

# Corn Dry-Milled Grit and Flour Fractions Exhibit Differences in Amylopectin Fine Structure and Gel Texture<sup>1</sup>

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## ABSTRACT

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Corn starch amylopectin (AP) fine structure and gel textural properties of dry-milled grit and flour fractions were investigated in 10 corn cultivars. Amylopectin was isolated by fractionating the starch derived from these two milled fractions using size-exclusion chromatography (SEC). Fine structure was characterized by SEC after thorough debranching with pullulanase. SEC revealed three major fractions of debranched AP from the grit and flour portion. Amylopectin in the grit portion had a significantly higher proportion of long chains (DP<sub>n</sub> 70–75) and a postulated lower extent of chain branching than its flour counterpart. Texture profile

analysis showed that flour gels from the grit fraction had significantly higher values for hardness, gumminess, and springiness compared with gels from the floury fraction. Trends were similar for starch gels of the two dry-milled fractions, though only springiness was significantly different. The finding that differences in AP fine structure in dry-milled fractions relate to gel textural differences suggests that dry millers may be able to produce flours of different functionalities that would be suited for different end uses. Additionally, mixing the dry-milled flour fraction with a grit-derived flour would result in different product properties.

Dry milling of cereals involves removal of the bran and germ from the endosperm, which makes the dry-milled products more useful and palatable as foods, and increases their storability. Most of the corn processed by dry millers in the United States today is done through the use of tempering-degerming systems. The corn is tempered to a high moisture content (≈20%) for easy separation of germ from the endosperm before it is processed in a degerminator, a specialized attrition mill, to strip the bran and germ away from the endosperm. After sieving and aspiration, the prime product, large pieces of endosperm, and smaller particle size grits and flour are recovered. The principal products from the corn endosperm include flaking grits, corn grits, corn meal, and corn flours (Alexander 1994). Corn dry-milled products are used in various areas of the food industry. For example, grits are used for breakfast cereals and brewing, meal for dry baking mixes, and flour for binders, breadings, and batters. Corn flour and meal are also used as ingredients for extruded snack foods (Senti and Schaeffer 1972; Rooney 1994).

The influence of starch on the textural properties of cooked cereal products has gained much attention, with a significant amount of work focused on starch as the textural determinant in cooked rice (Juliano 1979; Sowbhagya et al 1987; Radhika et al 1993; Ong and Blanshard 1995; Ramesh et al 1999). Apparent amylose (AM) content has been thought to be the single most important predictor of rice cooking quality (Juliano 1979). But since rice cultivars with similar AM contents also exhibit differences in texture, elucidation of the fine structure of amylopectin (AP) and its relation to the texture of cooked rice has been the subject of a number of investigations (Takeda et al 1987; Sowbhagya et al 1987; Radhika et al 1993; Ong and Blanshard 1995). The use of debranching enzymes (pullulanase or isoamylase), followed by fractionation of the debranched glucans by size-exclusion chromatography (SEC) has become the standard procedure for determining the fine structure of AP (Hizukuri 1986). It was reported that the molecular composition of starch determined from SEC profiles of debranched AP correlated with cooked rice texture; hard cooking rice tended to have more long chains (DP 92–98) in the AP than did soft cooking rice, which had more short chains (DP ≤25) (Radhika et al 1993; Ong and Blanshard 1995).

The objective of this study was to investigate potential causal relationships between observed differences in dry-milled grit and flour fraction gel textures, and starch characteristics, including AM content and AP fine structure.

## MATERIALS AND METHODS

### Corn Cultivars

Ten corn cultivars (six inbreds, B73, Mo17, H125, H122w, H126w, HSyn99; and four crosses, H125 × Mo17, H125 × LH216, H126w × H122w, FR819 × H126 w) were obtained from the Purdue University ARC Farm, West Lafayette, IN, 1993 and 1994 crop years.

