

This article is from the  
March-April 2011 issue of

# CEREAL CHEMISTRY<sup>®</sup>

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# Endogenous and Enhanced Oxidative Cross-Linking in Wheat Flour Mill Streams

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## ABSTRACT

Cereal Chem. 88(2):217–222

A better understanding of the variation in oxidative cross-linking potential among flour mill streams can improve flour quality through the formulating of superior flour blends, and hence end-product attributes. The purpose of this research was to study the variation of oxidative cross-linking among 10 Miag Multomat pilot mill streams from 31 hard, soft and club wheat cultivars using Bostwick viscosity measurements. Flour slurries were made with either water alone (to measure the endogenous oxidative cross-linking viscosity) or with added hydrogen peroxide-peroxidase (to measure the enhanced oxidative cross-linking viscosity). Hard cultivars tended to produce more viscous flour slurries for both water and

peroxide-peroxidase viscosity measurements in most mill streams. Mill streams with high oxidative cross-linking potential were those with the largest differences between water and peroxide-peroxidase viscosity including 1st Break, 1st and 2nd Middlings, and 1st Midds Redust. Conversely, mill streams 3rd Break and 4th and 5th Middlings were the least likely to form oxidative cross-links. The ability to form oxidative cross-links is dependent on the availability of ferulic acid and tyrosine residues. Therefore, the arabinoxylan and protein polymers in mill streams that have a high oxidative cross-linking potential have a structure that is more conducive to forming oxidative cross-links.

Wheat (*Triticum aestivum* L.) grain processed by roller milling produces mill streams with distinct functional characteristics. These functional characteristics are dependent on several factors including but not limited to wheat cultivar, kernel hardness, tempering conditions, and mill settings. Break and early reduction mill streams are primarily composed of pure, starchy endosperm. Later reduction streams tend to have higher ash content due to bran contamination. The concentration of protein and ash increases as extraction rate increases (Hinton 1959; Nelson and McDonald 1977; Posner and Hibbs 1997). To produce a commercial flour, selected mill streams with similar functional characteristics are combined to produce flour blends destined for a particular end-use; blends can produce a wide range of flour grades. The flour grades include high purity blends (low ash) such as fancy patent, first patent, and short patent as well as lower purity (high ash) blends like fancy clear and first clear (Posner and Hibbs 1997).

High-ratio cakes, pastry products, white salted noodles and Japanese sponge cakes are produced from lower extraction, lower ash, patent flours. Other products such as bread, cookies, crackers, and pancakes are generally produced from higher extraction straight-grade flours. Consequently, flour stream blending is based on the desired characteristics needed to produce a certain product. The blending process can benefit from a greater understanding of the functional characteristics of each flour stream. Further, more optimal blending of functional flour fractions may eliminate or reduce the need for further treatments and hence decrease labeling requirements. One minor constituent that significantly affects the functional characteristics of flours is arabinoxylans.

Arabinoxylans are nonstarch polysaccharides that comprise ≈2–6% of the wheat kernel. These polymers are structural components of cell walls; they form a matrix with protein to entrap cellulosic material as well as glucomannans (Stone 2006). Similar to protein and ash, arabinoxylans are not uniformly distributed in the wheat kernel (Ramseyer et al 2011). Arabinoxylans are more

highly concentrated in the bran, pericarp, seed coat, and aleurone layers (Pomeranz 1988). The concentration of arabinoxylans in the central endosperm is significantly less compared to the outer layers of the wheat kernel. Furthermore, arabinoxylans are also not homogenous in physical conformation.

Generally, arabinoxylan conformation consists of a  $\beta$ ,1-4-xylose backbone with arabinose residues branching from the 2- or 3-carbon position. More specifically, arabinoxylans from the outer layers of the wheat kernel have a higher degree of arabinose substitution compared to the inner endosperm arabinoxylans (MacArthur and D'Appolonia 1975; Ciacco and D'Appolonia 1982; Delcour et al 1999). The degree, pattern, and frequency of arabinose substitution on the xylose backbone affects the water extractability. Thus, total arabinoxylan (TAX) content can be empirically separated into water-extractable arabinoxylan (WEAX) or water-unextractable arabinoxylan (WUAX) fractions.

