

Oxidative Gelation Measurement and Influence on Soft Wheat Batter Viscosity and End-Use Quality

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ABSTRACT

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Viscosity is an important end-use attribute for some soft wheat flour formulations. Specifically, in formulations with minimal gluten development, such as batters (as in cake, pancake, and doughnut) and coatings (as in tempura), viscosity is important to leavening gas retention and flow characteristics. Current tests for predictors of viscosity leave considerable unexplained variation. The potential for water-extractable arabinoxylans to form oxidative gels through ferulic acid dimerization may represent an important component of viscosity variation. A method was developed to identify variation in viscosity due to oxidative gelation. This method, comparing viscosity of flour slurries made with water, a peroxide-peroxidase

system, and a system with xylanase, indicated that two, and likely three, types of oxidative gelation were contributing to viscosity. Predicted viscosity due to inter-arabinoxylan gelation through ferulic acid dimerization, di-tyrosine formation among proteins, and ferulic acid-tyrosine bond formation varied among wheat cultivars. Oxidative gel formation increased batter viscosity probably due to water sequestration; this effect was correlated with reduction in the sugar snap cookie spread (diameter). Results indicate that oxidative gelation is an important contributor to batter viscosity and also contributes to the quality attributes of dough systems.

Wheat flour product formulations encompass a large range in water content, from relatively dry, stiff doughs to low viscosity batters. In bread doughs, sufficient water and mechanical work are used to develop gluten and form an elastic network capable of holding fermentation gases. In many soft wheat products, such as cookies, and batter-based products such as cakes or donuts, the desired product performance and consistency is attained through the use of chemical leavening. Extensive gluten development in soft wheat flour products is generally undesirable because it reduces textural quality. Consequently, gluten development is minimized in most soft wheat products and other factors become more important as influences on end-use quality. For product formulas with a high water content such as cakes, coatings, pancakes, waffles, wafers, donuts, etc., batter viscosity is one of these critical factors (Morris and Rose 1996). Batters for coatings (e.g., tempura batter) must be viscous enough to adhere to the product but without clumping or sheeting off. Pancake and donut batters must be viscous enough to retain leavening gasses and prevent settling but without being so viscous as to inhibit flow and spread. Batter viscosity also relates to the control or degree of water sequestration.

Variation in batter viscosity has, in part, been attributed to arabinoxylans (also known as pentosans, hemicellulose, or nonstarch polysaccharides). Arabinoxylans consist of a β -1,4 xylose backbone, variously substituted at the 2- and 3-carbon position with arabinose. The degree, pattern, and frequency of the substitutions determine the water extractability of the polysaccharide. As such, arabinoxylans can be empirically separated into water-extractable (water-soluble) and water-unextractable (water-insoluble) fractions (Courtin and Delcour 2002). The water-unextractable fraction can interact with $\approx 10\times$ its weight in water in such a way that water activity is reduced. In addition to interacting with water directly through hydrogen bonding, water-extractable arabinoxylans can form gels. Water-extractable arabinoxylan (WEAX) has varying numbers of unlinked ferulic acid moieties esterified to the arab-

inose side groups. Under a conducive chemical environment, usually involving the presence of free radicals, dimerization between ferulic acid moieties results in a large network of arabinoxylan polymers (Morita et al 1974; Neukom and Markwalder 1978; Vinkx et al 1991). The resulting matrix entraps or sequesters water leading to a gel (Izydorczyk et al 1991; Carvajal-Millan et al 2005). Similar free-radical-induced cross-linking can also occur between tyrosine residues contained in proteins both among proteins (Neukom and Markwalder 1978; Oudgenoeg et al 2001; Tilley et al 2001; Wang et al 2002; Takasaki et al 2005) and between arabinoxylans and proteins through ferulic acid-tyrosine esterification (Neukom and Markwalder 1978; Oudgenoeg et al 2001; Wang et al 2002).

The potential for oxidative gels to form in aqueous environments has long been known. Durham (1925) published a paper indicating that an unidentified water-soluble fraction from wheat flour formed a gel when exposed to hydrogen peroxide. Further work (Morita et al 1974; Ciacco and D'Appolonia 1982) identified the water-soluble fraction as being composed of arabinoxylans, and the mechanism as ferulic acid cross-linking.

Water-extractable arabinoxylan and protein polymers create viscous suspensions at low concentration due to their large size (Izydorczyk and Biliaderis 1992; Lu et al 2005) but form oxidative gels under appropriate conditions that exhibit shear-thinning (Izydorczyk et al 1991; Lu et al 2005). This rheological trait indicates that their impact would likely be less important in mechanically mixed doughs (e.g., pan bread) and more important in cake and other batter formulations where there is less shear thinning. Further, the effect of di-cysteine bonds in gluten formation likely overshadows the effect of di-tyrosine bonds in bread dough formation.

