Influence of Cultivar and Environment on Water-Soluble and Water-Insoluble Arabinoxylans in Soft Wheat

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ABSTRACT

Arabinoxylans are hydrophilic nonstarch polysaccharides found in wheat grain as minor constituents. Arabinoxylans can associate with large amounts of water through hydrogen bonding and can form oxidative gels. These properties are important factors in end-use quality of wheat. The objective of this study was to delineate the influence of wheat cultivar and growing environment on variation in water-soluble (WS-AX), water-insoluble (WI-AX), and total (TO-AX) arabinoxylan contents of flour and whole grain meal. This study included seven spring and 20 winter soft white wheat cultivars grown in 10 and 12 environments, respectively (each evenly split over two crop years). Univariate analysis of variance (ANOVA) and multivariate analysis of variance with canonical analysis (MANOVA) was used to evaluate sources of variation. Variation in arabinoxylan contents and absolute amounts (xylose equivalents) among the two cultivar sample sets (spring and winter) was similar, and both cultivar and environment were significant sources of variation. The cultivar-by-environment interaction was relatively unimportant. Results indicate that the variation in arabinoxylan content is primarily influenced by cultivar and secondarily influenced by environment. Within arabinoxylan fractions, WS-AX content is primarily influenced by genotype, while WI-AX content is more greatly influenced by the environment.

Many intrinsic and extrinsic factors influence the biochemical composition of wheat (*Triticum aestivum* L.) grain and thus largely determine its end-use quality. These influencing factors can be grouped according to whether they originate from the genetic makeup of the plant (i.e., genotype or cultivar) or from the environment (all external conditions under which the plant grows). Cultivar and environment each influence wheat composition to varying degrees. Peterson et al (1992 and references therein) showed that, overall, environment has a greater influence than does cultivar on the quality attributes of wheat. Examples of specific quality traits in wheat that have been evaluated based on varietal and environmental influences include protein content, kernel texture (hardness), kernel color, and flour swelling volume (FSV) (Pomeranz et al 1985; Bassett et al 1989; Peterson et al 1992; Mariani et al 1995; Morris et al 1997; Zhu and Khan 2001; Matus-Cádiz et al 2003). Studies have shown that protein content in wheat is influenced more by the environment than by cultivar (Pomeranz et al 1985; Mariani et al 1995; Zhu and Khan 2001) and more specifically nitrogen availability in soil (Peterson et al 1992). Kernel texture, when including both soft and hard texture classes, is influenced more by cultivar (Pomeranz et al 1985). However within a texture class (soft or hard), environment has a greater influence on kernel texture (Bassett et al 1989). Matus-Cádiz et al (2003) found that kernel color varied significantly among hard white wheat cultivars. Morris et al (1997) investigated the environmental and genotypic influences on FSV. FSV is an indicator of flour quality for use in white salted Asian noodles. It was demonstrated that FSV was influenced primarily by cultivar and secondarily by environment.

Compared with other biochemical components, the influence of cultivar and environment on arabinoxylans is not well established. Arabinoxylans are an important subset of a larger group of nonstarch polysaccharides referred to as pentosans. Arabinoxylans constitute a large proportion (≈85%) of the nonstarch polysaccharides found in wheat grain (Mares and Stone 1973). The putative function of arabinoxylans in mature plants is to provide structural support to cell walls through hydroxy-cinnamate cross-linking (Carpita and Gibeaut 1993). The backbone conformation of arabinoxylans is a random coil with varying degrees of flexibility (Dervilly et al 2000; Courtin and Delcour 2002) including β(1→4)-linked D-xylopyranosyl units with α-L-arabinofuranoside residues variously substituted at the 2- and 3-carbon position. Ferulic acid can be ester-linked to the C (*O*)-5- of arabinose residues (Courtin and Delcour 2002 and references therein). Cross-linking between these ferulic acid residues to form diferulic acid cross-links or ferulic acid and tyrosine/protein cross-links largely determines solubility. The frequency of the arabinose residues on the xylose backbone further contributes to solubility/insolubility (Neukom et al 1967; Neukom 1976; Andrewortha et al 1979; Fincher and Stone 1986; Courtin and Delcour 2002) and results in the ability to partition arabinoxylans into two arbitrary classes: water-soluble (WS-AX) and water-insoluble arabinoxylans (WI-AX). These two main classifications of arabinoxylans are similar in gross composition. The flexible unsubstituted regions of the arabinoxylan molecule can associate with itself or with other arabinoxylan molecules through hydrogen bonding, causing aggregation and insolubility (Andrewortha et al 1979; Courtin and Delcour 2002).

