch concentrate ranged from 5.2 to 9.5 N.

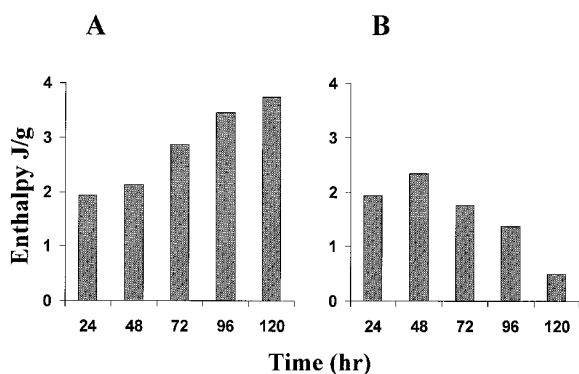


Fig. 7. Enthalpy values of retrograded starch from nonwaxy barley rescanned after storage for up to 120 hr at 4°C at 24-hr intervals (A) or rescanned repeatedly at 24-hr intervals for up to 120 hr (B).

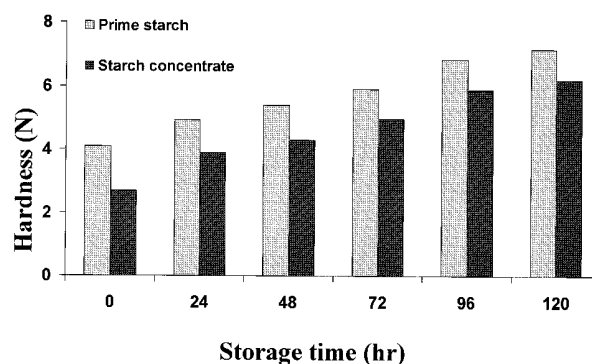


Fig. 9. Hardness of gels prepared from prime starch and starch concentrate from nonwaxy barley, measured with a texture analyzer equipped with a disk.

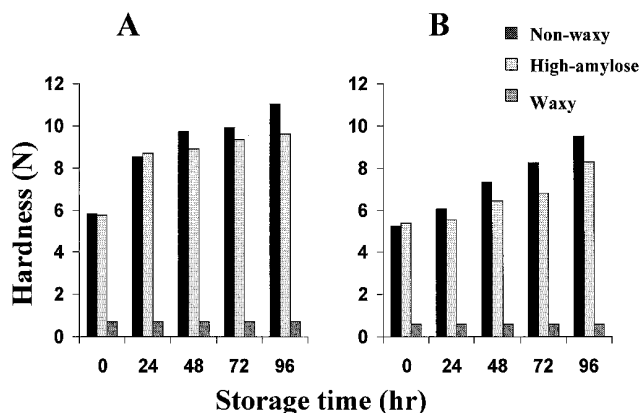


Fig. 8. Hardness of gels prepared from prime starch (A) and starch concentrate (B) measured with a texture analyzer equipped with a plunger.

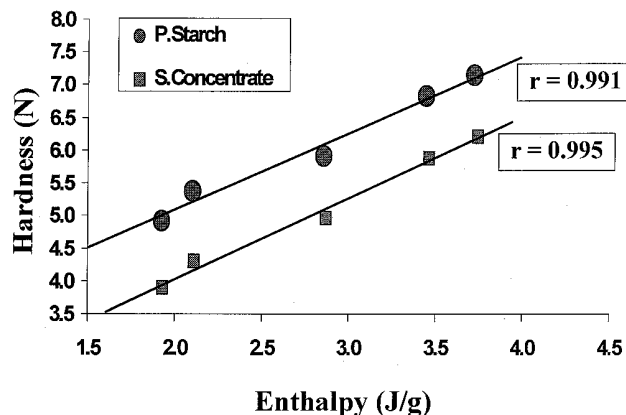


Fig. 10. Correlations between the enthalpy value of prime starch and hardness of gels from prime starch and starch concentrate from nonwaxy barley.

The texture of strong gels from nonwaxy and high-amylose barley was measured by a disk. The whole cylindrical gel was compressed without breaking the gel. The hardness of gel from starch of nonwaxy barley increased during storage from 4.1 to 7.2 N, whereas hardness of gel from high-amylose starch increased from 6.6 to 9.2 N. The higher relative value of gel hardness from high-amylose starch could be due to an almost two times higher amylose content in high-amylose starch than in nonwaxy starch, as determined by colorimetric method.