Arabinoxylans from both water-unextractable and water-extractable fractions have ferulic acid residues esterified at the C (O)-5-position to a variable number of the arabinose residues. Ferulic acid residues of WUAX are covalently cross-linked to other cell wall materials and are unavailable for further chemical reactions (Mares and Stone 1973). Ferulic acid residues of WEAX are unlinked and can participate in chemical reactions (Fausch et al 1963). In the presence of free radicals, oxidative cross-linking between adjacent unlinked ferulic acid residues occurs to produce large networks of WEAX (Fausch et al 1963; Morita et al 1974; Neukom and Markwalder 1978; Vinkx et al 1991).

In a batter food system, this network entraps water, resulting in the formation of a shear-thinning gel (Izydorczyk et al 1991). The availability and concentration of unlinked ferulic acid residues is an important factor that affects the gel characteristics (Carvajal-Millan et al 2005; Niño-Medina et al 2010). A similar oxidative cross-linking mechanism can also occur between adjacent unlinked tyrosine residues on proteins, as well as between ferulic acid and tyrosine residues and result in an arabinoxylan-protein network (Neukom and Markwalder 1978; Oudgenoeg et al 2001; Tilley et al 2001; Wang et al 2002; Takaski et al 2005).

Oxidative cross-linking of polymers affects the end-use quality of wheat flour. Bettge and Morris (2007) studied the oxidative cross-linking (also known as oxidative gelation) viscosity of soft and club wheat flours and related the findings to variation in sugar-snap cookie diameter. Kweon et al (2009) found that oxidative gelation viscosity increased as a consequence of extensive chlorination in soft wheat flour. Sarker et al (1998) found that oxidative cross-linking between wheat arabinoxylans and protein increased foam stability and concluded that the increased foam stability

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may be of significant importance in gas retention during bread-making. Oxidative cross-linking also occurs between WEAX and gluten proteins (Neukom and Markwalder 1978; Hosenev and Faubion 1981; Oudenoeg et al 2001; Wang et al 2004) and results in decreased dough/gluten extensibility (Wang et al 2002). Tilley et al (2001) postulated that oxidative cross-linking between tyrosine moieties is important for gluten structure and function in breadmaking.

Currently, the information available on the functional properties of arabinoxylans in different wheat mill streams is incomplete. Previous research has primarily focused on the differences in structural composition and distribution of arabinoxylans in mill streams (Loska and Shellenberger 1949; D'Appolonia and MacArthur 1975; MacArthur and D'Appolonia 1977; Hashimoto et al 1987; Hartunian-Sowa 1997; Loosveld et al 1997; Lempereur et al 1998; Delcour et al 1999; Every et al 2002; Dornez et al 2006; Wang et al 2006; Ramseyer et al 2011). A limited number of studies have focused on the functional characteristics of arabinoxylans among mill streams. Ciacco and D'Appolonia (1982) isolated and purified arabinoxylans from one hard red spring cultivar; the gelling capacity of arabinoxylans was determined for seven selected mill streams. The arabinoxylans from the flour streams representing the inner portion of the kernel had higher intrinsic viscosity, were less branched, and had higher gelling capacity than the flour streams representing the outer portion of the kernel.

The oxidative cross-linking of arabinoxylans and proteins in mill streams among a collection of red and white, spring and winter, hard and soft, and club wheat cultivars has not been reported. Understanding variation among the functional characteristics of different mill streams will improve stream utilization and end-use quality. Therefore, the purpose of this research was to study the variation in oxidative cross-linking among mill streams of genetically pure hard, soft and club wheat cultivars using Bostwick viscosity measurements.

## MATERIALS AND METHODS

### Wheat Samples

Thirty-one genetically pure grain lots representing advanced wheat lines and commercial cultivars common to the western U.S. and harvested in 2009 were obtained from the U.S. Department of Agriculture, Agriculture Research Service, Western Wheat Quality Laboratory in Pullman, WA (Ramseyer et al 2011). The samples represented six classifications of wheat: soft white winter, soft white spring, winter club, hard white winter, hard red winter, and hard white spring.

### Milling

The thirty-one samples were milled on a Miag Multomat pilot mill. Milling produced 10 flour streams and four feed streams; only the flour streams were collected and analyzed for this study, resulting in 310 unique samples. The 10 flour streams were organized by the order in which they came off the mill: first break (B1), second break (B2), grader (GR), third break (B3), first middlings (M1), first middling redust (M1RD), second middlings (M2), third middlings (M3), fourth middlings (M4), and fifth middlings (M5) (Fig. 1 in Ramseyer et al 2011).