### Dry Milling

The modified short-flow corn dry-milling method of Strohshine et al (1986) was used. Corn was stored at ≈ 13% moisture content. Before milling, 600 g of each cultivar was raised to 18% moisture and held for 18 hr, and finally to 20% moisture and held for 1 hr. The horizontal drum degerminator used by Strohshine et al (1986) was operated at 2,150 rpm. After degermination, the product was screened for 30 sec on a 3½-W sieve. The stock remaining on the sieve was given a second pass through the degerminator, and the combined stock was dried in a 45°C drying oven for 1 hr. The dried degermed stock was separated by screening on 3½-, 5-, 7-, 10-, and 16-W sieves for 1 min on the shaking device. Individual fractions (3½- to 10-W) were aspirated on a Bates model BG Laboratory aspirator (Brookshire, Texas) to remove hull material.

### Flour Sample Preparation of Grit and Flour Fractions

In this study, the large flaking grit on the 3½-W sieve was used as the grit fraction, and the flour that passed through 16-W sieve was used as the flour fraction. The proportion of large grits ranged from 50.5 to 62.8% among the cultivars. Large grit yield significantly correlated ( $r = 0.875$ ) with grain hardness determined by the Stenvert hardness test method (Pomeranz et al 1985). Large grits were ground to flour using a Buhler Miag mill (model MLI-204, Minneapolis, MN) at the widest gap setting, and then passed through a Tecator Cyclotec 1093 sample mill (Hoganas, Sweden) using a 0.5-mm mesh screen. The flour fraction was ground directly in the Cyclotec mill.

### Amylose Content

*Apparent amylose.* A simplified colorimetric procedure to determine apparent AM in corn starch was used (Knutson and Grove 1994). Samples (50 mg) were combined with 0.5 mL of 3M CaCl<sub>2</sub>, shaken thoroughly, and let stand for 10 min. To the slurry was added 5 mL of I<sub>2</sub>-DMSO (90% in water) solution (containing 6 × 10<sup>-3</sup> M iodine). The mixture was stirred and sonicated in a 65–70°C water bath for

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30 min. An aliquot (100  $\mu$ L) was diluted with 1 mL of I<sub>2</sub>-DMSO solution. Water (8 mL) was added to form the blue AM-iodine complex, and solutions were measured at 600 nm against a reagent blank. Unknowns were compared to a standard curve.

**SEC amylose.** Amylose content was also determined from SEC chromatograms by dividing the amount of starch under the AM peak by the peak area for starch in all fractions according to Jane and Chen (1992).

### Preparation of Starch Samples for SEC

Flour from the two dry-milled fractions (grit and flour) was defatted by washing with 80% (v/v) methanol (1:4, flour to 80% methanol) five times before starch isolation. Starch was extracted from the defatted flour fractions using a modification of Watson's wet-milling method (1964). The defatted fractions were first steeped overnight in a water bath (50°C) in 0.54% sodium bisulfite (NaHSO<sub>3</sub>). After decanting the soak solution, the precipitates were ground in a Waring blender with 50 mM NaCl. The slurry was passed through a 170-mesh sieve and washed with water several times until no whitish material indicating presence of starch was retained on the sieve. This was followed by shaking for 10 min in toluene and water (1:10, v/v), then centrifuging at 1,000  $\times$  g for 10 min. The supernatant was discarded and the toluene wash was repeated five times (until the toluene phase was clear). The protein on the top of the starch pellet was scraped, the starch was repeatedly washed with 70% ethanol and centrifuged (1,000  $\times$  g, 10 min), then decanted until there was no protein (yellow color) showing. Starch isolation was completed by washing with acetone (1:5, v/v) and air-drying in a hood overnight. After extraction, according to the method of Radhika et al (1993), starch from the two dry-milled fractions was defatted on a Soxhlet apparatus for 18–20 hr with 85% methanol and the moisture content equilibrated ( $\approx$ 12%).

