

Soft Wheat Starch Pasting Behavior in Relation to A- and B-type Granule Content and Composition¹

S. V. Shinde,² J. E. Nelson,³ and K. C. Huber^{3,4}

ABSTRACT

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Flours of two soft wheat cultivars were fractionated into native, prime, tailing, A-, and B-type starch fractions. Starch fractions of each cultivar were characterized with respect to A/B-type granule ratio, amylose content, phosphorus level (lysophospholipid), and pasting properties to investigate factors related to wheat starch pasting behavior. While both cultivars exhibited similar starch characteristics, a range of A-type (5.7–97.9%, db) and B-type granule (2.1–94.3%, db) contents were observed across the five starch fractions. Though starch fractions displayed only subtle mean differences (<1%) in total amylose, they exhibited a range of mean phosphorus (446–540 µg/g), apparent amylose (18.7–23%), and lipid-complexed amylose (2.8–7.5%) values, which were significantly

correlated with their respective A- and B-type granule contents. A-type (compared with B-type) granules exhibited lower levels of phosphorus, lipid-complexed amylose, and apparent amylose, though variability for the latter was primarily attributed to starch lipid content. While starch phosphorus and lipid-complexed amylose contents exhibited negative correlation with fraction pasting attributes, they did not adequately account for starch fraction pasting behavior, which was best explained by the A/B-type granule ratio. Fraction A-type granule content was positively correlated with starch pasting attributes, which might suggest that granule size itself could contribute to wheat starch pasting behavior.

Wheat endosperm contains two distinct populations of starch granules, A- and B-type, which may be classified according to time of biosynthesis, size, and shape. Synthesis of A-type granules begins four days after anthesis with granule growth and development continuing over the next 20 days (Bechtel et al 1990). In contrast, B-type granule synthesis is initiated at 10 days after anthesis with significant granule growth beginning 20 days thereafter. The report of two granule-bound starch biosynthetic proteins preferentially associated with A-type granules (while largely absent in B-type granules) has provided additional evidence that the two granule types represent separate and distinct populations (Peng et al 2000). A third wheat starch granule class, C-type, which is initiated at 21 days after flowering, was initially reported by Bechtel et al (1990). While it is not known whether C-type granules themselves represent a distinct granule class or that they simply constitute a later initiation of B-type granules, a majority of published studies have considered wheat starch to consist of two granule types (Soulaka and Morrison 1985; Peng et al 1999; Stoddard 1999).

With regard to morphology, A-type granules are larger sized (>10 µm) and lenticular shaped, while B-type granules are smaller sized (<10 µm) and spherical shaped (Evers et al 1974; Peng et al 1999). While A-type granules typically represent the greatest proportion of endosperm starch by weight (50–90%), B-type granules predominate numerically (as high as 99%) (Dengate and Meredith 1984; Soulaka and Morrison 1985; Bechtel et al 1990; Wooton et al 1993; Raeker et al 1998; Stoddard 1999; Peng et al 1999).

Aside from their differential prevalence within wheat endosperm, A- and B-type granule fractions differ with regard to chemical composition, gelatinization behavior, and pasting properties. A-type starch granules presumably possess slightly higher apparent (Kulp 1973; Meredith 1981; Soulaka and Morrison 1985) and total (Soulaka and Morrison 1985; Raeker et al 1998; Peng et al 1999) amylose contents compared with the B-type counterparts, though several authors have observed no such differences between the two granule types (Bathgate and Palmer 1972; Evers et al 1974). Higher levels of lipid and lipid-complexed amylose have been gen-

erally associated with the B-type granules (Eliasson and Karlsson 1983; Soulaka and Morrison 1985; Raeker et al 1998), though opposing reports also exist (Wong and Lelievre 1982a; Panozzo and Eagles 1998).

Starch swelling, gelatinization, and pasting properties are influenced by both amylose and lysophospholipid (LPL) contents (Eliasson and Karlsson 1983; Tester and Morrison 1990; Morrison 1995; Zeng et al 1997; Lin and Czuchajowska 1998). Tester and Morrison (1990) demonstrated a greater swelling capacity for A-type, while Wong and Lelievre (1982a) reported a greater swelling tendency for the smaller B-type granules. A third study (Kulp 1973) described similar swelling behaviors for small granule and prime starch fractions over 60–90°C. For the majority of published studies, B-type granules were more resistant to gelatinization (Bathgate and Palmer 1972; Kulp 1973; Wong and Lelievre 1982a; Eliasson and Karlsson 1983; Peng et al 1999), though several researchers have observed identical gelatinization behavior for the two granule types (Ghiasi et al 1982; Soulaka and Morrison 1985). There are several conflicting reports regarding the pasting properties of A- and B-type starch granules. Medcalf and Gilles (1968) reported a higher peak viscosity for the large granule fraction, but a greater hot paste stability and total setback for the small granule fraction. Data from Kulp (1973) was inconclusive with respect to peak viscosity differences between small granule and prime starch fractions, but reported a lower hot paste stability and final viscosity for the small granule fraction. Using a Rheotest 2 rotary viscometer, a small granule wheat starch exhibited lower peak and final viscosity, but similar breakdown, compared with a large granule fraction (Fortuna et al 2000). In direct contrast to the aforementioned studies, Sebecic and Sebecic (1999) and Peterson and Fulcher (2001) reported negative correlations between flour A-type granule content and flour peak viscosity.

The wide range of A- and B-type granule contents, compositions, and properties within these reports might be attributable to differences in wheat class and cultivar, starch isolation procedures, granule separation techniques, or modes of granule size measurement. As details regarding the recovery and purity of the separated A- and B-type granule fractions are provided to varying degrees within the cited literature, it is difficult to resolve many of the conflicting reports. Careful consideration of these variables will be necessary to provide additional knowledge of A- and B-type granule populations. The present study will characterize the composition and properties of five starch fractions (native, prime, tailing, A-, and B-type) isolated from two soft white wheat cultivars grown at multiple locations. It was anticipated that the five starch

¹ University of Idaho Agric. Exp. Stn. Paper 02B01.

² J.R. Simplot Co., Food Group, Caldwell, ID 83601-1059.

³ Department of Food Science and Toxicology, University of Idaho, P.O. Box 441053, Moscow, ID 83844.