The WS-AX and WI-AX are localized in different areas of the cell wall. WS-AX are weakly bound to the outside of the cell wall and the WI-AX are covalently bound within the cell walls (Mares and Stone 1973; Iiyama et al 1994; Courtin and Delcour 2002). Wang et al (2006) determined that endosperm arabinoxylans differ in their molecular structure, resulting in differences in extractability when compared with arabinoxylans localized in the aleurone.

Arabinoxylans possess hydrophilic characteristics, leading to a competition with other components for water in food systems (Izydorczyk and Biliaderis 1995). The effect of arabinoxylans on water relations in dough has been well established (Jelaca and Hlynka 1971; Kim and D'Appolonia 1977; Yin and Walker 1992). The amount of water retained by WI-X and WS-AX, determined from farinograph absorption, was 6.7 to 9.9 and 3.5 to 6.3 times the weight in water, respectively (Jelaca and Hlynka 1971; Kim and D'Appolonia 1977). Furthermore, arabinoxylans have the unique ability to sequester water through oxidative gelation. Under oxidizing conditions, and with the initiation of a free radical, adjacent arabinoxylan molecules can form a covalent cross-link between aromatic rings of ferulic acid residues (Geissmann and Neukom 1973; Figueroa-Espinoza and Rouau 1998). The resulting gel can sequester up to 100 g of water/g of arabinoxylan polymer (Izydorczyk et al 1990).

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Considering the influence that arabinoxylans have on water relations, either through hydrogen bonding or oxidative gelation, and that food processing properties are dependent on water content and availability, arabinoxylans undoubtedly exert an effect on the end-use quality of wheat. Food properties that are influenced by water content include rheological properties, microbial growth, osmotic pressure, enzyme activity, and molecular mobility (Walstra 2003). Arabinoxylans have been studied for their effects on the rheological properties in dough (Jelaca and Hylnka 1971, 1972; Kim and D'Appolonia 1977; Michniewicz et al 1991; Yin and Walker 1992; Biliaderis et al 1995) and on quality traits that are influenced by molecular mobility of water such as starch retrogradation and gluten yield (Izydorczk and Biliaderis 1992; Biliaderis et al 1995; Wang et al 2003). In aqueous solutions, arabinoxylans form highly viscous solutions (Izydorczyk and Biliaderis 1992; Sarker et al 1998) especially when compared with other polysaccharides (Izydorczyk and Biliaderis 1995). Arabinoxylans also increase dough viscosity and dough development time (Jelaca and Hylnka 1971; Michniewicz et al 1991; Billiaderis et al 1995). An increase in starch retrogradation was observed due to increased moisture content (Zeleznak and Hoseney 1986). Because arabinoxylans can bind or sequester a relatively large amount of water, causing an increase in moisture content, the molecular mobility of starch components may increase, resulting in an increased rate of starch retrogradation (Michniewicz et al 1992; Billiaderis et al 1995). Arabinoxylans also influence gluten formation and may decrease gluten yield (Wang et al 2003). Water-unextractable solids, which would include WI-AX, indirectly decreased gluten yield by competing for water during gluten formation, limiting the molecular mobility and ability of gliadin and glutenin proteins to form gluten.