### SEC of Native Starch

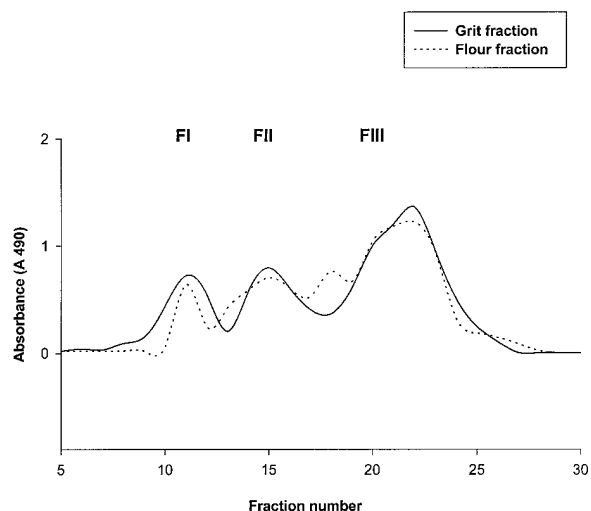
According to the method of Jane et al (1992), a column (2.6 cm i.d.  $\times$  90 cm) of Sepharose CL-2B (Pharmacia, Sweden) was used for characterizing native starches derived from dry-milled grit and flour fractions. Samples were chromatographed at room temperature at a flow rate of 0.3 mL/min in the ascending mode with 0.02% sodium azide aqueous solution as the eluent. Defatted starch (1%) was stirred with DMSO (90%, v/v) in a boiling water bath for 1 hr and at room temperature for another 24 hr to ensure complete dispersion. The starch solution aliquot (15 mg/1.5 mL, dry starch basis, DMSO, 90%, v/v) was mixed with absolute ethanol (5–6 mL) to precipitate the starch, which was recovered by centrifugation at 5,000  $\times$  g for

20 min. The precipitated starch was redissolved in boiling water (5 mL) and stirred for 20 min, then centrifuged (5,000  $\times$  g, 30 min) to remove the insoluble residue. The supernatant then was injected into the column. Fractions (3 mL) were collected for total carbohydrate and iodine staining tests. Total carbohydrate was determined by the phenol-sulfuric acid method (Dubois et al 1956) as measured at 490 nm against glucose standards. To the aliquots (2.5 mL) were added 0.2 mL of 0.2% iodine solution (2 g of iodine and 20 g of potassium iodide in 1L of aqueous solution), then solutions were measured at 630 nm. The minimum value from iodine staining was used to differentiate the end of the eluted AP peak from the AM peak of the starch profile. Starch from each dry-milled fraction of each corn cultivar was fractionated twice by SEC.

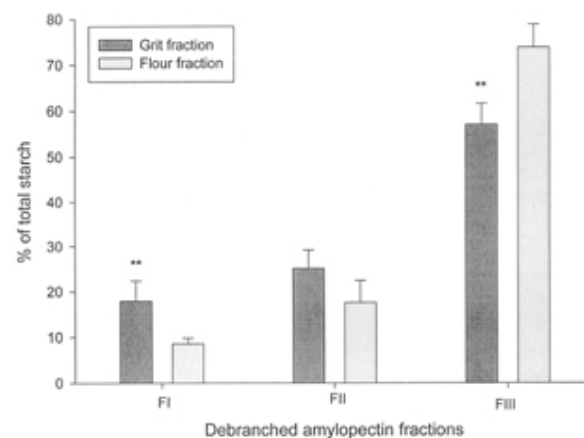
### SEC of Pullulanase-Debranched Amylopectin