Limited research on the influence of arabinoxylans and viscosity has been conducted. Izydorczyk et al (1991) and Moore et al (1990) measured viscosity using capillary viscometers or spindle-and-plate or spindle-and-cylinder type instruments that produce variable amounts of shear. However, variation in viscosity due to arabinoxylans was observed, even using the higher shear instruments. Trough-style consistometers (Bostwick) measure viscosity as flow distance under low shear conditions that better measure oxidative gels associated with batter systems that can exhibit shear thinning. Other current tests for flour constituents that contribute to viscosity include the lactic acid solvent retention capacity (SRC) and SDS-sedimentation tests which identify the contribution of protein components (Approved Methods 56-11 and 56-70, respectively), and sucrose SRC (Approved Method 56-11) (AACC International 2000) that identifies the contribution of arabinoxylans and, to an extent, gliadins (Slade and Levine 1994a,b).

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Thus, arabinoxylans, proteins, and their oxidative gels are likely to directly affect the batter properties (ability to cling to batter-coated products, the ability to be pumped, etc.) and indirectly affect product quality and geometry (due to decreased flow in the baking process). Obtaining the correct range of batter viscosity is crucial in preventing settling during holding (e.g., pancake batter in a restaurant setting between orders) or loss of leavening gas. Direct effects on end-product quality are apt to be seen as sticky-textured products due to sequestered water. Effects on lower moisture content cookies would also likely be observed due to reduced dough plasticity, through sequestration of sugar syrup, or through increased bake-out time and resulting problems with product checking (stress fractures induced in low-moisture products due to over-baking to reduce moisture content).

Consequently, a better understanding of the physical-chemical components contributing to viscosity has the potential to guide breeding programs in the development of wheat cultivars that will perform in end-use applications without the need for processing aids (e.g., hydrolytic enzymes such as xylanases), thereby reducing costs, shortening ingredient labels, and increasing consumer satisfaction. In this research, the ability of arabinoxylans and proteins to influence batter viscosity either directly or through their potential to form oxidative gels was quantified through the use of a new test method.

MATERIALS AND METHODS

This research used 240 winter-sown soft white common and club wheat samples from the 2003-harvest crop year advanced breeding nurseries (late-generation, F_6 , breeding program lines). The nurseries were selected to provide material that possesses a range of end-use quality that is reduced in released cultivars as a result of breeding selection. The nurseries were grown in three locations: Pendleton, OR, Moro, OR, and Pullman, WA.

Single Kernel Characterization System (SKCS) hardness was measured with the Perten SKCS 4100 (Perten Instruments, Springfield, IL). Milling was conducted on a modified Quadrumat Sr mill as described by Jeffers and Rubenthaler (1979). Ash was measured after combustion in a Leco TGA-601 and protein in a Leco FP-528 (Leco Corp., St. Joseph, MI, USA). Cookie spread was measured using Approved Method 10-52 (AACC International 2000) for sugar-snap cookies.

Quantitation of WEAX was performed using a modification of the phloroglucinol-based method of Douglas (1981). Duplicate flour samples (125 mg) were weighed into 50-mL tubes and suspended, by vortexing in 25 mL of water. Two 1-mL aliquots each containing 5 mg of suspended flour was removed immediately and dispensed into stoppered 15-mL reaction tubes. A total of four samples, representing the total arabinoxylan content resulted from two duplicate aliquots. The slurries were mixed for 30 min at room temperature ($\approx 22^\circ\text{C}$) and centrifuged at $2,500 \times g$ for 10 min. Duplicate 1-mL supernatant aliquots from each replicate were transferred into 15-mL stoppered reaction tubes. The resulting four samples represented the soluble arabinoxylan content. An additional 1 mL of water was added to each reaction tube to bring the volume to 2 mL. Reaction reagent (10 mL of stock reagent made of 110 mL of glacial acetic acid, 2 mL of concentrated hydrochloric acid, 5 mL of 20%, w/v, phloroglucinol in absolute ethanol, 1 mL of 1.75%, w/v, glucose) was added and the samples were boiled for 25 min. The reaction tubes were then quenched in a cold water bath with ice. Absorbance of the samples was read at 552 and 510 nm as soon as practical after cooling. The absorbance reading at 510 nm was subtracted from 552 nm to mathematically remove the influence of hexose sugars. Arabinoxylan content was calculated as mg xylose equivalents after conversion using a xylose standard concentration series (xylose standards run in triplicate of 0, 0.05, 0.10, 0.15, and 0.20 mg) and expressed as mg xylose equivalents (Douglas 1981).

An oxidative gelation test was developed that measures the combined impact of free radicals (endogenous or induced) and the arabinoxylan-ferulic acid-protein complexes on viscosity. The basic method involves weighing 10 g of flour (14% mb) into a 50-mL conical-bottom, screw capped, polypropylene tube (05-539-9, Fisher Scientific, Hampton, NH, or similar). Solutions appropriate to the test were added, the tube was briefly vigorously shaken by hand to disperse the flour, and the suspension was hydrated for 20 min on an end-over-end rotating shaker (Barnstead/Thermolyne Labquake shaker, Fisher Scientific, Hampton, NH). At the end of the 20-min hydration, additional reagents were added as appropriate, and the slurry was dispensed into the reservoir of a Bostwick consistometer (VWR International, West Chester, PA). The reservoir gate was tripped after 1 min, allowing the reservoir to empty, and the distance the slurry flowed was measured at 40 sec. Preliminary research (data not shown) obtained viscosity readings at 20, 40, and 60 sec and the results indicated that the largest range of viscosity results occurred at 40 sec. If allowed to run for 60 sec, flour-water slurry viscosities reached a maximum value of 17.0 cm, and xylanase-treated flour slurries reached a maximum value of 17.4 cm. Values at 40 sec were ≈ 0.5 – 0.8 cm less than those observed at 60 sec, indicating that the data were not censored by potential flow distance, only nearing the maximum potential flow. All viscosity measurements were conducted at 21°C and were replicated for each sample and treatment.