⁴ Corresponding author. Phone: 208-885-4661. Fax: 208-885-2567. E-mail: huberk@uidaho.edu.

fractions would provide a range of granule size distributions for novel investigation of the contribution of granule type and chemical composition to wheat starch properties. Starch fractions within a flour will be characterized with regard to chemical attributes (amylose, LPL level), granule size content (A/B-type ratio), and pasting properties to elucidate primary factors contributing to wheat starch pasting behavior.

MATERIALS AND METHODS

Material Sources

Two soft white wheat cultivars, Madsen and Lewjain, each grown at five different Idaho locations (Moscow, Bonners Ferry, Parma, Tensed, Tammany), were selected for study. Wheat for each cultivar-location combination was milled to straight-grade flour on a Quadramat Sr. mill (Brabender, Hackensack, NJ) according to Approved Method 26-31 (AACC 2000), and represented the sources of all isolated starch for the study.

Native Starch Isolation

Native starch, which will be defined as the entire starch fraction present within a flour, was isolated from flour for each cultivar-location combination similar to the method of Reddy and Seib (1999). Flour (60.0 g, db) was suspended in a solution of 0.02M HCl (600 mL) with stirring (10 min). Sodium bisulfite (0.3 g) and thiomersal (0.006 g) were added to the slurry, and adjusted to pH 7.5 using Tris-(hydroxymethyl)aminomethane. In addition, a solution of protease (0.3 g, Sigma, P-5147) was prepared in 0.02M HCl (10 mL), stirred (5 min) gently to avoid frothing, and added to the flour slurry. The combined suspension was incubated 24 hr at 4°C with continuous stirring. After the incubation period, the starch slurry was subdivided equally among three centrifuge bottles, and centrifuged at 2,500 × g (15 min). The subsequent supernatants were discarded, and the resultant pellet in each bottle was resuspended in a solution of cesium chloride (80%, w/v; 50 mL), and centrifuged at 2,500 × g (15 min), after which the supernatants were discarded. Additional cesium chloride solution (30 mL) was added to each centrifuge bottle, and the centrifugation procedure was repeated once more to yield a clean white starch pellet (supernatants again discarded). The starch pellet was washed extensively with deionized water (100 mL × 3), filtered through a 70-µm sieve to remove any remaining fiber or bran material, and centrifuged (2,500 × g, 15 min) to obtain a purified native starch fraction. Obtained starch was suspended in absolute ethanol, recovered on a Büchner funnel, and allowed to air-dry.

Prime and Tailing Starch Isolation

Flour for each cultivar-location combination was fractionated into prime and tailing starch fractions according to the method outlined by Yamazaki et al (1977) with some minor modifications. Flour (50.0 g, db) was combined with deionized water (35 mL) and mixed (3 min) in an ultra power mixer (Kitchen Aide Corp., St. Joseph, MI). Following initial mixing, additional deionized water (65 mL) was added, and the resultant dough was subjected to further mixing (3 min), transferred to a Waring blender, and blended (1 min) at high speed. The blended slurry was centrifuged (2,500 × g, 15 min) to yield prime (lower pellet layer) and tailing (upper pellet layer) starch fractions. The supernatant was discarded, while the upper gluten and tailing starch layers were separated from the prime starch layer with a spatula. The prime starch fraction was further purified by centrifugation through cesium chloride solution, washed extensively with deionized water, and recovered by centrifugation as previously outlined for the native starch fraction. The purified prime starch fraction was suspended in absolute ethanol, recovered on a Büchner funnel, and allowed to air-dry.

The combined tailing starch and gluten fractions remaining after prime starch isolation were suspended in 0.02M HCl (400 mL) followed by the addition of sodium bisulfite (0.25 g) and thiomersal (0.005 g). The suspension was stirred on a magnetic stirrer (10 min) to break up the gluten mass, after which the slurry was adjusted to pH 7.5 with the addition of Tris-(hydroxymethyl) aminomethane. Protease solution (0.2 g of protease in 10 mL 0.02M HCl) was prepared as previously described for native starch isolation and added to the starch-gluten slurry followed by incubation for 24 hr at 4°C. After 24 hr, the slurry was centrifuged (2,500 × g, 15 min) to recover starch, while the supernatant was discarded. Tailing starch was further purified by centrifugation through cesium chloride solution, washed extensively with deionized water, and recovered by centrifugation as previously described for the native starch fraction. Recovered starch was suspended in ethanol, collected on a Büchner funnel, and air-dried.

Fractionation of A- and B-Type Starch Granules

For each cultivar-location combination, a portion of the isolated native starch was fractionated into respective A- and B-type granule populations using a combination of centrifugation and microscreening techniques. Using a modification of the method described by Peng et al (1999), native starch (2.5 g, db) was suspended in aqueous sucrose solution (80%, w/v; 20 mL) within a plastic tube (28.8 × 106.7 mm, Nalgene Corp., NY), and centrifuged at 18 × g (5 min), after which supernatant 1 (B-type granules) was collected and set

TABLE I
Mean Yields of Starch Fractions^a

Cultivar	Starch Yield				
	Native ^b	Prime ^b	Tailing ^b	A-Type ^c	B-Type ^c
Madsen	83.0 ± 1.0	47.0 ± 4.2	29.4 ± 4.8	64.9 ± 1.4	20.6 ± 1.2
Lewjain	83.8 ± 1.6	48.6 ± 3.5	23.9 ± 4.1	63.6 ± 2.3	23.5 ± 2.8

^a Values are mean ± standard deviation calculated across five growing locations.

^b g/100 g of flour.

^c g/100 g of native starch.

TABLE II
Mean Chemical Composition of Starch Fractions Isolated from Madsen and Lewjain Wheat Flours^a

Fraction	Madsen					Lewjain				
	N (%)	P (µg/g)	AAM	TAM	ΔAM	N (%)	P (µg/g)	AAM	TAM	ΔAM
A-type	0.06b	446a	22.6d	26.9c	4.3b	0.06a	448a	22.1c	26.6b	4.5b
Prime	0.04ab	453a	23.0d	25.8ab	2.8a	0.04a	455a	22.3c	25.6a	3.3a
Native	0.03a	506b	21.9c	26.5bc	4.6b	0.02a	472a	22.2c	27.2b	5.0b
Tailing	0.15c	522b	18.7a	25.2a	6.5c	0.15b	508c	19.0a	26.5b	7.5d
B-type	0.05ab	497b	19.6b	26.0b	6.4c	0.05a	540b	20.1b	26.5b	6.4c

^a AAM, apparent amylose; TAM, total amylose; ΔAM = TAM — AAM. Value means calculated across five growing locations. Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

aside. The resultant pellet was resuspended in fresh sucrose solution (20 mL), and recentrifuged (18 × g, 2.5 min) to yield supernatant 2 (also B-type granules). The same pellet was further centrifuged (18 × g, 1.25 min) three additional times through fresh sucrose solution to generate supernatants 3, 4, and 5 (contained mixtures of A- and B-type granules), and a final pellet (A-type granules).