Hardness of gel prepared from prime starch and starch concentrate of nonwaxy barley measured by a disk are presented in Fig. 9. When storage time was increased, gel hardness increased significantly for both starch and starch concentrates. However, starch concentrate produced a softer gel, due to the presence of other components. A statistically significant positive correlation was obtained between hardness and storage time of gels prepared from starch and starch concentrate for nonwaxy ($r = 0.997$) and high-amylose ($r = 0.964$) barley. The gradual increase in gel hardness (starch and starch concentrate) could be due mainly to retrogradation of amylopectin, especially because changes in gel hardness were greater for nonwaxy than for high-amylose gel. Therefore, a relationship should exist between starch gel hardness and DSC enthalpy values during storage.

The correlations between DSC values (J/g) and gel hardness (N) for prime starch ($r = 0.991$) and starch concentrate ($r = 0.995$) of nonwaxy barley are shown in Fig. 10. The strong correlation between starch enthalpy values and hardness of starch concentrates indicates that the texture of starch gel concentrate is due mainly to its starch content, structure, and functionality (Table II). The fact that this relationship is found for starch concentrate might be of particular importance for the food industry, because it favors the application of barley starch concentrate without the necessity of using the wet fractionation process to isolate starch.

ACKNOWLEDGMENTS

This research is part of the IMPACT (International Marketing Program for Agricultural Commodities and Trade) activities at Washington State University, Pullman, and was supported financially by the Washington Barley Commission.

LITERATURE CITED

- Aastrup, S. 1983. Selection and characterization of low β -glucan mutants in barley. *Carlsberg Res. Commun.* 48:308-316.
- Ahluwalia, B., and Ellis, E. E. 1984. A rapid and simple method for the determination of starch and β -glucan in barley and malt. *J. Inst. Brew.* 90:254-260.
- Åman, P., Hesselman, K., and Tilly, A.-C. 1985. The variation in chemical composition of Swedish barleys. *J. Cereal Sci.* 3:73-77.
- American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9th ed. Method 08-01, approved April 1961, revised October 1981 and October 1986; Method 30-25, approved April 1961, revised October 1976 and October 1981, reviewed October 1994; Method 44-15A, approved October 1975, revised October 1981 and October 1994. The Association: St. Paul, MN.
- Bach Knudsen, K. E., Åman, P., and Eggum, B. O. 1987. Nutritive value of Danish-grown barley varieties. I. Carbohydrates and other major constituents. *J. Cereal Sci.* 6:173-186.
- Baik, B.-K., and Czuchajowska, Z. 1997. Barley in udon noodles. *Food Sci. Technol. Int.* 3:423-435.
- Bamforth, C. W. 1983. *Penicillium funiculosum* as a source of β -glucanase for the estimation of barley β -glucan. *J. Inst. Brew.* 89:391-392.
- Bhatty, R. S. 1993. Nonmalting uses of barley. 1993. Pages 355-417 in: *Barley Chemistry and Technology*. A. W. MacGregor and R. S. Bhatty, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Bradbury, A. G. W., and Bello, A. B. 1993. Determination of molecular size distribution of starch and debranched starch by a single procedure using high-performance size-exclusion chromatography. *Cereal Chem.* 70:543-547.
- Cheigh, H.-S., Ryu, C. H., and Kwon, T. W. 1976. Preparation and evaluation of dried noodles using barley-wheat and barley-soybean flours. *Korean J. Food Sci. Technol.* 8:236-242.
- Cheigh, H.-S., Snyder, H. E., and Kwon, T. 1975. Rheological and milling characteristics of naked and covered barley varieties. *Korean J. Food Sci. Technol.* 7:85-90.
- Czuchajowska, Z., and Pomeranz, Y. 1989. Differential scanning calorimetry, water activity, and moisture contents in crumb center and near-crust zones of bread during storage. *Cereal Chem.* 66:305-309.
- Czuchajowska, Z., and Pomeranz, Y. 1993. Protein concentrates and prime starch from wheat flours. *Cereal Chem.* 70:701-706.
- Czuchajowska, Z., and Smolinski, S. 1997. Instrumental measurements of raw and cooked gluten texture. *Cereal Foods World* 42:526-532.
- Czuchajowska, Z., Sievert, D., and Pomeranz, Y. 1991. Enzyme-resistant starch. IV. Effect of complexing lipids. *Cereal Chem.* 68:537-542.
- Czuchajowska, Z., Szczodrak, J., and Pomeranz, Y. 1992. Characterization and estimation of barley polysaccharides by near-infrared spectroscopy. I. Barleys, starches and β -d-glucans. *Cereal Chem.* 69:413-418.
- Erdogdu, N., Czuchajowska, Z., and Pomeranz, Y. 1995. Wheat flour and defatted milk fractions characterized by differential scanning calorimetry. I. DSC of flour and milk fractions. *Cereal Chem.* 72:70-75.
- Granfeldt, Y., Liljeberg, H., Drews, A., Newman, R., and Bjorck, I. 1994. Glucose and insulin responses to barley products: Influence of food structure and amylose-amylopectin ratio. *Am. J. Clin. Nutr.* 59:1075-1081.
- Hanashiro, I., Abe, J., and Hizukuri, S. 1996. A periodic distribution of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydr. Res.* 283:151-159.
- Henry, R. J. 1987a. Pentosan and (1 \rightarrow 3),(1 \rightarrow 4) β -glucan concentrations in endosperm and whole grain of wheat, barley, oats and rye. *J. Cereal Sci.* 6:253-258.
- Henry, R. J. 1987b. Variation in the carbohydrate composition of barley. Pages 763-766 in: *Barley Genetics V. Proc. 5th Int. Barley Genet. Symp.* S. Yasuda and T. Konishi, eds. Sanyo Press, Okayama, Japan.
- Kim, Y. S., Ahn, S. B., Lee, K., and Lee, S. R. 1973. Development of composite flours and their products utilizing domestic raw materials. III. Noodle-making and cookie-making tests with composite flours. *Korean J. Food Sci. Technol.* 5:25-32.
- Knutson, C. A., and Grove, M. J. 1994. Rapid method for estimation of amylose in maize starches. *Cereal Chem.* 71:469-471.
- Kobayashi, S., Schwartz, S. J., and Lineback, D. R. 1985. Rapid analysis of starch, amylose and amylopectin by high-performance size-exclusion chromatography. *J. Chromatogr.* 319:205-214.
- Lin, P.-Y., and Czuchajowska, Z. 1997. Starch properties and stability of club and soft white winter wheats from the Pacific Northwest of the United States. *Cereal Chem.* 74:639-646.
- Lloyd, J. B., and Whelan, W. J. 1969. An improved method for enzymic determination of glucose in the presence of maltose. *Anal. Biochem.* 30:467-469.
- 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.* 61:222-228.
- McDonald, A. M. L., and Stark, J. R. 1988. A critical examination of procedures for the isolation of barley starch. *J. Inst. Brew.* 94:125.
- Melland, R., Newman, R. K., McGuire, C. F., and Eslick, R. F. 1984. The effects of bleach treatment on pasta made from a series of barley genotypes. *Cereal Res. Commun.* 12:201-207.
- Morrison, W. R., Scott, D. C., and Karkalas, J. 1986. Variation in the composition and physical properties of barley starches. *Starch/Staerke* 38:374-379.
- Newman, R. K., and Newman, C. W. 1991. Barley as a food grain. *Cereal Foods World* 36:800-805.
- Newman, R. K., McGuire, C. F., and Newman, C. W. 1990. Composition and muffin-baking characteristics of flours from barley cultivars. *Cereal Foods World* 35:563-566.
- Newman, R. K., Newman, C. W., McGuire, C. F., and Han, X. 1992. Preparation of Chinese steamed bread with hard white winter wheat and waxy barley flours. Pages 118-125 in: *Proc. 1994 Int. Symp. Exhibition New Approaches Prod. Food Stuffs Intermediate Prod. Cereal Grains Oil Seeds*. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Prosky, L., Asp, N. G., Schweizer, T. F., Devries, J. W., and Forda, I. 1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *J. Assoc. Anal. Chem.* 71:1017-1023.
- Ryu, C.-H., Cheigh, H.-S., and Kwon, T. W. 1977. A note on the preparation and evaluation of Ramyon (deep fat fried instant noodle) using