The test procedures used were 1) hydration with water (25 mL) for 20 min, and poured into the Bostwick consistometer; 2) hydration with hydrogen peroxide solution (25 mL of 75 ppm H_2O_2 in water) (Hoseney and Faubion 1981) for 20 min followed by the addition of horseradish peroxidase (60 μL of 1 purpurogallin unit μL^{-1}) (type II, no. P8250, Sigma), the tube was inverted three times, and poured into the Bostwick consistometer; 3) hydration with xylanase solution (25 mL of 2U Bioxylanase 10 L) (endo-1,4- β -D xylanase; IUB 3.2.1.8, Kerry Bio-Science, Tralee, Co. Kerry, Ireland) for 20 min, and poured into the Bostwick consistometer; and 4) hydration with xylanase solution (25 mL of 2U) for 20 min, followed by the addition of peroxidase (65 μL of 3% hydrogen peroxide to yield 75 ppm), the tube was inverted three times, 60 μL of peroxidase was added, the tube was inverted three more times, and then poured into the Bostwick consistometer. The peroxide +peroxidase treatment will be referred to only as “peroxidase” for simplicity. The various treatments provided the following analyses: 1) water, to measure the combined viscosity and endogenous oxidative gelation of the flour; 2) peroxide-peroxidase, to measure enhanced viscosity resulting from the oxidative gelation potential of the flour; 3) xylanase, to measure viscosity of the flour slurry resulting from the endogenous oxidative gelation of proteins without the contribution to gelation by arabinoxylans; and 4) xylanase-peroxidase to measure the enhanced viscosity resulting from the oxidative gelation potential of proteins without the contribution to oxidative gelation by arabinoxylans.

All statistical analyses were performed using PC-SAS statistical software (v9.0, SAS Institute, Cary, NC). Primarily, a general linear model approach was used for ANOVA and mean separations. Proc REG, with a “maxr” selection stipulated, was used for exploration of the most influential model components.

RESULTS AND DISCUSSION

The set of 240 soft white common and club winter wheat breeding lines and cultivars exhibited grain, flour, and baking characteristics typical for Pacific Northwest breeding populations and locations (Table I). SKCS kernel hardness, with a mean of 33.9, is typical of PNW soft white wheats, which are generally harder than soft wheats from the eastern United States (Morris et al 2005). Similarly, flour protein and ash contents were in the range normally encountered in advanced soft white winter wheat breeding populations. Due to growing environment, protein content varied

among the nurseries. The mean protein content of nurseries from Pendleton, OR, was 9.8% ($n = 88$), Moro, OR, was 7.5% ($n = 77$), and Pullman, WA, was 7.1% ($n = 75$). The mean and range of flour WEAX was consistent with previous studies (Finnie et al 2006). The cookie diameters, ranging from 8.39 to 10.01, indicated a range from poor to excellent, based on our evaluation experience, and were consistent with a significant amount of genetic variation at this stage of the breeding process.

Bostwick consistometer readings for water (measuring viscosity resulting from endogenous flour constituents and the combined oxidative gelation potential of the arabinoxylans and proteins, initiated by endogenous free radicals) ranged from 9.8 to 16.5 cm with a mean of 14.0 cm (Table I). A distance of 17.5 to 18 cm is near the practical limit of flow, for the volume of flour-solvent slurries used. Endogenous free radicals are derived from enzymatic- or auto-oxidation of triglycerides to fatty acids and are present in flour that has been aged for any length of time, as were the samples used in this research (Tsen and Hlynka 1961; Clayton and Morrison 1972; Garcia et al 2002; Reichenauer and Goodman 2003). Although a slow process that is dependent on storage conditions, especially temperature and flour moisture content, free radicals are inevitably generated. Reichenauer and Goodman (2003) indicated that a rise in free radical content occurs over a period of four to six months, after which no further significant increase is observed. In this research, all of the flour samples were analyzed between 8 and 11 months after milling. Variability in the concentration of free radicals, arabinoxylans, and proteins among samples leads to variation in water viscosity due to some oxidative gelation. But cross-linking and gelation are not as complete as they could be with additional free radicals; the difference between water viscosity (mean = 14.0 cm at 9.8–16.5 cm) and the

peroxide-peroxidase viscosity (mean = 12.2 cm at 3.8–15.8 cm) (Table I).

Consistometer readings for peroxide (enhanced combined oxidative gelation potential of the arabinoxylans and proteins, with free radicals supplied by the peroxide-peroxidase system) had a mean of 12.2 cm at 3.8–15.8 and an average decrease of 1.8 cm (Table I). Batter viscosity of some of the cultivars increased much more, due to conditions that enhanced oxidative gelation. The overall decrease in batter flow was expected because the concentration of free radicals present in the system to initiate oxidative gelation was high for all samples and caused the much enhanced gelation capacity, manifested as viscosity (Faubion and Hosney 1990). Some samples were more prone to form an oxidative gel than were others with the minimum flow distance decreasing from 9.8 cm for water viscosity to 3.8 cm for peroxide viscosity. Water and peroxide consistometer values correlated at only $r = 0.55$ (Table II), indicating that samples that were low in endogenous flow (water viscosity) were not always those with the enhanced potential for gelation (peroxidase viscosity).