Purified B-type granules within supernatants 1 and 2 were combined and recovered by centrifugation (2,500 × g, 15 min). The final pellet, which consisted of purified A-type granules, was also retained. Both collected starch fractions (final pellet, combined supernatants 1 and 2) were each washed extensively with deionized water (50 mL × 3), resuspended in an excess of absolute ethanol, recovered on a Büchner funnel, and allowed to air-dry.

Supernatants 3, 4 and 5, which contained a mixture of A- and B-type granules, were combined and collected by centrifugation. The combined starch material was washed extensively with deionized water (30 mL × 3), resuspended in deionized water, and filtered on a 10-µm precision screen (ATM Corp., Milwaukee, WI) enhanced with mechanical vibration (to augment flow) to recover both A-type (overs) and B-type (throughs) starch granules. Additional deionized water was added during the filtration step as necessary to facilitate the process. Purified A-type (overs) and B-type (throughs) granules were each recovered by centrifugation (2,500 × g, 15 min) and dried as mentioned above.

After drying, like fractions were combined to yield final A-type (final pellet + sieve overs) and B-type (supernatants 1 and 2 + sieve throughs) starch granule fractions. Although the outlined fractionation method is described on the basis of a single tube, one replicate for a cultivar-location combination typically consisted of four to six tubes, which allowed 10–15 g of native starch to be fractionated simultaneously.

Assessment of Granule Size Distribution

Granule size distributions for all isolated starch fractions (native, prime, tailing, A-, B-type) were determined similar to the method of Dengate and Meredith (1984) using a Coulter Counter (model TAI; Beckman Coulter, Miami, FL) equipped with a 200-µm orifice probe. A starch suspension (2.5%, w/v) was prepared in deionized water, after which an aliquot (15 µL) was transferred to sodium chloride electrolyte solution (1% w/v; 200 mL) with continuous stirring. The starch suspension was examined on a 16 channel

analyzer that had been calibrated previously with standardized latex spheres over a diameter of 0.63–80.6 µm. A 10-µm cutoff was used as a threshold diameter for differentiating A- and B-type starch granule populations.

General Analyses

Moisture content of starch fractions was determined according to AOAC Method 925.09 (AOAC 1990), while starch protein content was estimated by nitrogen combustion (%N × 5.70) (Approved Method 46.30, AACCC 2000). Starch phosphorus content was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) as described by Anderson (1996) to approximate starch LPL content. Flour starch content was assessed using Megazyme test kits (Wicklow, Ireland) (Approved Method 76-13, AACCC 2000).

Amylose Determination

Apparent (AAM), total (TAM), and lipid-complexed (ΔAM) amylose contents were determined for isolated starch fractions (native, prime, tailing, A-, B-type) representing each cultivar-location combination. Assays were conducted according to the colorimetric method reported by Morrison and Laignelet (1983).

Starch Pasting Properties

Pasting characteristics of the wheat flours and the native, prime, tailing, A-, and B-type starch fractions for each cultivar-location combination were determined according to the parameters described by Batey et al (1997) using the Rapid Visco Analyzer (RVA) (Newport Scientific, NSW, Australia). Starch (3.0 g, db) or flour (3.5 g, db) was weighed into a RVA canister followed by addition of deionized water or silver nitrate solution (0.012M), respectively, to achieve a final slurry weight of 29.0 g for analysis.

Experimental Design

To ensure that isolated starch fractions were representative and to facilitate calculation of starch fraction yields, native starch isolation from flour was conducted in triplicate for each cultivar-location combination, while isolation of prime and tailing starch fractions was performed in duplicate. After calculating starch fraction yields, replicate isolations of starch were pooled to generate a single source of native, prime, and tailing starch for each cultivar-

TABLE III
Correlation Coefficients (r) Among Starch Granule Types (A or B), Chemical Composition, and Pasting Attributes for Madsen and Lewjain Starch Fractions^{a,b}

	B-Type	P	AAM	TAM	ΔAM	Peak	Trough	Final	Breakdown	TS
Madsen										
A-type	-0.99*	-0.50*	0.77*	0.29*	-0.65*	0.87*	0.67*	0.89*	0.45*	0.79*
B-type		0.50*	-0.76*	-0.29*	0.65*	-0.87*	-0.67*	-0.88*	-0.45*	-0.79*
P			-0.54*	ns	0.45*	-0.50*	ns	-0.39*	-0.50*	-0.49*
AAM				0.43*	-0.83*	0.65*	0.42*	0.66*	0.38*	0.65*
TAM					ns	ns	ns	ns	0.25*	0.27*
ΔAM						-0.55*	-0.45*	-0.60*	-0.25*	-0.52*
Peak							0.52*	0.90*	0.72*	0.95*
Trough								0.80*	ns	0.38*
Final									0.38*	0.84*
Breakdown										0.79*
Lewjain										
A-type	-0.99*	-0.70*	0.70*	ns	-0.64*	0.95*	0.66*	0.89*	0.63*	0.78*
B-type		0.70*	-0.70*	ns	0.64*	-0.94*	-0.65*	-0.88*	-0.64*	-0.77*
P			-0.59*	ns	0.58*	-0.62*	-0.44*	-0.56*	-0.41*	-0.49*
AAM				ns	-0.82*	0.63*	0.48*	0.63*	0.42*	0.55*
TAM					0.48*	ns	ns	ns	ns	ns
ΔAM						-0.60*	-0.48*	-0.60*	-0.38*	-0.50*
Peak							0.58*	0.91*	0.76*	0.89*
Trough								0.78*	ns	0.34*
Final									0.49*	0.84*
Breakdown										0.82*

^a AAM, apparent amylose; TAM, total amylose; ΔAM = TAM – AAM; TS, total setback.