A better understanding of the influence of environment and wheat cultivar on WS-AX and WI-AX could reduce variation in arabinoxylans, thereby improving quality. Lempereur et al (1997) investigated the genetic and environment influences on WS-AX and TO-AX in durum wheat (*T. turgidum* ssp. *durum* [Desf.] Husn.) and concluded that both cultivar and environment influence WS-AX and TO-AX. Hong et al (1989) and Saulnier et al (1995) found that cultivar played an important role in WS-AX content of *T. aestivum*. However, the limited number of environments, two for Hong et al (1989) and "not shown" by Saulnier et al (1995), indicated that further research would provide more insight into the relative influence of cultivar and environment. Andersson et al (1992) studied the influence of cultivar and environment on nonstarch polysaccharide content in wheat flour and concluded that environment had the greater influence. Other cereal grains such as barley and rye have been studied for the influence of cultivar and environment on total arabinoxylan (TO-AX) content. In barley, Henry (1986) found that both cultivar and growing environment influenced TO-AX content. Different results were demonstrated for rye, where no cultivar influence was found, but year and location influences were significant (Saastamoinen et al 1989).

Given the potential impact of arabinoxylans on the end-use quality in wheat, determination of sources of variation influencing arabinoxylan content and their distribution in the kernel would be beneficial. This objective of this study was to delineate the influence of cultivar and growing environment on WS-AX, WI-AX, and TO-AX content in soft wheat.

MATERIALS AND METHODS

Soft White Wheat Flour and Meal Samples

Soft white wheat samples were obtained from an ongoing cultivar and environment study conducted by the USDA-ARS Western Wheat Quality Laboratory in Pullman, WA. Depending on growth habit, the samples were organized into two sample sets: spring or winter. The spring sample set contained seven wheat cultivars: Alpowa, Alturas, Eden, Fielder, Nick, WA7920, and Zak, each grown in 10 unique environments. An environment is defined here as a unique variable combining the planting location and year. The spring wheat environments were Dayton, Fairfield, Farmington, Lamont and Mayview, WA, 2003; Dayton, Horse Heaven, Lind, Pullman, and St. John, WA, 2004. The winter wheat sample set contained 20 cultivars: ARS00173, ARS00226, ARS00235, ARS96277, Bruehl, Brundage, Coda, Dune, Edwin, Eltan, Finch, Hiller, ID587, Madsen, Masami, OR990055, ORCF101, Rely, Simon, and Stephens, each grown in 12 unique environments. The winter wheat environments were Almira, Lamont, Lind, Mayview, Moses Lake, and Pullman, WA, 2002-03; Colton, Creston, Dusty, Lamont, Lind, and Moses Lake, WA, 2003-04. All samples were of commercial quality ranging in test weight from 74.4 to 82.9 kg/hL (mean 78.6), and protein content (14% wb) from 67 to 147 g/kg (mean 103).

All wheat samples (600 g each, then tempered to 13% fresh wt basis) were milled into flour on a modified Quadrumat milling system (Jeffers and Rubenthaler 1977) and also ground (no tempering) into meal with a cyclone mill (Udy Corp., Boulder, CO) through a 0.5-mm screen. The resulting flours and meals were analyzed for moisture content (Approved Method 44-16, AACC International 2000) with ranges of 12.2–13.3% and 9.0–10.0%, respectively.

Arabinoxylan Determination

A colorimetric method described by Douglas (1981) that measures pentose sugar content in wheat flour was modified to measure both WS-AX and TO-AX content from wheat flour and wheat meal. The Douglas (1981) method was based on a method described by Dische and Borenfreund (1957) that was subsequently modified by Cracknell and Moye (1970) for use in cereal products. For our modification to the Douglas (1981) method, 125 mg of flour or meal was placed in a 50-mL graduated conical screw-cap polypropylene tube (Fisher Scientific, Pittsburgh, PA) to which was added 25 mL of H_2O. The tubes were vortexed, producing a sample suspension. Immediately after vortexing, 1 mL of sample suspension was quickly removed and pipetted into a stoppered reaction tube (Pyrex tube, 16 × 100 mm, screw cap with PTFE liner). The sample suspension (1 mL) contained 5 mg of sample and was used to determine TO-AX content. The original sample suspension was then placed on a laboratory rocker (model AR-100, PGC Scientific, Gaithersburg, MD) for 30 min. After this hydration/extraction period, the sample was centrifuged for 10 min at 2,500 × *g*. After centrifugation, 1 mL of supernatant was removed and pipetted into a stoppered reaction tube, similar to the method above. The supernatant aliquot thus represented a 5-mg equivalent of sample for determination of WS-AX content. For each sample, flour or meal, two sample suspensions were made. From each sample suspension two 1-mL aliquots were removed, resulting in four sample replicates for TO-AX content, two from the first sample suspension and two from the second sample suspension. For each centrifuged sample suspension, two 1-mL aliquots of the supernatant were removed, resulting in four sample replicates for WS-AX content, two from the first sample suspension and two from the second sample suspension. Once the sample aliquots were collected, 1 mL of H_2O was added to bring the final volume to 2 mL. Arabinoxylan determination then followed the method iterated by Douglas (1981). Our modification provided both WS-AX and TO-AX to be determined from the same original sample suspension.