Xylanase treatment (identical to the water consistometer procedure but with xylanase added to hydrolyze WEAX, thus measuring the endogenous gelation potential of protein only) produced a consistometer range of 12.3–17.0 cm with a mean of 15.1 cm (Table I). The enzymatic hydrolysis of the arabinoxylans effectively eliminated arabinoxylan participation in oxidative gelation and led to a decrease in viscosity (increase in mean flow), as well as increases in both minimum and maximum flow, relative to water viscosity measurements. Some di-ferulate bonds were undoubtedly formed but gels involving multiple networks of long arabinoxylan chains could either not form or were hydrolyzed. Thus batter flow was not constrained and therefore increased. Some increase in viscosity occurred due to endogenous protein cross-linking (Oudgenoeg et al 2001; Tilley et al 2001; Takasaki et al 2005), otherwise all samples would have had a reading of ≈ 17 cm, and this was not the case. The extent of xylanase hydrolysis was not ascertained but was clearly sufficient to significantly reduce viscosity.

Xylanase treatment followed by peroxidase treatment had flows of 10.4–17.0 with a mean of 14.6 cm (Table I). This treatment should measure only the protein fraction's enhanced ability to form a gel. With the arabinoxylans enzymatically hydrolyzed, the only polymers that should remain to form a gel were the unhydrolyzed proteins. When compared with results from the xylanase-only treatment, it was apparent that additional gelation was occurring even after the hydrolysis of gel-forming arabinoxylans. The xylanase treatment viscosity had a correlation with the water treatment viscosity of $r = 0.42$ (Table II), indicating a loss of gelation potential. Also the xylanase treatment viscosity was correlated with the peroxidase treatment viscosity at only $r = 0.20$ (Table II), indicating an even greater loss of gelation potential. It

TABLE I
Summary Statistics for 240 Soft White and Club Wheat Samples

	Mean	Minimum	Maximum	SD
SKCS hardness ^a	33.9	13.7	52.1	8.13
Flour protein (%) ^b	8.2	6.0	13.5	1.47
Flour ash (%; 14% mb)	0.38	0.26	0.50	0.053
Total arabinoxylans (%)	1.55	1.00	2.36	0.251
WEAX (%) ^c	0.45	0.22	0.83	0.111
Cookie diameter (cm)	9.46	8.39	10.01	0.304
Bostwick consistometer (cm)				
Water	14.0	9.8	16.5	1.18
Peroxide-peroxidase	12.2	3.8	15.8	2.01
Xylanase	15.1	12.3	17.0	0.91
Xylanase+peroxide-peroxidase	14.6	10.4	17.0	1.20

^a Measured by Perten Single Kernel Characterization System 4100.

^b $N \times 5.7$, 14% mb.

^c Water-extractable arabinoxylans.

TABLE II
Correlation Coefficients^a for Grain and Flour Characteristics and Gelation Characteristics of 240 Soft White and Club Wheat Samples Measured by Bostwick Consistometer^a

	Flour Ash (%)	WEAX (%) ^b	Water (cm) ^c	Peroxide-Peroxidase (cm) ^c	Xylanase (cm) ^c	Xylanase+Peroxide-Peroxidase (cm) ^c	SKCS Hardness ^d	Cookie Diameter (cm)
Flour protein (%)	0.38	0.33	-0.56	-0.52	-0.42	-0.76	0.46	-0.72
Flour ash (%)		0.14	-0.09ns	-0.53	0.06ns	-0.21	0.28	-0.35
WEAX (%) ^b			-0.54	-0.47	-0.24	-0.31	0.24	-0.47
Water (cm)				0.55	0.42	0.67	-0.41	0.70
Peroxide-peroxidase (cm)					0.20	0.43	-0.37	0.65
Xylanase (cm)						0.51	-0.27	0.41
Xylanase+peroxide-peroxidase (cm)							-0.38	0.72
SKCS hardness								-0.55

^a All correlations significant at $P < 0.001$; ns, not significant at $P < 0.05$.

^b Water-extractable arabinoxylans.

^c Bostwick Consistometer values.

^d Kernel hardness measured by Perten Single Kernel Characterization System 4100.

appeared that the oxidative gel normally observed from peroxidase treatment was prevented from forming because the polymeric xylose backbones that interconnect through di-ferulic acid bridges were hydrolyzed by the xylanase.

WEAX concentration influenced water viscosity (Table II and Fig. 1A) (Izydorczyk et al 1991, 1992; Martinant et al 1998). More viscous batters were correlated with greater WEAX concentration, though considerable variation existed ($r = -0.54$). When the peroxidase system was used, maximizing the potential for gelation in the batter, the relationship was maintained ($r = -0.47$) but much more variation was observed (Table II and Fig. 1B). As expected, treatment with xylanase substantially reduced the correlation between viscosity and WEAX content to $r = -0.24$. WEAX content had essentially little relationship with viscosity after treatment with xylanase (Table II and Fig. 1C). However, when the peroxidase was coupled with xylanase digestion, the variation in viscosity increased (Fig. 1D), though the correlation remained nearly the same ($r = -0.31$; Table II) as with xylanase viscosity. Although the molecular size of WEAX and its ability to be extracted into the water phase of the system influenced viscosity, factors other than WEAX were apparently also contributing to viscosity.