^b *, significant at $P < 0.05$; ns = not significant; $n = 75$.

location combination to be used for all further analyses. Fractionation of native starch into A- and B-type starch granule populations was replicated six times for each cultivar-location combination. Repetitions were bulked to achieve final fraction compositions. All starch analyses described above were conducted in triplicate (unless specified otherwise) for native, prime, tailing, A-, and B-type starch fractions of each cultivar-location combination. To facilitate statistical comparison of the various starch fractions, experimental data were pooled across growing location (each growing location was considered a replicate) to calculate fraction mean values for each cultivar. Using the Statistical Analysis System (v. 8.1, SAS Institute, Cary, NC), significant differences between starch fractions were determined using analysis of variance (ANOVA), while a least significant difference (LSD) test was used to differentiate fraction means. Pearson's correlation analysis examined relationships among the various starch characteristics and related these characteristics to observed starch fraction pasting properties.

RESULTS AND DISCUSSION

Starch Fraction Yields and A- and B-type Granule Contents

Average yields of native, prime, tailing, A-, and B-type starch fractions calculated for Madsen and Lewjain are shown in Table I, while the respective mean A- and B-type granule contents of each fraction are illustrated in Fig. 1 for Lewjain. Starch fraction A/B-type granule ratios are presented on a volume basis, which is presumed to be equivalent or interchangeable with weight basis (Soulaka and Morrison 1985). All stated values and percentages presented are reported on a dry weight basis unless specified otherwise. Mean yields of native starch yields were virtually identical for both cultivars and represented $\approx 98\%$ recovery of the total starch present in flours. Native starch A- and B-type granule contents within our study were intermediate compared with the wide range of values previously reported (Dengate and Meredith 1984; Wooton et al 1993; Stoddard 1999).

Average prime and tailing starch fraction yields for the two cultivars are presented in Table I. Combined prime and tailing yields represented 85–90% of the total starch present in parent flours. As anticipated, the prime starch fractions possessed higher mean proportions of A-type granules and lower mean percentages of B-type granules compared with the tailing starch fractions, which exhibited higher mean percentages of B-type and a lower mean percentages of A-type granules (Fig. 1). While there are no quantitative reports in the literature regarding the A- and B-type starch granule contents of prime and tailing starch fractions, Endo et al (1988) reported a higher average granule diameter range for prime starch (16–25 μm) compared with tailing (8–9 μm) starch. Pomeranz (1988) reported a similar qualitative observation based on microscopic examination. As the prime and tailing starch isolation process recovered 85–90% of the indigenous starch present within a flour, we investigated whether the isolation process had resulted in disproportionate losses of either A- or B-type granules. Comparison of the mean A/B-type granule ratios of the collective

prime and tailing starch fractions (1.85 Madsen and 2.02 Lewjain) and the native starch fractions (1.88 Madsen and 2.39 Lewjain) provided evidence that the indigenous A/B-type granule ratios within the original native starches was adequately conserved in the isolated prime and tailing starches.

Yields of A- and B-type starch granule fractions (Table I) (isolated from a portion of the native starch fractions) compared favorably with previously reported values (Evers et al 1974; Evers and Lindley 1977; Soulaka and Morrison 1985). Collective mean yields of A- and B-type starch granule fractions within this study accounted for $\approx 85\text{--}89\%$ of the original native starch used for fractionation. Nevertheless, highly pure A- and B-type starch granule fractions were obtained from isolated native starches (Fig. 1). The purity of the A- and B-type fractions confirmed the efficacy of the separation procedure, which involved a combination of centrifugation and microsieving techniques. Previous studies that used microsieving (nylon mesh sieve) for fractionation experienced relatively poor separation (B-type granule fractions $<70\%$ purity) as suggested by Soulaka and Morrison (1985) and Peng et al (1999). This study employed an improved microsieving technique utilizing a precision sieve that yielded highly pure and distinct A- and B-type granule populations. However, a portion of starch (11–15%) was unrecovered during the fractionation process. The mean ratios of A/B-type granules in the native starch fraction for both cultivars (1.88 Madsen and 2.39 Lewjain) were lower than those of the collective A- and B-type isolated starch fractions (2.96 Madsen and 2.81 Lewjain), suggesting that predominantly B-type granules were lost during the fractionation process. Nevertheless, purified B-type granule fractions exhibited normal granule size distributions, and possessed starch granules over the entire 1–10 μm range (data not shown).

In summary, starch recoveries and purities for the various starch fractions (native, prime, tailing, A-, and B-type) appeared to be acceptable and representative. Furthermore, isolated starch fractions possessed varying proportions of A- and B-type granules and provided an effective model for investigating the impact of granule type on the chemical composition and pasting properties of wheat starch.

Chemical Composition of Starch Fractions

All starch fractions were reasonably pure as indicated by low mean nitrogen contents (Table II). While starch nitrogen levels within all fractions were relatively low, the tailing starch fractions generally exhibited the highest nitrogen levels compared with the other starch fractions.

As lysophospholipid (LPL) accounts for 86–94% of total starch lipids in wheat starch (Soulaka and Morrison 1985; Raeker et al 1998), total starch phosphorus levels were determined to approximate the relative LPL contents of the various starch fractions (Table II). The A-type starch granules of both Madsen and Lewjain exhibited

TABLE IV
Mean Rapid Visco Analyser (RVA) Pasting Characteristics
of Starch Fractions Isolated from Lewjain^a

Fraction	Viscosity (RVU)				TS
	Peak	Trough	Final	Breakdown	
A-type	341a	194b	435a	147a	241a
Prime	299b	223a	427a	76b	204b
Native	267c	224a	403b	43d	179c
Tailings	215d	183b	318c	32d	135e
B-type	193e	130c	295c	63c	165d

^a Pasting value means calculated across five growing locations. TS, total setback. Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

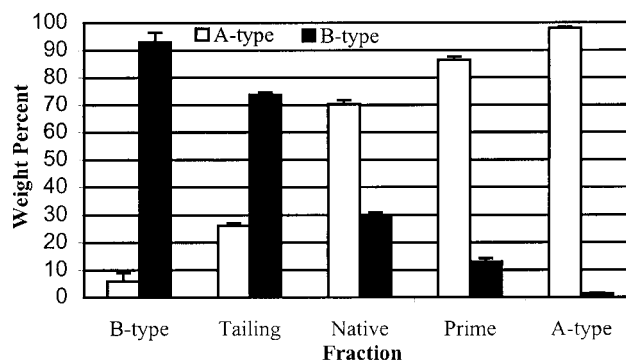


Fig. 1. Mean A- and B-type granule contents of starch fractions isolated from Lewjain wheat flour. A similar profile was exhibited for starch fractions of Madsen.