### Proximate Analysis

The 310 flour stream samples were analyzed in duplicate for protein, moisture, and ash. Moisture and ash were measured in a thermogravimetric oven (TGA-601, Leco, St. Joseph, MI). Protein was determined by the Dumas combustion method (AACC Approved Method 46-30.01) (Leco model FP-528). Single kernel characterization system (SKCS) hardness on the parent grain lots was measured with a SKCS 4100 (Perten Instruments, Springfield, IL).

### Arabinoxylan Determination

Flour streams were analyzed for water-extractable arabinoxylan content (WEAX). A colorimetric method described by Douglas (1981) that measures pentose sugar content in wheat flour after hydrolysis was modified to measure WEAX from wheat flour (Finnie et al 2006). WEAX content was calculated as mg of xylose equivalents after conversion using a xylose standard curve and expressed as mg of xylose equivalents (Douglas 1981).

### Bostwick Viscosity Determination

The viscosity of flour-water slurries was measured as flow distance with a Bostwick consistometer (VWR International, West Chester, PA). Mill stream samples were stored at room temperature ( $\approx 21^\circ\text{C}$ ) for eight weeks and then stored at  $-20^\circ\text{C}$  until viscosity measurements were obtained. Flow distance was determined using a modification of the method described by Bettge and Morris (2007). Flour samples (10 g, 14% moisture content) were weighed into 50-mL conical screw-cap polypropylene tubes (No. 05-539-9, Fischer Scientific, Pittsburgh, PA or equivalent). Deionized water (25 mL) was added to hydrate the flour samples, the tube was shaken briefly and vigorously by hand to disperse the flour. The suspension was hydrated for 20 min on a laboratory rocker (AR-100, PGC Scientific, Gaithersburg, MD) at room temperature ( $21^\circ\text{C}$ ). At the end of 20 min, additional reagents were added as appropriate, and the slurry was dispensed into the reservoir of a Bostwick consistometer. The reservoir gate was tripped after 2 min, allowing the reservoir to empty, and the distance the slurry flowed was measured after 40 sec. All viscosity measurements were conducted at  $21^\circ\text{C}$  and were replicated for each sample and treatment.

Treatment conditions used were 1) hydration with water (25 mL) for 20 min; 2) hydration with water (24.835 mL) for 20 min plus addition of 65  $\mu\text{L}$  of 3% (v/v) hydrogen peroxide and 60  $\mu\text{L}$  of horseradish peroxidase (1 purpurogallin unit/ $\mu\text{L}$ ). These treatment conditions provided the following analyses: 1) water, to measure the combined viscosity and endogenous oxidative cross-linking of WEAX and protein; 2) 3% hydrogen peroxide-horseradish peroxidase (POx), to measure the enhanced viscosity resulting from the oxidative cross-linking potential of WEAX and protein.

### Statistical Analyses

All statistical analyses were performed using PC-SAS statistical analysis software (v9.2, SAS Institute, Cary, NC). Primarily, a general linear model approach was used for ANOVA followed by Duncan's multiple comparison for testing mean differences. Type III sums of squares were reported. Proc CORR was used for determination of correlation coefficients. Proc REG with "maxr" was used to determine the most influential model components.

## RESULTS

The sources of variation for water-extractable arabinoxylans (WEAX), protein, water viscosity and peroxide-peroxidase (POx) viscosity are summarized in Table I. The two-way ANOVA models for WEAX, protein, water viscosity, and POx viscosity were robust in that 98–99% of the total variation ( $R^2$ ) was explained. The main effects, flour stream and cultivar, were significant sources of variation ( $P < 0.0001$ ) for WEAX, protein, water viscosity, and POx viscosity. Interactions between the main effects were also significant but the contribution of each of the interaction terms to the overall ANOVA models was minor compared to the main effects. Therefore, mill stream by cultivar interactions were considered to be a relatively unimportant source of overall variation and are not discussed further here.

Mill streams were the greatest source of variation for all response variables, but differences among cultivars (grain lots) were also important (Table I). Of the polymers that can participate in oxidative cross-linking, protein was more highly influenced by mill

stream compared to WEAX. The cultivar effect was also greater for protein than for WEAX. Water viscosity was more highly influenced by mill stream than was POx viscosity. Cultivar influenced POx viscosity but was less influential for water viscosity.