Visual examination of the relationship between protein content and water viscosity showed a similar relationship to that of WEAX and water viscosity, and similar correlations at $r = -0.54$ and $r = -0.56$, respectively (Table II and Figs. 1A and 2A). Protein, as has been shown (Bresson and Barmore 1955), also affects viscosity. Hence SRC and SDS-sedimentation tests have been used to measure the impact of protein on viscosity. However, neither of these two techniques fully explains variation in batter viscosity.

When peroxidase was added to the system to induce enhanced gelation capacity, two groups of points seemed to form trends (circled groups in Fig. 2B). One group was more highly correlated with protein content (the group on the main regression line), whereas another grouping seemed to exist, descending more steeply from the main trend and showing a greater increase in viscosity due to gelation. The overall correlation between flour protein and the peroxidase treatment viscosity was $r = -0.52$ (Table II). When treated with xylanase, the correlation between viscosity and protein content was reduced to $r = -0.42$. Again, endogenous gelation potential existed among proteins, even after the hydrolysis of the

WEAX gel-forming fraction, and certainly to a greater extent than existed with the WEAX fraction alone (Fig. 1C vs. Fig. 2C).

However, when the slurry was treated with peroxidase after incubation with xylanase, the correlation with protein improved to $r = -0.76$ (Fig. 2D). The figure illustrates the enhanced potential viscosity of the batter due to protein gelation, in the absence of enhanced arabinoxylan gelation potential. The group of samples that reacted to gelation but was not well-correlated with protein, shown in Fig. 2B (the group in the lower left quadrant of the chart), was much more highly correlated with protein content after xylanase treatment removed the potential for arabinoxylan cross-linking to form a gel. The results imply that oxidative gels formed among arabinoxylans that greatly increased viscosity in some samples. When the arabinoxylan gelation potential was mostly removed by xylanase treatment, the correlation between viscosity and protein content was markedly improved because only protein remained as gel-forming polymers.

Taken as a whole, these observations demonstrate that not only was an oxidative gel being formed among arabinoxylans but among proteins as well, through the oxidative gelation mechanisms of di-ferulic acid and di-tyrosine linkages described above. Further, each form of oxidative gelation contributed individually to overall viscosity due to both endogenous free radicals, and the results of the peroxide viscosity systems, where free radicals were provided in plentitude. Intrinsically, proteins and WEAX influenced viscosity through their molecular size and concentration. But when free radicals were present, the potential for modification of batter viscosity (thickening due to oxidative gelation) and water sequestration resulted, affecting end-use quality. Increased viscosity due to oxidative gelation is difficult to predict through conventional analysis with methods such as SRC or SDS-sedimentation tests. More of the variation in the viscosity of batters or slurries can be explained through the mechanism of oxidative gelation but the overall viscosity of batters is the result of a culmination of interactions among protein and WEAX contents, the tyrosine or ferulic acid content, and the availability and concentration of endogenous free radicals in the flour. Measuring both water and peroxide viscosity provides information on endogenous and maximum potential overall oxidative gelation. For more information about the oxidative gelation potential of either proteins or arabinoxylans, treatment with xylanase, with and without peroxide may be helpful.

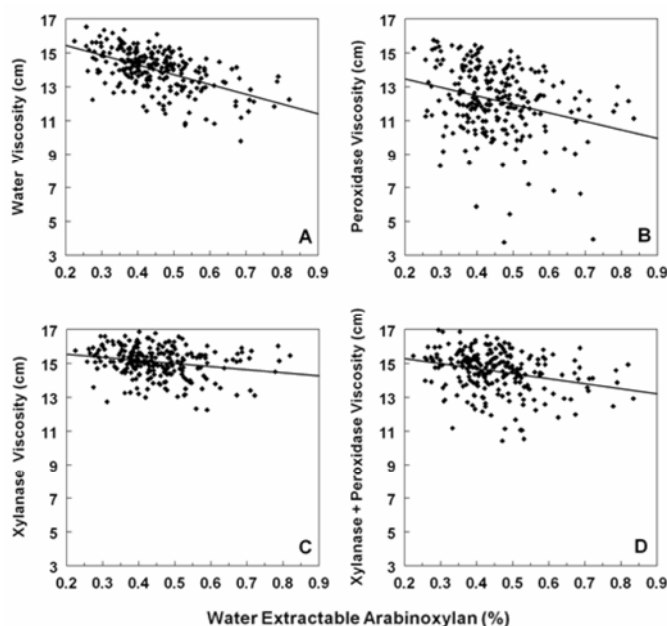


Fig. 1. Scatter plots of water-extractable arabinoxylan (WEAX) content and water viscosity (A); peroxide-peroxidase viscosity (B); xylanase viscosity (C); and xylanase followed by peroxide-peroxidase viscosity (D).