significantly lower phosphorus contents compared with the B-type granules, corroborating the previous report of Soulaka and Morrison (1985). Likewise, the prime starch fractions, which consisted predominantly of A-type granules, exhibited lower mean phosphorus contents compared with tailing starch fractions, which possessed a majority of B-type starch granules. The native starch fractions generally exhibited intermediate phosphorus values compared with the other starch fractions. Overall, a negative correlation was evident between starch phosphorus levels and A-type granule content for both cultivars, indicating that larger granules generally possessed lower LPL contents (Table III). Thus, wheat starch phosphorus contents were directly affected by granule type.

Mean apparent (AAM), total (TAM), and lipid-complexed (Δ AM) amylose contents were determined for each starch fraction (Table II). Only a subtle difference ($\approx 1\%$) was observed between the TAM contents of A- and B-type granule fractions for Madsen, whereas no such difference was observed for Lewjain. This finding parallels previous conflicting reports in which some researchers reported a higher TAM content for A-type granules (Soulaka and Morrison 1985; Peng et al 1999), and others found no such difference (Evers et al 1974). Soulaka and Morrison (1985) suggested a relatively minor TAM differential ($<2\%$) between the A- and B-type granules. Peng et al (1999) reported a relatively large difference in TAM (4–10%) among A- and B-type granules, which they attributed to the high purity (89–100%) of fractionated A- and B-type granules. However, the present study, which also attained comparably pure A- and B-type granule fractions detected only minor TAM differences between the two fractions. While variability among cultivars is certain, our findings, in conjunction with the majority of previous studies, suggest that the differences between the A- and B-type fractions with regard to TAM are relatively minute.

Prime starch from Lewjain exhibited a lower mean TAM content compared with tailing starch, although no such difference was observed for Madsen (Table II). TAM content of the starch fractions was correlated positively with Δ AM for Lewjain, while no such correlation was evident for Madsen (Table III). No additional significant correlation was observed between TAM and any other starch chemical characteristic. To conclude, only subtle differences were observed among the TAM contents of the various starch fractions, implying that TAM content was not likely largely responsible for observed differences in the pasting properties of starch fractions.

In contrast to the TAM values, the five starch fractions exhibited a wider range of mean AAM contents (Table II). The A-type granule fractions for Madsen and Lewjain exhibited significantly higher mean AAM contents compared with the B-type granule fractions in harmony with the previous report of Soulaka and Morrison (1985). Similarly, prime starch fractions, which possessed a high proportion of A-type granules, also exhibited higher mean AAM contents in contrast to the tailing starch fractions. Native starch fractions possessed intermediate mean AAM contents, while tailing

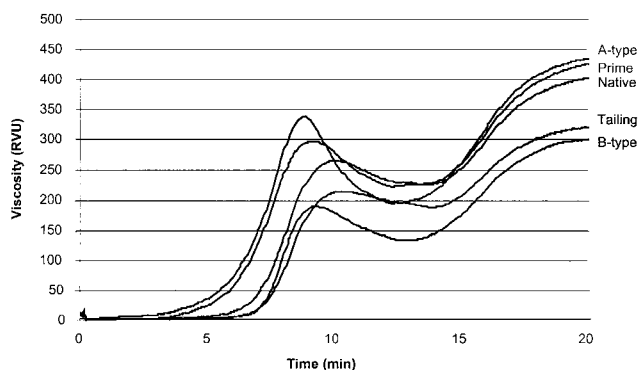


Fig. 2. Mean Rapid Visco Analyser (RVA) pasting profiles of starch fractions isolated from Lewjain (starch paste concentration 10.3% w/w).

and B-type granule fractions, which possessed the highest proportions of B-type granules, exhibited the lowest mean AAM contents (Table II). In addition, AAM content for Madsen and Lewjain correlated positively with A-type and negatively with B-type granule fractions (Table III), indicating that AAM fraction contents were influenced substantially by A/B-type granule ratios. Further, AAM content correlated negatively with starch phosphorus levels and Δ AM for both cultivars (Table III), whereas TAM content lacked consistent, strong correlation with any single starch characteristic.

Fractions displayed a wide range of mean Δ AM values (Table II). Mean lipid-complexed amylose contents were higher in the B-type compared with the A-type starch granule fractions of both cultivars (Table II). Native starch fractions exhibited intermediate mean Δ AM values, which did not differ statistically from those of the A-type granule fractions. Prime starch fractions displayed the lowest mean Δ AM values of any fraction, while tailing starches represented the opposite extreme. Differences in Δ AM levels among the fractions were somewhat attributable to fraction A- and B-type granule contents, as Δ AM correlated negatively with A-type and positively with B-type granule content in both cultivars (Table III). As expected, starch fractions with higher Δ AM values typically exhibited higher phosphorus contents (Table II), which was supported by positive correlation between Δ AM and phosphorus content for both cultivars (Table III). As Δ AM content was inversely correlated to AAM content for both cultivars (Table III), starch fractions with lower AAM contents generally exhibited higher values for lipid-complexed amylose. As starch fractions previously varied minimally in terms of their actual (total) amylose contents, differences in AAM content among the fractions appeared to be primarily a function of starch Δ AM levels. Thus, differences in LPL and Δ AM contents might offer some explanation for the variable pasting behaviors of the native, prime, tailing, A-, and B-type starch granule fractions.