According to a modification of Biliaderis et al (1981), the AP fraction was collected, rapidly frozen at  $-80^{\circ}\text{C}$ , and then freeze-dried. Amylopectin (10 mg) was debranched with 50  $\mu$ L of pullulanase (EC 3.2.1.41) from *Enterobacter aerogenes* (Megazyme, Australia) in 0.2M acetate buffer (5 mL, pH 5.5) for 48 hr in a 40°C water bath according to the method of Radhika et al (1993). The digestion was determined to be complete, as longer digestion times did not reveal any differences in chromatograms. The enzyme action was stopped (boiling water bath, 10 min), and the solution was centrifuged in a microcentrifuge (8,160  $\times$  g, 20 min). About 4.5 mL of the solution was fractionated by descending SEC on a 1.6 i.d.  $\times$  53 cm Biogel P-10 (Bio-Rad Laboratories, CA) column, using 0.02% sodium azide aqueous solution as the eluent (0.2 mL/min), and 3.5 mL fractions were collected at room temperature. The fractions (0.5 mL) were analyzed for total carbohydrate by the phenol-sulfuric acid method as described above. Elution fractions (0.5 mL) were analyzed for reducing value by a modified Nelson-Somogyi's reducing sugars method (Nelson 1944). The heating time with the Somogyi reagent was extended to 30 min (Hizukuri et al 1970). The average chain length (DP<sub>n</sub>) of debranched AP was determined by dividing total carbohydrate by its reducing value. Wavelength of maximum absorption ( $\lambda_{\text{max}}$ ) was determined by scanning iodine-treated solutions of total carbohydrate peak elution fractions. An index of the extent of chain branching of AP was shown by the molar ratio of two chain populations (III-chains and II-chains) and calculated assuming a symmetrical weight distribution of chains around the peak fractions (Biliaderis et al 1981):

$$\text{Molar ratio of III-chains to II-chains} = \frac{\text{total carbohydrate of III-chains} / \text{total carbohydrate of II-chains}}{\times \text{DP of II-chains} / \text{DP of III-chains}}$$



**Fig. 1.** Representative elution pattern of pullulanase-debranched corn amylopectin.



**Fig. 2.** Mean distribution of debranched amylopectin chains (%) in corn dry-milled fractions representing 10 cultivars. Dry-milled fractions that are significantly different from each other are indicated by \*\* ( $P < 0.01$ ).

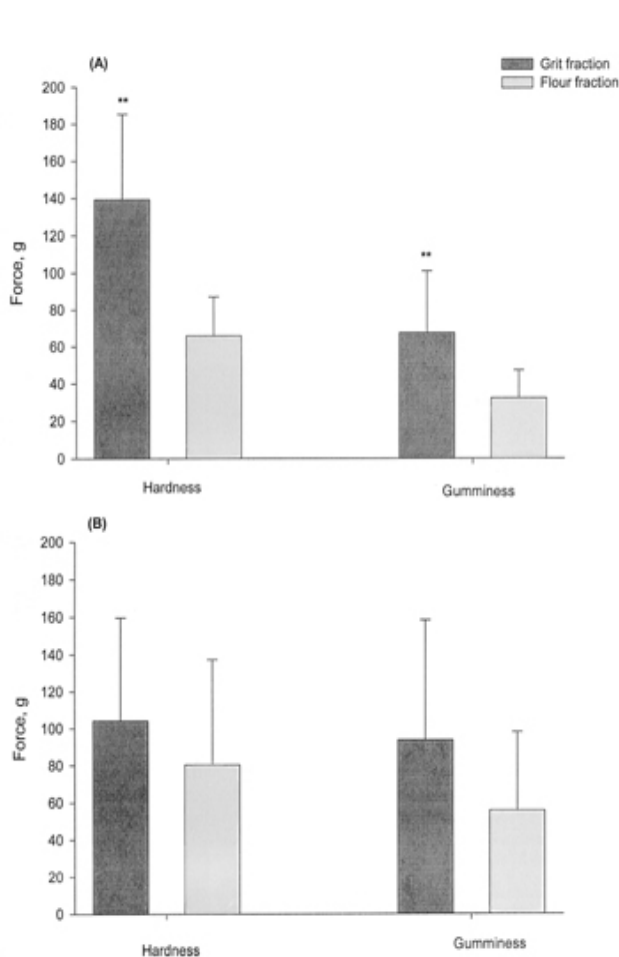
### Texture Profile Analysis (TPA)