Because hard and soft wheat cultivars have different milling and functional properties, correlation coefficients were calculated among the viscosity measurements and the kernel texture (SKCS) of the parent grain lots for each of the flour fractions (i.e., across cultivars). Water viscosity was significantly negatively correlated ( $P < 0.05$ ) with kernel hardness in mill streams B1, B2, GR, B3, and M1RD at  $r = -0.84$ . Water viscosity of M1, M2, and M3 were also significantly correlated with kernel hardness:  $r = -0.48$ ,  $-0.75$ , and  $-0.74$ , respectively. This indicates that hard wheat flour slurries are generally more viscous than soft wheat flour slurries. Water viscosity was not significantly correlated with kernel hardness in streams M4 and M5 ( $P > 0.05$ ).

POx viscosity was correlated with kernel hardness in mill streams B2, GR, B3, M1RD, M2, and M3 from  $r = -0.57$  to  $-0.87$ . In these flour streams, hard wheat flour slurries tended to be more viscous than soft wheat flour slurries. POx viscosity was not significantly correlated with kernel hardness in mill streams B1, M1, M4, and M5 ( $P > 0.05$ ).

As indicated above, water viscosity and POx viscosity were not significantly correlated with kernel hardness in flour streams M4 and M5. This suggests an independence of both kernel texture and market class characteristics in these flour streams in relationship to slurry viscosity. POx viscosity correlations with kernel hardness were different from water viscosity correlations in two distinct ways. First, the correlation coefficients generally decreased in magnitude between water viscosity and POx viscosity among the mill streams except for streams B3 and M3. Second, POx viscos-

ity was not significantly correlated with kernel hardness in flour streams B1 and M1 while water viscosity was significantly correlated with kernel hardness in these streams.

The distribution of WEAX, protein and viscosity measurements for flour mill streams (across cultivars) is summarized in Table II. The mill streams are arranged in the order in which they were collected off the mill. The distribution for WEAX and protein was reported by Ramseyer et al (2011) and discussed therein.

Bostwick consistometer measurements for water viscosity among flour streams (across cultivars) ranged from 5.2 to 13.5 cm. Water viscosity measurements were more highly similar among flour streams B1, B2, GR, B3, M1, M1RD, M2, and M3 (10.8–13.5 cm, Table II). Flour streams GR and M1 were not significantly different ( $P < 0.05$ ). Flour streams M4 and M5 were considerably more viscous (less flow) compared to the other eight flour streams (8.2 and 5.2 cm, respectively).

The range for POx viscosity among all flour streams was 3.9–10.9. POx viscosity was similar among flour streams B1, B2, GR, M1, M1RD, M2, M3, and M4 with a mean range of 5.8–8.4 cm (Table II). Flour streams B2 and M3 were not significantly different ( $P < 0.05$ ). Flour streams B3 and M5 were notably different from the other flour streams in terms of POx viscosity. B3 had the greatest POx flow distance at 10.9 cm, whereas M5 had the smallest POx flow distance at 3.9 cm.

Figure 1 shows water viscosity (viscosity resulting from endogenous flour constituents and the combined oxidative cross-linking potential of WEAX and protein from endogenous free radicals) and POx viscosity (viscosity resulting from enhanced combined oxidative cross-linking potential of WEAX and protein with free radicals supplied by the hydrogen peroxide-peroxidase) among mill streams. Mill stream B1 exhibited the second greatest

**TABLE I**  
Sources of Variation for Water-Extractable Arabinoxylans, Protein, Water Viscosity, and Peroxide-Peroxidase Viscosity Among Mill Streams from 31 Varietal Grain Lots<sup>a</sup>

	df <sup>b</sup>	WEAX <sup>c</sup>	Protein	Water Viscosity	POx Viscosity <sup>d</sup>
Whole model $R^2$	309	0.98	0.99	0.99	0.99
Mill stream $F$ -value	9	898	9294	2972	1872
Variety $F$ -value	30	171	1265	374	724
Interaction $F$ -value	270	14	140	31	87

<sup>a</sup> All  $F$ -values are significant at  $P < 0.0001$ .

<sup>b</sup> Degrees of freedom.

<sup>c</sup> Water-extractable arabinoxylans.

<sup>d</sup> Peroxide-peroxidase viscosity.