Starch Pasting Properties

Each individual starch fraction (native, prime, tailing, A-, B-type) exhibited a distinct RVA pasting profile as illustrated for Lewjain (Fig. 2; Table IV). Because starch fractions differed significantly in terms of their chemical compositions and A/B-type starch granule contents, both factors were investigated for explanation of starch fraction pasting behavior. A significant positive correlation was observed between AAM content and peak, trough, final, breakdown, and total setback viscosities for both cultivars (Table III). Both fraction peak and final starch pasting viscosities increased with increasing AAM values, while no meaningful correlation was observed between TAM content and starch pasting attributes. In stark contrast, Zeng et al (1997) reported high negative correlations for both AAM (-0.91) and TAM (-0.95) contents with peak paste viscosities of

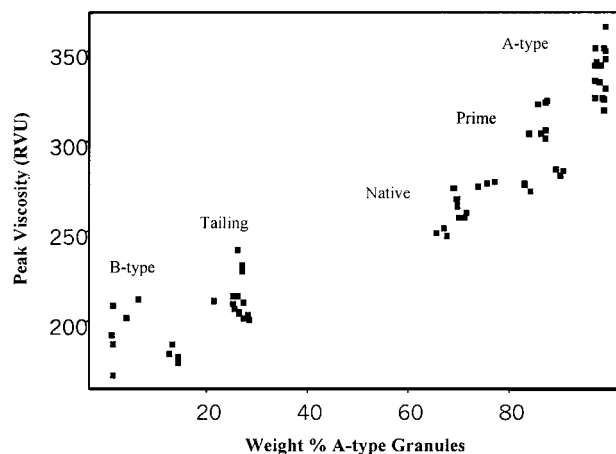


Fig. 3. Relationship between A-type granule content and Rapid Visco Analyser (RVA) peak viscosity of starch fractions of Lewjain.

isolated prime starches. The apparent contradiction between this and the Zeng et al (1997) studies may be reconciled through examination of the two experimental approaches. Zeng et al (1997) employed a set of genotypically diverse wheat cultivars that provided a range of TAM contents (22.8–28.2%), while our study utilized two soft white wheat genotypes that exhibited only a narrow span of total amylose contents (25.2–27.2%) across the isolated starch fractions. Though the native, prime, tailing, A-, and B-type fractions within this study possessed a wider range of AAM contents (18.7–23.0%), AAM differences among the fractions were, in actuality, more a function of variable Δ AM levels. Thus, relationships between AAM content and starch fraction pasting characteristics within our study reflect primarily an effect of starch lipid rather than amylose content per se.

In agreement with others that have suggested an inhibitive effect of lipid-complexed amylose on starch swelling, gelatinization, and pasting behavior (Eliasson and Karlsson 1983; Tester and Morrison 1990; Morrison 1995), Δ AM and phosphorus levels correlated negatively with peak, trough, final, breakdown, and total setback viscosities for both cultivars (Table III). Furthermore, starch Δ AM, phosphorus, and AAM contents exerted effects of nearly identical magnitude on starch pasting characteristics (Table III), which would be expected as they represent the same effect within our sample set. While starch lipids may be primarily responsible for the differential swelling tendencies of wheat A- and B- type starch granules (Tester and Morrison 1990), at best, they accounted for less than half of the variation observed in starch fraction peak viscosities (R^2 0.25–0.42), and explained even a lesser extent of the variability associated with other starch pasting characteristics. Thus, explanation of starch fraction pasting behavior is not fully accounted for by the effect of starch lipids alone, and requires that additional factors be taken into consideration.

Explanation of starch fraction pasting characteristics was dramatically improved through correlation with granule type. A-type granule content correlated positively with peak, trough, final, breakdown, and total setback viscosities for both cultivars (Table III). B-type granule content displayed an opposite effect (though of similar magnitude) on the starch pasting characteristics. Differences among the fractions with respect to peak paste and final viscosities, in particular, appeared to be largely a function of the fraction A- and B-type granule content as indicated by the approximately linear relationships between granule type and pasting characteristic values (Fig. 3 and 4; Table III). The relatively weaker correlation coefficients observed between granule type and trough and breakdown RVA attributes was due to the nonlinear nature of these relationships (data not shown). While the data plots for peak (Fig. 3) and final (Fig. 4) viscosities do not represent a continuous span of A/B-type granule contents (requiring cautious interpretation), there is a definite effect associated with granule type. The ability of granule

type to better account for and explain starch fraction pasting characteristics (compared with LPL content) should come as no surprise because the correlation with granule type also would be expected to account for LPL or any other inherent chemical differences that exist between the A- and B-type starch granules. Nevertheless, differences between the pasting behaviors of the A- and B-type starch granules may not be due solely to their respective compositional differences but could be a function of granule size itself.

Wheat starch A- and B-type granules differ considerably in terms of size, specific surface area, and swelling capacity (Soulaka and Morrison 1985; Tester and Morrison 1990; Fortuna et al 2000). B-type granules, which are smaller in size, have larger specific surface areas compared with the A-type granules. Thus, at equal weights, the B-type granules would be expected to possess a greater or denser packing ability and occupy a relatively smaller volume compared with the A-type granules. As starch rheological behavior is influenced by particle size, suspensions containing particles of larger size (occupying a greater volume fraction) tend to be more viscous compared with those of smaller size (Wong and Lelievre 1981, 1982b), even at identical concentrations. Hence, starch suspensions with high proportions of A-type granules might be expected to exhibit higher viscosity than those with high B-type granule contents when compared on an equal weight basis. The relationships between granule type and starch pasting characteristics might suggest that granule size itself provides significant contribution to wheat starch fraction pasting properties, although additional chemical and physical differences (amylopectin chemical structure, granule crystallinity, etc.) between the two granule types not investigated in this study could yet account for their differential rheological behaviors. Furthermore, as the rigidity of swollen granules has also contributed to starch paste properties (Eliasson and Bohlin 1982; Ring 1985; Steeneken 1989), it is not known whether differences in the stiffness of A- and B-type swollen granules or granule remnants might offer some additional explanation for wheat starch fraction pasting behavior.

In summarizing fraction RVA pasting profiles and further relating them to starch chemical and physical characteristics, starch fractions with significant B-type granule contents (B-type, tailing, and native starch fractions) exhibited delayed RVA gelatinization times compared with those with higher A-type granule contents (prime and A-type starch fractions) (Fig. 2). Ironically, the native starch fraction, in spite of its predominant A-type starch content (weight basis), tended to follow the pasting curves of the B-type and tailing starch fractions rather than the prime and A-type starch fractions. This phenomenon was likely a function of starch LPL, which causes a delayed gelatinization through complexation with starch amylose (Eliasson et al 1981). As the RVA does not provide accurate determination of starch gelatinization temperatures at rapid heating rates, starch fractions might be better differentiated (on the basis of time to gelatinization) at slower heating rates. On this basis, it might be possible to further differentiate the fractions (with respect to time to gelatinization) according to their respective LPL contents.