Corn gels were prepared from defatted starches and flours (9.5 g, dry weight basis) derived from the two dry-milled fractions by cooking a slurry (14%, w/v) on a hot plate at maximum heat for 3 min with continuous manual stirring. Pastes were poured into a plastic syringe (12 cm<sup>3</sup>) and injected into cylindrical polypropylene tubes (diameter 14 mm, height 16 mm). After covering with a moisture barrier plastic film (Saran Wrap) and cooling for 1 hr at room temperature, corn gels were removed from the casting model and analyzed.

Samples were subjected to a two-stroke test using a cylindrical probe (diameter 2 cm, height 5 cm) at a test speed of 5 mm/sec, and control force of 5 g using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) with accompanying computer software. Six replicate samples were tested for each corn sample. The texture parameters of hardness or force necessary to attain a given deformation, gumminess (quantity to simulate the energy required to disintegrate a semisolid sample to a steady state of swallowing), springiness (rate at which a deformed sample goes back to its undeformed condition after the deforming force is removed), and cohesiveness (quantity to simulate the strength of the internal bonds making up the body of a sample) were obtained.

### Statistics

The paired *t*-test was used to determine statistical differences between values for dry-milled grit and flour fractions (SigmaStat package, Jandel Scientific). The level of significance was established at the 95% confidence limit ( $P < 0.05$ ).



**Fig. 3.** Mean hardness and gumminess values of defatted flour (A) and starch (B) gels from corn dry-milled fractions representing 10 cultivars. Dry-milled fractions that are significantly different from each other are indicated by \*\* ( $P < 0.01$ ).

## RESULTS AND DISCUSSION

### SEC Fractions of Starch

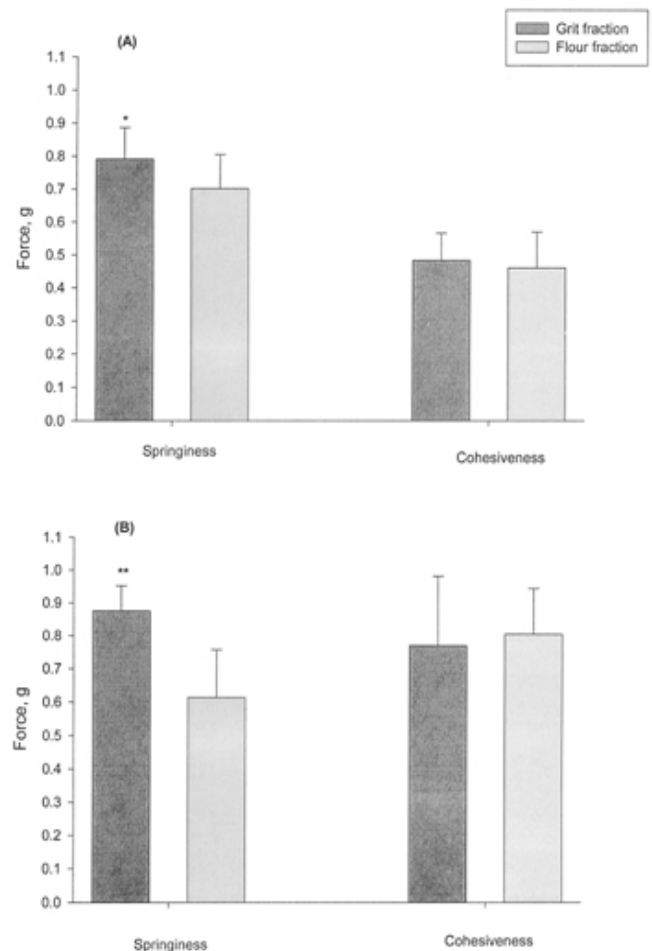
Corn starch derived from grit and flour dry-milled portions separated into the typical two fractions by Sepharose CL-2B: a larger gel-excluded peak (AP) and a smaller gel-included peak (AM, *not shown*). All starches showed similar elution patterns both in the two dry-milled fractions and among the 10 cultivars. Amylopectin fractions were collected for further study of fine structure.