**TABLE II**  
Mean and Range for Water-Extractable Arabinoxylans, Protein, and Viscosity Measurements in Flour Mill Streams<sup>a</sup>

Mill Streams <sup>b</sup>	WEAX (%) <sup>c</sup>		Protein (%) <sup>d</sup>		Water (cm) <sup>e</sup>		POx (cm) <sup>f</sup>	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
B1	0.43g	0.28–0.78	8.21i	5.01–14.55	13.1b	8.0–16.4	6.3f	0.5–13.3
B2	0.39h	0.25–0.72	10.35e	7.02–16.77	12.5c	7.5–15.3	8.2c	0.9–11.9
GR	0.44fg	0.29–0.75	9.88f	6.69–14.37	12.3d	8.4–14.6	8.4b	0.5–12.3
B3	0.37i	0.25–0.62	11.68c	7.52–18.79	13.5a	8.9–16.9	10.9a	5.6–13.8
M1	0.44f	0.26–0.89	8.41h	6.46–12.31	12.4d	8.8–14.8	7.7d	0.3–12.4
M1RD	0.48e	0.32–0.87	9.31g	6.62–15.44	11.8e	7.8–14.6	7.5e	0.4–11.1
M2	0.51d	0.35–0.91	9.35g	6.94–12.17	10.8g	7.9–12.9	5.8h	0.3–9.8
M3	0.55c	0.37–1.08	10.49d	7.22–13.07	11.6f	8.4–14.0	8.2c	0.5–12.9
M4	0.68b	0.45–1.08	13.45b	9.76–18.66	8.2h	4.6–12.9	6.1g	2.4–10.4
M5	0.77a	0.53–1.19	15.52a	6.04–19.11	5.2i	0.8–10.5	3.9i	0.5–6.9

<sup>a</sup> Mean values followed by the same letter in a column are not significantly different at  $P < 0.05$ . Range corresponds to the two most extreme varietal grain lots for that mill stream.

<sup>b</sup> B1, first break; B2, second break; GR, grader; B3, third break; M1, first middlings; M1RD, first middlings redust; M2, second middlings; M3, third middlings; M4, fourth middlings; M5, fifth middlings.

<sup>c</sup> Water-extractable arabinoxylans calculated as mg/g of xylose equivalents.

<sup>d</sup> Protein ( $N \times 5.7$ , 14% mb).

<sup>e</sup> Water viscosity Bostwick consistometer values.

<sup>f</sup> Peroxide-peroxidase viscosity Bostwick consistometer values.

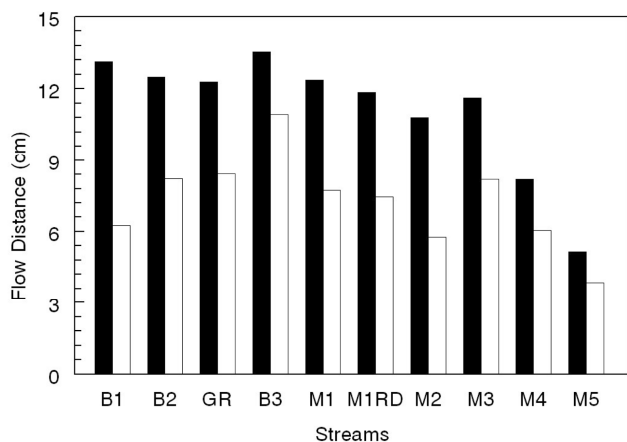
flow distance for water viscosity (13.1 cm) but had a markedly decreased flow distance for POx viscosity (6.3 cm). The difference between water viscosity and POx viscosity for mill stream B1 was 6.8 cm. Mill stream B3 was distinctly different from B1 in that B3 had the least viscous slurry for water viscosity (13.5 cm) and for POx viscosity (10.9 cm) of all the streams. Consequently, B3 only had a 2.6 cm reduction in flow distance between water viscosity and POx viscosity. Other mill streams had intermediate differences between water viscosity and POx viscosity, ranging from 3.4 cm for M3 to 5.0 cm for M2 (Fig. 1). Mill streams M4 and M5 had the smallest differences between water and POx viscosity at 2.1 and 1.3 cm, respectively.

Individual samples were classified with high oxidative cross-linking potential when the difference between water viscosity and POx viscosity was  $\geq 5$  cm; 19 of 31 varietal grain lots had at least one mill stream with high oxidative cross-linking potential. Notable cultivars with multiple mill streams with high oxidative cross-linking potential were 3 soft white winter cultivars: KWP006 (mill streams B1, B2, GR, M1, M1RD, M2, and M3), OR2040726 (streams B1, B2, M1, M1RD, M2) and Xerpha (streams B1, B2, GR, M1, and M2); and one hard white winter cultivar, Silver (streams B1, B2, GR, M1, M1RD, M2, and M3)

Furthermore, 53 of 310 individual samples were classified as having high oxidative cross-linking potential ( $\geq 5$  cm difference between water and POx viscosity). A majority of the samples with high oxidative cross-linking potential were found in mill stream B1 (16 samples) and M2 (11 samples). Other mill streams with high oxidative cross-linking potential were M1 (8 samples), M1RD (6 samples), B2 (5 samples), M3 (4 samples), and GR (3 samples). Mill streams B3, M4, and M5 did not have any samples that were classified as having high oxidative cross-linking potential.