RVA viscosity attributes of the various fractions generally decreased in the order A-type > prime > native > tailing > B-type (Figs. 2–4; Table IV). As starch fraction pasting behavior was largely a function A/B-type granule ratio, the A-type granule fraction displayed higher values for peak, final, breakdown, and total setback viscosities compared with the B-type granule fraction. The A-type granules likely displayed higher peak viscosities (relative to B-type granules) on account of their larger swollen mass, which was likely related to both their lower starch LPL content and larger granule size. The greater hot paste stability of B-type granules relative to that of the A-type granules could be attributable to higher LPL and lipid-complexed amylose contents that consequently stabilize starch granule structure (Dengate 1984). Han and Hamaker (2001) also reported hot paste stability was highly correlated with amylopectin chemical structure (long chain length provides greater resistance to breakdown), though this aspect was not investigated

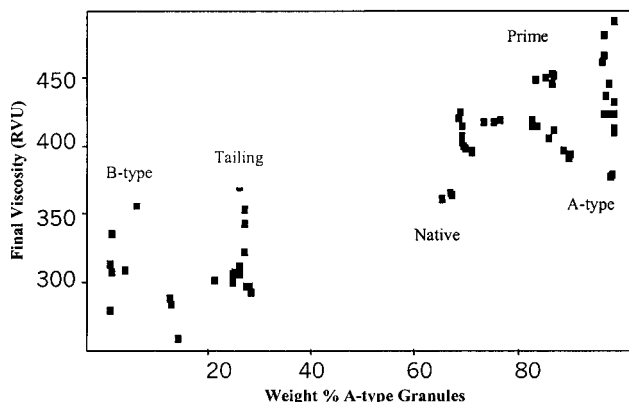


Fig. 4. Relationship between A-type granule content and Rapid Visco Analyser (RVA) final viscosity of starch fractions of Lewjain.

within our study. Higher total setback and final viscosity values exhibited by the A-type granule fraction were likely influenced by starch LPL values. Lin and Czuchajowska (1998) reported a decreased rate of retrogradation for starch pastes with high LPL contents. Native lipids in starches inhibit gel strength by creating barrier or steric hinderances that interfere with amylose reassociation (Takahashi and Seib 1988). Finally, subtle differences (<1%, observed only for Madsen) in TAM content between the two granule types might have also contributed to the higher total setback value of the A-type granules.

While cultivar A/B-type granule ratios are likely under genetic control (Stoddard 2000), native starch A- and B-type granule contents and pasting properties varied significantly across growing location for each cultivar (data not shown); this phenomenon was also observed by Panozzo and Eagles (1998). Of the various chemical and physical starch attributes analyzed within our study, granule type provided the best explanation for fluctuations in native starch and flour pasting properties across the five growing locations. For both cultivars, native starch A-type granule content was significantly correlated with native starch peak (0.67 Madsen and 0.69 Lewjain), trough (0.67 Madsen and 0.76 Lewjain), and final (0.72 Madsen and 0.63 Lewjain), as well as flour peak (0.26 Madsen and 0.66 Lewjain), trough (0.82 Madsen and 0.63 Lewjain), and final (0.68 Madsen and 0.63 Lewjain) viscosities in relation to growing environment. In a previous report, Wootten et al (1998) reported significant correlation between small granule starch content and starch pasting properties among 16 Australian wheat cultivars. While not the primary focus of our study, Lewjain native starch, with higher A-type granule and lower LPL contents relative to that of Madsen, also exhibited higher pasting characteristic values. Preliminary evidence might suggest that starch A/B-type granule ratios could offer some explanation for both cultivar and environmental-based fluctuations in starch pasting behavior.

SUMMARY AND CONCLUSIONS

Starch fractions (native, prime, tailing, A-, and B-type) that exhibited a range of A- and B-type granule contents, differed primarily with regard to the state of their amylose (free vs. lipid-complexed) rather than their actual amylose contents. A-type granules possessed lower levels of lipid-complexed amylose and LPL compared with B-type granules. As all fractions contained variable proportions of A- and B-type starch granules, fraction chemical composition was significantly affected by the respective A/B-type granule ratio.

However, LPL and Δ AM levels alone did not solely account for A- and B-type granule pasting behavior. Fraction A/B-type granule ratio offered the best explanation of wheat starch pasting behavior, as starch fractions with higher A-type (or lower B-type) granule contents generally exhibited higher values for primary pasting characteristics. Thus, fluctuations in starch A/B-type granule ratios could lead to variable pasting behaviors, which may prove important to the manufacture of consistent, commercial starches from wheat.

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LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 26-31, 46-30, and 76-13. The Associ-