### Amylose Content of Starch

Mean AM contents of starches of the two dry-milled fractions of the 10 cultivars were similar whether measured colorimetrically (grit 31.2%, flour 30.4%) or by the SEC method (grit 31.4%, flour 29.6%). South et al (1991) reported that a more accurate AM content can be obtained from SEC data than from iodine affinity or other iodine complex methods. This is because the occurrence of short chain-length AM underestimates the apparent AM content and AP with long external chains overestimates the apparent AM content (Shannon and Garwood 1984). Values from this study, however, were very similar to each other.

### Fine Structure of Amylopectin from Grit and Flour Portions

SEC-debranched AP chain length profiles from the dry-milled grit and flour portions of one representative corn cultivar (B73) are presented in Fig. 1. Three chain length fractions (FI, FII, and FIII) were found in both the grit and flour portions. An extra fraction that eluted between FII and FIII was also observed in the flour.



**Fig. 4.** Mean springiness and cohesiveness values of defatted flour (A) and starch (B) gels from corn dry-milled fractions representing 10 cultivars. Dry-milled fractions that are significantly different from each other are indicated by \* ( $P < 0.05$ ) and \*\* ( $P < 0.01$ ).

The extra fraction was present in flour portions of all 10 corn cultivars, though the amount varied widely. A small shoulder on FIII of the grit portion also was observed among the cultivars. For purposes of this study, the extra fraction and shoulder were combined with the large FIII peak.

### Carbohydrate Distribution of Fractions

Figure 2 shows the mean carbohydrate proportions of the debranched amylopectin fractions in the two dry-milled portions of the 10 corn cultivars. AP from the grit fraction had a significantly higher proportion of longest chains (FI 10–24%) and lower proportion of shortest chains (FIII 51–63%) than its flour counterpart (FI 2–14%, FIII 61–82%) ( $P < 0.01$ ). No significant differences were observed between the grit and flour fractions in the mean amounts of FII. This is the first known report of differences in AP fine structure found within milled fractions. The grit and flour dry-milled fractions are mainly derived from different locations within the kernel (outer and inner endosperm, respectively). This suggests that the differences in AP structure may be spatially determined, perhaps at different synthesis times during seed development. Supporting our study, Cagampang and Kirleis (1985) found differences in starch properties between vitreous and floury sorghum endosperm, and speculated that vitreous endosperm starch contained higher molecular weight components than larger granules from the floury endosperm.

Hizukuri and coworkers (1989) reported that the iodine affinity of rice AP is dependent on the proportion of the longest chain fraction. They suggested that this long and linear AP fraction would show functional properties approaching that of AM, with a tendency toward rapid retrogradation.

### DP<sub>n</sub> and λ<sub>max</sub> of Fractions

Enzyme-debranched AP chains have been characterized and identified based on DP<sub>n</sub> and λ<sub>max</sub> (Hizukuri 1986; Hizukuri et al 1989; Radhika et al 1993). Debranched AP chains from the grit and flour dry-milled portions were characterized as fraction I (FI) with DP<sub>n</sub> (70–75) and λ<sub>max</sub> (610–640) as long chains; fraction II (FII) with DP<sub>n</sub> (40–50) and λ<sub>max</sub> (550–610) as intermediate chains; and fraction III (FIII) with DP<sub>n</sub> (15–30) and λ<sub>max</sub> (420–530) as short chains. Among the 10 cultivars, there was a range of average chain length for AP with higher values in the grit fraction (25–34) than in its flour fraction counterpart (19–28). Kalichevski and coworkers (1990) studied AP from various botanical sources that differed in fine structure. They concluded that the reduced rate of retrogradation in cereal AP is linked to shorter average chain lengths. In corn therefore, the higher average chain length in the grit fraction may give a higher tendency toward retrogradation.