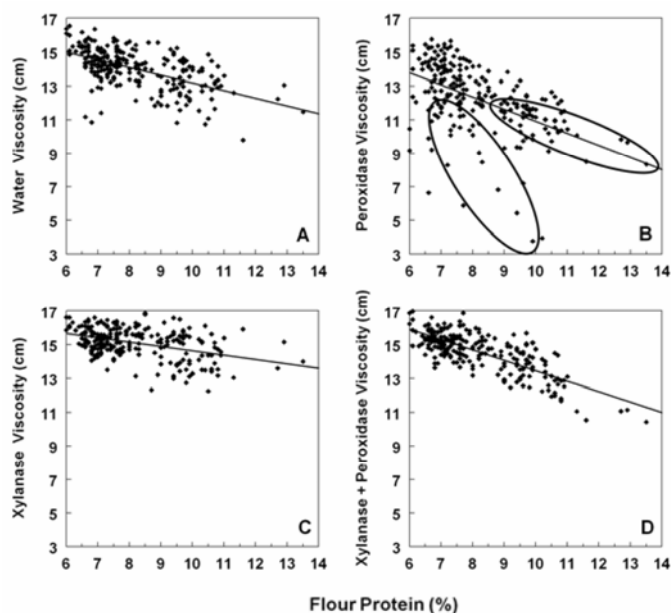


Fig. 2. Scatter plots of flour protein content and water viscosity (A); peroxide-peroxidase viscosity (B); xylanase viscosity (C); and xylanase followed by peroxide-peroxidase viscosity (D).

It appeared that oxidative gelation also had an effect on end-product quality when sugar-snap cookie diameter was examined in light of the oxidative gelation viscosity test. The results suggested that oxidative gel from either WEAX or protein could form, sufficient to sequester water and affect water relationships within the dough system.

Protein and WEAX have long been associated with cookie quality but the relationship has unexplained variation remaining (Gaines 1990 and Jeltema et al 1983, respectively). Figure 3A shows the relationship between protein and cookie diameter ($r = -0.72$; Table II). Certainly a relationship exists wherein smaller cookies are associated with higher protein content. The presence of endogenous free radicals has the potential to initiate oxidative gelation within cookie dough systems but in an uncontrolled manner that depends on free radical, WEAX, and protein concentration. WEAX content showed the same sort of relationship with cookie diameter but to a lesser extent where $r = -0.47$ (Fig. 3B and Table II).

The relationship between cookie diameter and the viscosity tests was greater (in terms of correlations) than simple protein or WEAX content. Water viscosity was correlated with the cookie diameter at $r = 0.70$ (Table II and Fig. 3C). The endogenous potential for oxidative gelation appeared to be associated with cookie baking quality.

Figure 3D indicates that arabinoxylan and protein oxidative gelation may be a part of the unexplained variation in cookie diameter that is not predicted by either WEAX or protein content. The correlation is slightly reduced ($r = -0.65$; Table II) but a pattern similar to that in Fig. 2B (protein content vs. peroxide viscosity) is evident. Two “families” of points emerge: one family likely associated with protein oxidative gelation; the other associated with arabinoxylan oxidative gelation. Xylanase treatment showed no strong relationship ($r = -0.41$; Table II) that could be explained by either protein or by WEAX (Fig. 3E). The relationship between cookie diameter and gelation-caused viscosity was reduced ($r = -0.69$ and -0.65 for water and peroxidase vs. $r = -0.41$ for xylanase).

However, when slurries were treated with xylanase, followed by peroxidase, the effect of protein gelation and lack of arabinoxylan gelation potential could be seen. Again, in the relationship between protein and the xylanase+peroxidase system (Fig. 2D), there was a clearer relation between the residual protein gelation and cookie diameter of $r = -0.72$ (Fig. 3F; Table II). The relationship between cookie diameter and peroxidase consistency was similar to that of the relationship between peroxidase consistency and flour protein (Fig. 2D and Fig. 3F). Gelation did appear to have a significant effect on the ability of cookie dough to spread and to explain some of the variation in cookie diameter unexplained by either WEAX or protein content alone.

To examine the combined effect of factors contributing to end-use quality as measured by cookie diameter, a statistical model was created, taking only the best combination of independent variables. The model was constructed less to attempt to predict cookie-baking quality than to examine the combined impact of influential components. Independent model variables used in examining contributions were kernel hardness, protein content, ash content, WEAX, total arabinoxylan content, water viscosity, peroxide viscosity, xylanase viscosity, and xylanase+peroxidase viscosity. Kernel hardness (SKCS) was used as a proxy for starch damage, an influence on cookie spread. Because starch damage represented a constant background effect, unaffected by the methods used here, and was represented by kernel hardness (the primary factor contributing to starch damage), the presence of SKCS values in the analysis was judged to be sufficient to model much of the effect of starch damage.

Three models of four, five, and six variables were best statistically in terms of maximizing F -values and R^2 values. All of the individual variables were significant at $P < 0.05$ and the overall model F -values were >100 . Further, R^2 values increased with each

additional variable, although the increase from the four- to five- and six-variable models explained $<2\%$ additional variation, indicating that model over-fit was occurring. But again, delineating the influential variables was the goal of this modeling exercise, not a predictive equation per se.