- ation: St. Paul, MN.
- AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, International, 16th ed. AOAC: Arlington, VA.
- Anderson, K. A. 1996. Micro-digestion and ICP-AES analysis for the determination of macro and micro elements in plant tissues. *Atom. Spectrosc.* 17:30-33.
- Batey, I. L., Gras, P. W., and Curtin, B. M. 1997. Optimization of Rapid-Visco Analyser test conditions for predicting Asian noodle quality. *J. Sci. Food Agric.* 74:503-508.
- Bathgate, G. N., and Palmer, G. H. 1972. A reassessment of the chemical structure of barley and wheat starch granules. *Starch* 24:336-341.
- Bechtel, D. B., Zayas, I., Kaleikau, L., and Pomeranz, Y. 1990. Size-distribution of wheat starch granules during endosperm development. *Cereal Chem.* 67:59-63.
- Dengate, H. N. 1984. Swelling, pasting and gelling of wheat starch. *Adv. Cereal Sci. Technol.* 6:49-82.
- Dengate, H. N., and Meredith, P. 1984. Variation in size distribution of starch granules from wheat grain. *J. Cereal Sci.* 2:83-90.
- Eliasson, A. C., and Bohlin, L. 1982. Rheological properties of concentrated wheat starch gels. *Starch* 34:267-271.
- Eliasson, A. C., and Karlsson, R. 1983. Gelatinization properties of different size classes of wheat starch granules measured with differential scanning calorimetry. *Starch* 35:130-133.
- Eliasson, A. C., Carlson, T. L. G., Larsson, K., and Mieziš, Y. 1981. Some effects of starch lipids on the thermal and rheological properties of wheat starch. *Starch* 33:130-134.
- Endo, S., Karibe, S., Okada, K., and Nagao, S. 1988. Comparative studies on the quality characteristics for prime starch and starch tailings. *Nippon Shokuhin Kogyo Gakkaishi* 35:813-822.
- Evers, A. D., and Lindley, J. 1977. The particle-size distribution in wheat endosperm starch. *J. Sci. Food Agric.* 28:98-102.
- Evers, A. D., Greenwood, C. T., Muir, D. D., and Venables, C. C. 1974. Studies on the biosynthesis of starch granules. 8. A comparison of the properties of the small and the large granules in mature cereal starches. *Starch* 26:42-46.
- Fortuna, T., Januszewska, R., Juszczyk, L., Kielski, A., and Palasinski, M. 2000. The influence of starch pore characteristics on pasting behavior. *Int. J. Food Sci. Technol.* 35:285-291.
- Ghiasi, K., Hosney, R. C., and Varriano-Marston, E. 1982. Gelatinization of wheat starch. III. Comparison by differential scanning calorimetry and light microscopy. *Cereal Chem.* 59:258-262.
- Gibson, T. S., Solah, V. A., and McCleary, B. V. 1997. A procedure to measure amylose in cereal starches and flours with concanavalin A. *J. Cereal Sci.* 25:111-119.
- Han, X. Z., and Hamaker, B. R. 2001. Amylopectin fine structure and rice starch paste breakdown. *J. Cereal Sci.* 34:279-284.
- Kulp, K. 1973. Characteristics of small-granule starch of flour and wheat. *Cereal Chem.* 50:666-679.
- Lin, P. Y., and Czuchajowska, Z. 1998. Role of phosphorus in viscosity, gelatinization, and retrogradation of starch. *Cereal Chem.* 75:705-709.
- Medcalf, D. G., and Gilles, K. A. 1968. The function of starch in dough. *Cereal Sci. Today* 13:382-392.
- Meredith, P. 1981. Large and small starch granules in wheat—Are they really different? *Starch* 33:40-44.
- Meredith, P., Dengate, H. N., and Morrison, W. R. 1978. The lipids of various sizes of wheat starch granules. *Starch* 30:119-125.
- Morrison, W. R. 1988. Lipids in cereal starches: A review. *J. Cereal Sci.* 8:1-15.
- Morrison, W. R. 1995. Starch lipids and how they relate to starch granule structure and functionality. *Cereal Foods World* 40:437-446.
- Morrison, W. R., and Laignelet, B. 1983. An improved colorimetric procedure for the determination of amylose in cereal and starches. *J. Cereal Sci.* 1:9-20.
- Panozzo, J. F., and Eagles, H. A. 1998. Cultivar and environmental effects on quality characters in wheat. I. *Starch. Aust. J. Plant Res.* 49:757-66.
- Peng, M., Gao, M., Abdel-Aal, E. S. M., Hucl, P., and Chibbar, R. N. 1999. Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chem.* 76:375-79.
- Peng M. S., Gao, M., Baga M., Hucl, P., and Chibbar R. N. 2000. Starch-branching enzymes preferentially associated with A-type starch granules in wheat endosperm. *Plant Physiol.* 124:265-272.
- Peterson, D. G., and Fulcher, R. G. 2001. Variation in Minnesota HRS wheats: Starch granule size distribution. *Food Res. Int.* 34:357-363.
- Pomeranz, Y. 1988. Composition and functionality of wheat flour com-

- ponents. Pages 231-241 in: *Wheat Chemistry and Technology*, Vol. 2, Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Raeker, M. O., Gaines, C. S., Finney, P. L., and Donelson, T. 1998. Granule size distribution and chemical composition of starches from 12 soft wheat cultivars. *Cereal Chem.* 75:721-728.
- Reddy, I., and Seib, P. A. 1999. Paste properties of modified starches from partial waxy wheats. *Cereal Chem.* 76:341-49.
- Ring, S. G. 1985. Some studies on starch gelation. *Starch* 37:80-83.
- Sebecic, Bl., and Sebecic, B. 1999. Wheat flour starch granule-size distribution and rheological properties of dough. 3. Amylographic measurements. *Starch* 51:441-444.
- Soulaka, A. B., and Morrison, W. R. 1985. The amylose and lipid contents, dimensions, and gelatinization characteristics of some wheat starches and their A- and B-granule fractions. *J. Sci. Food Agric.* 36:709-718.
- Steeneken, P. A. M. 1989. Rheological properties of aqueous suspensions of swollen starch granules. *Carbohydr. Polym.* 11:23-42.
- Stoddard, F. L. 1999. Survey of starch particle-size distribution in wheat and related species. *Cereal Chem.* 76:145-149.
- Stoddard, F. L. 2000. Genetics of wheat starch B-granule content. *Euphytica* 112:23-31.
- Takahashi, S., and Seib, P. A. 1988. Paste and gel properties of prime corn and wheat starches with and without lipids. *Cereal Chem.* 65:474-483.
- Tester, R. F., and Morrison, W. R. 1990. Swelling and gelatinization properties of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chem.* 67:551-557.
- Wong, R. B. K., and Lelievre, J. 1981. Viscoelastic behavior of wheat-starch pastes. *Rheol. Acta* 20:299-307.
- Wong, R. B. K., and Lelievre, J. 1982a. Comparison of the crystallinities of wheat starches with different swelling capacities. *Starch* 34:159-61.
- Wong, R. B. K., and Lelievre, J. 1982b. Rheological characteristics of wheat-starch pastes measured under steady shear conditions. *J. Appl. Polym. Sci.* 27:1433-1440.
- Wootton, M., Kensington, N. S. W., and Mahdar, D. 1993. Properties of starches from Australian wheats. 2. Some physicochemical properties. *Starch* 45:295-299.
- Wootton, M., Panozzo, J. F., and Hong, S. H. 1998. Difference in gelatinisation behaviour between starches from Australian wheat cultivars. *Starch* 50:154-158.
- Yamazaki, W. T., Donelson, J. P., and Kwolek, W. F. 1977. Effects of flour fraction composition on cookie diameter. *Cereal Chem.* 54:352-360.
- Zeng, M., Morris, C. F., Batey, I. L., and Wrigley, C. W. 1997. Sources of variation for starch gelatinization, pasting, and gelation properties of wheat. *Cereal Chem.* 76:63-71.

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