Biliaderis et al (1981) suggested that the molar ratio of AP III chains and II chains could be used as an index of the extent of chain branching. Our data showed a wide range of chain branching among cultivars revealed by the molar ratio index in the grit (6.0–10.5) and the flour fractions (6.1–15.1). Mean molar ratio index values were 7.6 for the grit fraction and 10.2 for the flour fraction. Using this approach, we speculate that AP from the flour fraction is more extensively branched compared with AP from the grit fraction. An increase in chain branching was proposed to lower the percentage of crystallinity in the starch granule with a resulting decrease in melting temperature (Biliaderis et al 1981). It is also likely to result in the formation of softer gels.

### TPA of Flour and Starch Gels

Mean values of the textural parameters hardness, gumminess, springiness, and cohesiveness of corn flour and starch gels from the dry-milled grit and flour fractions of the 10 corn cultivars are shown in Figs. 3 and 4. Defatted flour gels (Figs. 3A and 4A) from the grit fraction had significantly higher mean hardness, gumminess, and springiness compared with gels from the flour fraction. Starch gels (Figs. 3B and 4B) showed a similar trend. However, only the

mean springiness value in the grit fraction was statistically higher than in the flour fraction. There was substantial variation in gel textural properties among the 10 corn cultivars. Ranges in defatted flour gel hardness, gumminess, springiness, and cohesiveness values were 55.4–199.5, 8.2–117.8, 0.56–0.90, and 0.34–0.59 g, respectively, for the grit fraction and 22.2–98.3, 5.7–54.3, 0.45–0.79, and 0.25–0.61 g, respectively, for the flour fraction. Similarly, ranges of starch gel hardness, gumminess, springiness, and cohesiveness values were 41.3–205.9, 28.9–212.6, 0.73–0.95, and 0.38–0.95 g, respectively, for the grit fraction and 35.4–203.3, 17.6–125, 0.39–0.93, and 0.45–0.93 g, respectively, for the flour fraction. These data support that of Cagampang and Kirleis (1985), who found that sorghum starch gels made from vitreous endosperm were stiffer than gels from the flour fraction counterpart. They suggested that vitreous endosperm starch underwent a greater degree of retrogradation upon cooling, resulting in a stiffer alkali gel.

Our findings suggest a structure-function relationship between AP fine structure of the grit and flour dry-milled corn fractions and gel texture. Among the 10 cultivars, flour and starch gel hardness was always higher in the grit fraction than in the flour counterpart. AP from the grit fraction consistently had a higher proportion of long chains than AP from the flour fraction. The resulting postulated AP structures, with less branching in the grit fraction and more branching in the flour fraction, are speculated to contribute to the harder gel texture of the former and softer gel texture of the latter.

## CONCLUSIONS

Our findings indicate that fine structural differences in AP in the corn grit and flour fractions obtained from the dry-milling process relate to textural differences of the respective flour and starch gels. Harder gels from the grit fraction had a greater proportion of AP long chains and lesser proportion of short chains than did the softer gels from the flour fraction. This is the first report, to our knowledge, indicating spatial differences (outer vitreous versus inner floury endosperm) in AP fine structure within the corn kernel. Structural differences were consistent among the 10 corn cultivars examined.

The observed differences in AP fine structure and textural properties of the two dry-milled fractions may indicate some application to processes that use corn flours. Flours produced from grit fractions can be expected to behave differently than flour obtained directly from the dry-milling process. Blending of fractions, which sometimes occurs if directly obtained flour quantity is insufficient, should be done with the realization that functional properties will be changed.

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