In the four-variable model for cookie diameter, the most influential variables in ranked order by F -value were xylanase+ peroxidase viscosity (F -value = 60), peroxidase viscosity (F -value = 49), SKCS kernel hardness (F -value = 28), and water viscosity (F -value = 13). The statistical model indicated that in order of importance, protein gelation potential, the combined enhanced gelation potential of arabinoxylans and proteins, kernel hardness, and the combined oxidative gelation effect of intrinsic gelation, contributed to cookie quality. Other variables, including the best-correlated single variable, protein content, had no significant effect on the model. The ability of the proteins and arabinoxylans to form a gel had a greater correlation with cookie diameter than did the simple concentration of protein or arabinoxylans alone. The overall model accounted for about 72% of the variation in cookie diameter ($R^2 = 0.72$).

The five-variable model added flour protein as a significant variable but, as noted above, explained only an additional 1% variation (R^2 increase from 0.72 to 0.73), and its F -value was the smallest of the five variables. The six-variable model incorporated WEAX as a significant variable. But as noted before, this model was approaching the end of statistical utility in that its overall F -value was declining. Nevertheless, the addition of WEAX was the most interesting feature of this model. Intrinsic content of both protein and WEAX do indeed relate to cookie diameter but to a lesser extent than the effect imparted by the oxidative gelation of the two polymers. Functionally, the reduced availability of water or sugar syrup in the cookie dough due to formation of oxidative

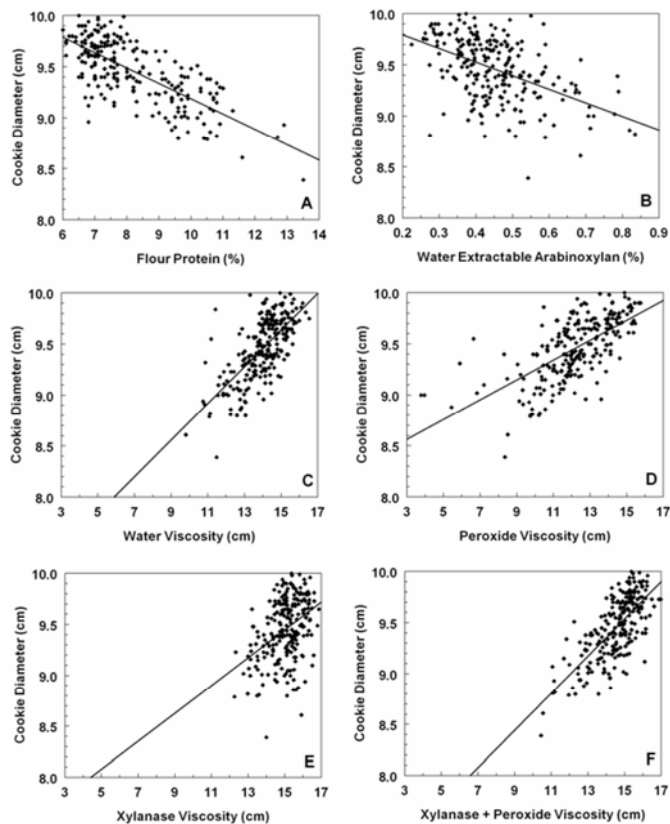


Fig. 3. Scatter plots of cookie diameter and flour protein (A); water-extractable arabinoxylan (B); water viscosity (C); peroxide-peroxidase viscosity (D); xylanase viscosity (E); and xylanase followed by peroxide-peroxidase viscosity (F).

gels has a large effect on the end-use quality of cookie that can be measured with the oxidative gelation test.

CONCLUSIONS

Oxidative gels were formed and measured in the method described here. Oxidative gelation occurred among arabinoxylan polymers, likely through di-ferulic acid bridges; among proteins, likely through di-tyrosine bridges and possibly among arabinoxylans and proteins through ferulic acid-tyrosine bridges. Oxidative gels appeared to likely reduce cookie diameter through enhanced water and sugar syrup sequestration but are more likely to have a much larger influence on the quality of high-moisture, batter-based products that are prepared under low shear-thinning conditions (e.g., pancakes, cake donuts, and other batter systems) where it was shown that oxidative gels increased viscosity, as measured by Bostwick consistometer.