Oxidative cross-linking that results in variation in flour slurry viscosity is influenced by WEAX and protein content (Bettge and Morris 2007). Across all flour streams and cultivars ( $n = 310$ ), WEAX content was correlated with water viscosity at  $r = -0.64$  (Fig. 2A). The correlation coefficient for WEAX and POx viscosity was lower in magnitude at  $r = -0.51$  (Fig. 2B). Protein content was highly correlated with water viscosity ( $r = -0.82$ ) (Fig. 3A), but less well correlated with POx viscosity ( $r = -0.39$ ) (Fig. 3B). Clearly, protein content was more influential for water viscosity, while WEAX content was more important for POx viscosity. However, the coefficient correlation for protein content had a much larger decrease in magnitude between water viscosity and POx viscosity than did WEAX content.

A statistical model was constructed to examine the relationship between WEAX and protein on water viscosity and POx viscosity. The model was primarily aimed at examining the combined impact of influential components and not necessarily to predict water



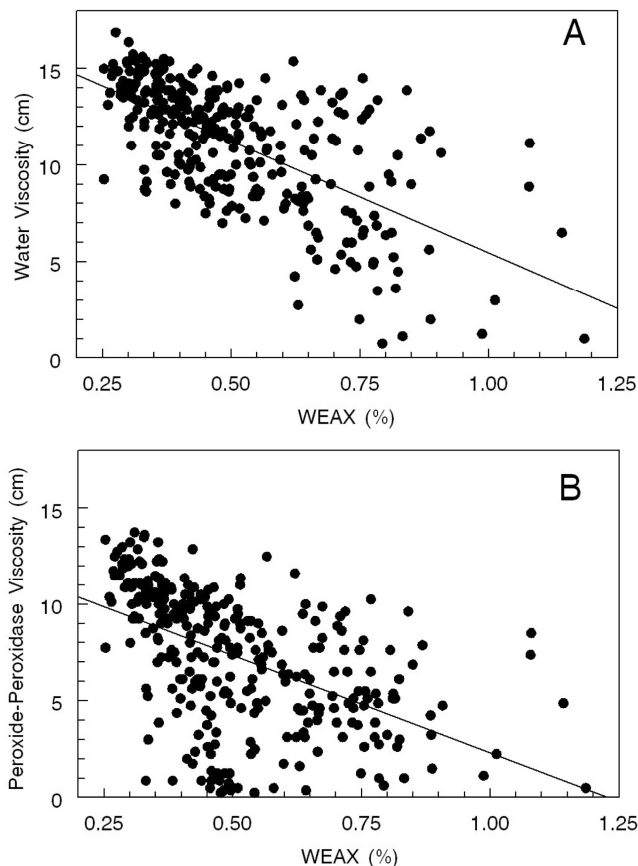
**Fig. 1.** Water viscosity (black bars) and peroxide-peroxidase (POx) (white bars) viscosity among wheat flour mill streams.

or POx viscosity. For water viscosity in a one-variable model, protein was the most influential component in that it explained  $\approx 68\%$  of the variation in water viscosity (adjusted  $R^2 = 0.68$ ,  $P < 0.001$ ). When WEAX was added to the model,  $\approx 76\%$  of the variation in water viscosity was explained. Conversely, the one-variable model for POx viscosity selected WEAX as the most influential component ( $R^2 = 0.26$ ;  $P < 0.001$ ); the two-variable model added protein, but only slightly increased the model fit ( $R^2 = 0.29$ ;  $P < 0.001$ ).

## DISCUSSION

Formulating superior flour blends requires detailed knowledge of the functional constituents in mill streams. Understanding the oxidative cross-linking potential of WEAX and protein in individual mill streams can contribute to the formulation of flour blends on a product-by-product basis and might reduce the need for additional chemical or enzymatic treatments. Oxidative cross-linking potential may also be useful for understanding or partially explaining the functional variation among various flour blends (e.g., straight-grade vs. patent flour).