- barley-wheat composite flours. *Korean J. Food Sci. Technol.* 9:81-83.
- Shuey, W. C., and Tipples, K. H., eds. 1980. *The Amylograph Handbook*. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Sievert, D., Czuchajowska, Z., and Pomeranz, Y. 1991. Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylo maize VII starch and enzyme-resistant starch residues. *Cereal Chem.* 68:86-91.
- Szczodrak, J., Czuchajowska, Z., and Pomeranz, Y. 1992. Characterization and estimation of barley polysaccharides by near-infrared spectroscopy. II. Estimation of total β -D-glucans. *Cereal Chem.* 69:419-423.
- Szczodrak, J., and Pomeranz, Y. 1991. Starch and enzyme-resistant starch from high-amylose barley. *Cereal Chem.* 68:589-596.
- Vasanthan, T., and Bhatta, R. S. 1996. Physicochemical properties of small and large granule starches of waxy, regular, and high-amylose barley. *Cereal Chem.* 73:199-207.
- Yuan, R. C., Thompson, D. B., and Boyer, C. D. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. *Cereal Chem.* 70:81-89.
- Zobel, H. F. 1988. Starch crystal transformations and their industrial importance. *Starch/Staerke* 40:1-7.

[Received January 26, 1998. Accepted June 4, 1998.]