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LITERATURE CITED

- AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Approved Methods 10-52, 56-11, and 56-70. The Association: St. Paul, MN.
- Bresson, C. R., and Barmore, M. A. 1955. Viscosity vs. protein and ash content of western wheat varieties. *Cereal Chem.* 32:144-152.
- Carvajal-Millan, E., Landillon, V., Morel, M.-H., Rouau, X., Doublier, J.-L., and Micard, V. 2005. Arabinoxylan gels: Impact of the feruloylation degree on their structure and properties. *Biomacromolecules* 6:309-317.
- Ciacco, C. F., and D'Appolonia, B. L. 1982. Characterization of pentosans from different wheat flour classes and of their gelling capacity. *Cereal Chem.* 59:96-99.
- Clayton, T. A., and Morrison, W. R. 1972. Changes in flour lipids during the storage of wheat flour. *J. Sci. Food Agric.* 23:721-736.
- Courtin, C. M., and Delcour, J. A. 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *J. Cereal Sci.* 35:225-243.
- Cyran, M., Courtin, C. M., and Delcour, J. A. 2003. Structural features of arabinoxylans extracted with water at different temperatures from two rye flours of diverse breadmaking quality. *J. Agric. Food Chem.* 51:4404-4416.
- Douglas, S. G. 1981. A rapid method for the determination of pentosans in wheat flour. *Food Chem.* 7:139-145.
- Durham, R. K. 1925. Effect of hydrogen peroxide on relative viscosity measurements of wheat and flour suspensions. *Cereal Chem.* 11:297-305.
- Finnie, S. M., Bettge, A. D., and Morris, C. F. 2006. Influence of variety and environment on water-soluble and water-insoluble arabinoxylans in soft wheat. *Cereal Chem.* 83:617-623.
- Gaines, C. S. 1990. Influence of chemical and physical modification of soft wheat protein on sugar snap cookie dough consistency, cookie size and hardness. *Cereal Chem.* 67:73-77.
- Garcia, R., Rakotzafy, L., Telef, N., Potus, J., and Nicolas, J. 2002. Oxidation of ferulic acid or arabinose-esterified ferulic acid by wheat germ peroxidase. *J. Agric. Food Chem.* 50: 3290-3298.
- Hoseney, R. C., and Faubion, J. M. 1981. A mechanism for the oxidative gelation of wheat flour water-soluble pentosans. *Cereal Chem.* 58:421-424.
- Izydorczyk, M. S., and Biliaderis, C. G. 1992. Effect of molecular size on physical properties of wheat arabinoxylan. *J. Agric. Food Chem.* 40:561-568.
- Izydorczyk, M. S., Biliaderis, C. G., and Bushuk, W. 1991. Physical properties of water-soluble pentosans from different wheat varieties. *Cereal Chem.* 68:145-150.
- Jeffers, H. C., and Rubenthaler, G. L. 1979. Effect of roll temperature on flour yield with the Brabender Quadrumat experimental mills. *Cereal Chem.* 54:1018-1025.
- Jeltema, M. A., Zabik, M. E. and Thiel, L. J. 1983. Prediction of cookie quality from dietary fiber components. *Cereal Chem.* 60:227-230.
- Martinant, J. P., Cadalen, T., Billot, A., Chartier, S., Leroy, P., Bernard, M., Saulnier, L., and Branlard, G. 1998. Genetic analysis of water-extractable arabinoxylans in bread wheat endosperm. *Theor. Appl. Genet.* 97:1069-1075.
- Moore, A. M., Martinez-Munoz, I., and Hoseney, R. C. 1990. Factors affecting the oxidative gelation of wheat water-solubles. *Cereal Chem.* 67:81-84.
- Morita, S.-I., Ito, T., and Hirano, S. 1974. A gel-forming polysaccharide containing ferulic acid in protein-free form present in aqueous extract of wheat flour. *Int. J. Biochem.* 5:201-205.
- Morris, C. F., Campbell, K. G., and King, G. E. 2005. Kernel texture differences among U.S. soft wheat cultivars. *J. Sci. Food Agric.* 85:1959-1965.
- Neukom, H., and Markwalder, H. U. 1978. Oxidative gelation of wheat flour pentosans: A new way of cross-linking polymers. *Cereal Foods World* 23:374-376.
- Oudgenoeg, G., Hilhorst, R., Piersma, S. R., Boeriu, C. G., Gruppen, H., Hessing, M., Voragen, A. G. J., and Laane, C. 2001. Peroxidase-mediated cross-linking of a tyrosine-containing peptide with ferulic acid. *J. Agric. Food Chem.* 49:2503-2510.
- Reichenauer, T. G., and Goodman, B. A. 2003. Free radicals in wheat flour change during storage in air and are influenced by the presence of ozone during the growing season. *Free Radical Res.* 37:523-528.
- Slade, L., and Levine, H. 1994a. Aspects of soft wheat quality of current interest to a cookie/cracker baker. I and II. *Cereal Foods World* 39:640.
- Slade, L., and Levine, H. 1994b. Structure-function relationships of cookie and cracker Ingredients. Pages 23-141 in: *The Science of Cookie and Cracker Production*. H. Faridi, ed. Chapman and Hall: New York.
- Takasaki, S., Kato, Y., Murata, M., Homma, S., and Kawakishi, S. 2005. Effects of peroxidase and hydrogen peroxide on the dityrosine formation and the mixing characteristics of wheat-flour dough. *Biosci. Biotechnol. Biochem.* 69:1686-1692.
- Tilley, K. A., Benjamin, R. E., Bagorogoza, K. E., Okot-Kotber, B. M., Prakash, O., and Kwen, H. 2001. Tyrosine cross-links: Molecular basis of gluten structure and function. *J. Agric. Food Chem.* 49:2627-2632.
- Tsen, C. C. and Hlynka, I. 1961. The role of lipids in oxidation of doughs. *Cereal Chem.* 39:209-219.
- Vinkx, C. J. A., Van Nieuwenhove, C. G. and Delcour, J. A. 1991. Physico-chemical and functional properties of rye nonstarch polysaccharides. III. Oxidative gelation of a fraction containing water-soluble pentosans and proteins. *Cereal Chem.* 68:617-622.
- Wang, M., Hamer, R. J., von Vliet, T., and Oudgenoeg, G. 2002. Interaction of water extractable pentosans with gluten protein: Effect of dough properties and gluten quality. *J. Cereal Sci.* 36:25-37.

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