A standard curve was produced using a stock solution of 10 mg of D-(+)-xylose (X-1500, Sigma) in 100 mL of H_2O. Triplicate standard samples were prepared using 0.0, 0.5, 1.0, 1.5, and 2.0 mL of xylose stock solution pipetted into stoppered reaction tubes representing 0.0, 0.05, 0.10, 0.15, and 0.20 mg of xylose, respectively, with the total volume brought to 2 mL with H_2O. Standard check samples were analyzed with each batch of flour and

meal samples. Arabinoxylan content was determined by the equation: Arabinoxylan content (mg/g of sample) = 1,000 × [($\Delta A_{552\text{-}510}$ sample) × {(xylose equivalent, mg) / ($\Delta A_{552\text{-}510}$ standard)} + (xylose equivalent intercept, mg)] and expressed as xylose equivalents. The xylose equivalent terms in the equation represent the standard curve regression slope and intercept.

Statistical Analysis

The two replicates (also considered repeated measures) from each sample suspension for TO-AX and supernatant for WS-AX were averaged, resulting in two observations for each sample. WI-AX content was derived from the difference between WS-AX content and TO-AX content. Normal data distribution, univariate and multivariate, was confirmed by inspecting a plot of residuals (histogram) and a normal probability plot (Proc Univariate, SAS Institute, Cary, NC). Univariate (ANOVA) and multivariate (MANOVA) analyses of variance (Proc GLM, SAS) were conducted with consideration of the experimental design and data organization. Cultivar was considered a fixed effect while environment was considered a random effect. Balanced data sets were constructed containing cultivar and environment. MANOVA was used to provide insight into the intercorrelations among the different variables (Steinhorst and Williams 1985). Canonical variable analysis was used in MANOVA to best explain group differences. Wilks's lambda test for significance was used in the MANOVA with a significance level of $P = 0.0001$. Due to the especially low error variance in this research, cultivar mean differences were more conservatively tested using Duncan's multiple range test with $\alpha = 0.001$ instead of the more common $\alpha = 0.05$.

RESULTS

The spring wheat cultivars had a slightly lower mean content of water-soluble arabinoxylan in flour compared with the winter wheat samples even though the spring wheat WS-AX minimum and maximum were slightly greater (Tables I and II). Within each spring or winter wheat sample set, WS-AX content of flour varied fourfold to fivefold, from ≈1–2 mg of xylose equivalents/g of flour up to ≈7–8 mg of xylose equivalents/g. The WS-AX content of meals was only modestly greater than the flours for both sample sets: ≈5 mg of xylose equivalents/g or ≈1 mg greater than the flours. Minimums and maximums for WS-AX content of meals were similarly ≈1 mg greater for each sample set compared with their respective flours. These differences are generalized, however. In the meals, the differences between minimum and maximum WS-AX contents were on the order of threefold. The greater differences among flours compared with meals may reflect intervarietal differences in milling.

The WI-AX content of flours was about twice that of WS-AX (Tables I and II). For meals, however, the difference was much greater, on the order of sevenfold or eightfold more WI-AX than WS-AX (mean values). From minimum to maximum, the difference in WI-AX of flours was about threefold, but only about twofold for meals. In absolute terms, the WI-AX content of flours was ≈5–14 mg of xylose equivalents/g and ≈25–60 mg of xylose equivalents/g of meal.