The endogenous (water viscosity) and enhanced (POx viscosity) oxidative cross-linking varied dramatically among flour mill streams, more so than among individual cultivars (Tables I and II, Fig. 1). This result is important since this study included a large number of pure varietal samples (31) and encompassed six distinct U.S. market classes or classifications: soft white winter, soft white spring, soft white winter club, hard white winter, hard red winter, and hard white spring. The correlation coefficients between kernel texture and viscosity measurements among mill streams indicated that hard wheat flours generally tend to be more viscous (less flow) than soft wheat flours for both water viscosity and POx viscosity.



**Fig. 2.** Scatter plots of WEAX content and water viscosity (A) and POx viscosity (B).

Certain mill streams exhibited a greater propensity for forming oxidative cross-links with enhanced levels of free radicals as evidenced by the difference between water viscosity and POx viscosity. A large difference between the two viscosity measurements indicates a greater potential to form oxidative cross-links among the WEAX and protein polymers. Also, some cultivars such as Silver (hard white winter), and the three soft white winter cultivars KWP006, Xerpha, and OR2040726 had a large number of flour streams with high oxidative cross-linking potential ( $\geq 5$  cm difference between water and POx viscosity). Mill streams B1 and M1 (which are composed mainly of endosperm from the inner portion of the kernel) had large differences in water and POx viscosity. A large proportion of the mill stream samples that had  $\geq 5$  cm difference between water viscosity and POx viscosity were from B1 and M1 as well as M2 and M1RD. Mill streams B3, M4, and M5 all contain a larger percentage of material from the outer layers of the wheat kernel (based on ash content and mill flow) and had the smallest differences in water versus POx viscosity. Of the mill stream samples that had  $\geq 5$  cm difference between water viscosity and POx viscosity, none were from B3, M4, or M5. A large flow difference between water viscosity and POx viscosity indicates that the structure of WEAX and protein was more conducive at forming oxidative cross-links when free radicals were plentiful. Alternatively, the structure of WEAX and protein polymers in mill streams B3, M4, and M5 was not as conducive to forming oxidative cross-linking when free radicals were plentiful.

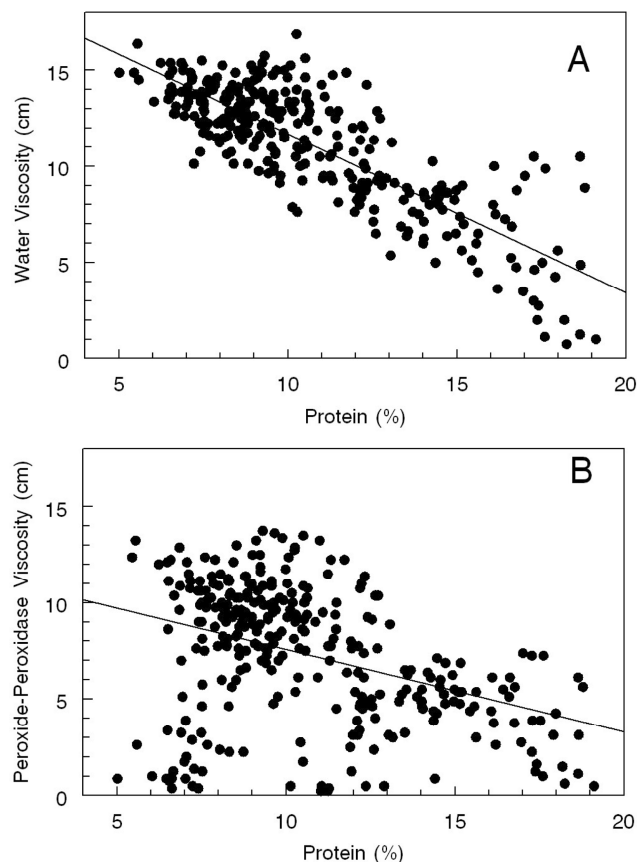
Not only does the structure of the protein or WEAX polymers need to have tyrosine or ferulic acid residues available to participate in oxidative cross-linking, but the environment needs to be chemically or enzymatically conducive as well. That is, excessive free radicals are required to catalyze the cross-linking reaction among ferulic acid and tyrosine residues to obtain the enhanced or maximum oxidative cross-linking potential. Water viscosity was used as a “control” treatment since free radicals were apparently limiting in the flour. Some endogenous free radicals were undoubtedly generated from enzymatic or auto-oxidation of triacylglycerides to fatty acid as they are present in flour that has been stored for any length of time, as were the samples in this study (Tsen and Hlynka 1961; Clayton and Morrison 1972; Garcia et al 2002; Reichenauer and Goodman 2003). The oxidation of triacylglycerides to fatty acids is a slow process that is dependent on several factors including storage temperature, moisture content, and structural conformation of the lipids. In this study, mill stream samples were aged for eight weeks at room temperature (21°C) before being analyzed. Differences in the concentration of free radicals, WEAX, and protein among samples may account for some variation in water viscosity due to cross-linking catalyzed by endogenous free radicals. However, cross-linking of WEAX and protein polymers was not as extensive as with the addition of supplementary free radicals (generated by peroxide-peroxidase). This difference in extent of cross-linking is evident in the difference between water viscosity and POx viscosity.

Ciacco and D’Appolonia (1982) observed that WEAX from mill streams representing the inner portion of the endosperm had a higher oxidative gelation capacity (oxidative cross-linking potential) than WEAX from mill streams containing a higher proportion of material from the outer layers of the wheat kernel. Our results are similar in that mill streams B1 and M1 (which represent the inner portion of the endosperm) had the largest differences in water vs. POx viscosity, and thus had among the highest oxidative cross-linking potential. Furthermore, previous research has shown that unlinked ferulic acid and tyrosine residues are required to form oxidative cross-links (Morita et al 1974; Neukom and Markwalder 1978; Vinkx et al 1991; Oudgenoeg et al 2001; Tilley et al 2001; Wang et al 2002; Takasaki et al 2005; Niño-Medina et al 2010). Therefore, the difference in water (endogenous) and POx (enhanced) viscosity is most likely dependent on the structural conformation of WEAX and protein polymers,

although the absolute concentration of WEAX and protein among mill streams is also important.

Across all mill streams and cultivars ( $n = 310$ ) water viscosity was more highly correlated with protein content and less highly correlated with WEAX content. This may be expected because protein content is greater than WEAX content (18–32 times greater among mill streams in this study). Therefore, the protein polymers likely have more influence on the endogenous viscosity. The one-variable statistical model indicated that protein content explained  $\approx 68\%$  of the variation in water viscosity across all mill streams and cultivars; however, when WEAX was added to the model,  $\approx 76\%$  of the total variation was explained. The 8% increase in total variation explained indicates that while WEAX is in relatively small quantities in mill streams compared to protein, the endogenous oxidative cross-linking of WEAX polymers is important for overall water viscosity. Obviously, WEAX and protein polymers are both important in determining the endogenous viscosity of wheat flour slurries.

For enhanced oxidative cross-linking viscosities, WEAX content was more highly correlated with POx viscosity and was the most influential component in the statistical model. Whereas the correlation coefficient for WEAX content decreased in magnitude between water and POx viscosity, it did not decrease nearly as much as the correlation coefficient for protein content. In general, higher WEAX content meant reduced flow for POx viscosity whereas higher protein content was not always associated with less flow. In the statistical models, WEAX content explained 26% of the total variation and when protein content was added to the model, total variation explained increased to only 29%. The variation in POx viscosity transcends gross concentration of polymers. Rather, a large portion of the unexplained variation is likely due to the structural conformation of the WEAX and protein polymers.



**Fig. 3.** Scatter plots of protein content and water viscosity (A) and POx viscosity (B).

As discussed previously, the availability of ferulic acid and tyrosine is a key factor in forming oxidative cross-links.

In conclusion, endogenous (water viscosity) and enhanced (POx viscosity) oxidative cross-linking varied greatly among wheat flour mill streams. Some mill streams were more likely to form oxidative cross-links than others. Mill streams with a large difference between water viscosity and POx viscosity indicates a greater potential to form oxidative cross-links. A large difference between the two viscosity measurements also indicates that the WEAX and protein polymers in the mill streams are conducive to forming oxidative cross-links when free radicals are plentiful. The differences in oxidative cross-linking potential among mill streams may partially explain functional variation among flour blends (e.g. patent vs. straight-grade) and subsequent end-use quality. However, improved quantification of bioactive compounds, especially ferulic acid, in wheat flour is needed to better understand and determine the structure and functional properties of WEAX.

#### ACKNOWLEDGMENTS

We gratefully acknowledge the technical staff of the USDA-ARS Western Wheat Quality Laboratory.

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[Received November 12, 2010. Accepted January 20, 2011.]