Total arabinoxylan content reflected the sum of the prior two fractions. Spring and winter wheat flours averaged 11.90 and 12.56 mg of xylose equivalent/g, respectively. Their meals contained, on average, 45.79 and 41.55 mg of xylose equivalents/g, respectively. As seen earlier with the WI-AX fraction, the range among samples within a set was approximately twofold.

These sample statistics indicated that individual samples of wheat cultivars grown over multiple environments and two crop years differed markedly. Yet, the spring and winter wheat sample sets were similar in arabinoxylan content (sample statistics). The next step was to understand how water-soluble, water-insoluble, and total arabinoxylan contents varied in these two sample sets according to cultivar, environment, and cultivar-by-environment interaction.

TABLE I
Sample Statistics[a] and *F*-Values from Mixed Model Analysis of Variance[b] for Cultivar and Environment Sources of Variation[c]

	Flour			Meal		
Statistic/Source	WS-AX	WI-AX	TO-AX	WS-AX	WI-AX	TO-AX
Minimum	1.87	4.79	7.98	2.87	27.34	31.55
Mean	3.96	7.94	11.90	5.02	40.77	45.79
Maximum	8.06	13.86	19.05	9.09	56.13	60.83
Cultivar	183.37	15.16	75.10	148.02	7.67	9.17
Environment	13.89	8.87	11.53	19.85	39.71	45.91
Interaction	4.83	1.38ns	1.28ns	3.90	1.30ns	1.35ns

[a] Sample statistics are expressed as mg equivalents of xylose/g of sample.
[b] *F*-values are significant at $P = 0.0001$; ns, not significant. *F*-values were derived from Type III sums of squares; the error term used for cultivar and interaction is the mean square error; the error term used for environment is the interaction mean square.
[c] Water-soluble, water-insoluble, and total arabinoxylan content of flour and meal from seven soft white spring wheat cultivars grown in 10 environments: WS-AX, water soluble arabinoxylan; WI-AX, water insoluble arabinoxylan; TO-AX, total arabinoxylan.

TABLE II
Sample Statistics[a] and *F*-Values from Mixed Model Analysis of Variance[b] for Cultivar and Environment Source of Variation[c]

	Flour			Meal		
Statistic/Source	WS-AX	WI-AX	TO-AX	WS-AX	WI-AX	TO-AX
Minimum	1.39	4.36	8.51	2.37	24.53	30.62
Mean	4.30	8.26	12.56	4.94	36.61	41.55
Maximum	7.22	13.13	16.93	7.41	57.46	63.56
Cultivar	154.82	33.38	36.45	23.54	13.38	10.95
Environment	33.47	28.65	42.34	11.75	17.04	16.36
Interaction	8.54	1.96	2.13	4.49	2.16	2.34

[a] Sample statistics are expressed as mg equivalents of xylose/g of sample.
[b] *F*-values are significant at $P = 0.0001$; ns, not significant. *F*-values were derived from Type III sums of squares; the error term used for cultivar and interaction is the mean square error; the error term used for environment is the interaction mean square.
[c] Water-soluble, water-insoluble, and total arabinoxylan content of flour and meal from 20 soft white winter wheat cultivars grown in 12 environments: WS-AX, water-soluble arabinoxylan; WI-AX, water-insoluble arabinoxylan; TO-AX, total arabinoxylan.

ANOVA results for the spring and winter wheat sample sets are presented in Tables I and II, respectively. Data were determined to be normally distributed as judged by a plot of the residuals. The overall model R^2 values for the ANOVA indicated good model fit (R^2 0.80–0.97). Both main effects, cultivar and environment, were significant at $P = 0.0001$ for water-soluble, water-insoluble, and total arabinoxylans for both sample sets. The interactions between cultivar and environment were significant for the spring wheat WS-AX fraction and for all the winter wheat arabinoxylan fractions. Even though the interactions were statistically significant, the contribution of the interactions to the overall ANOVA models was relatively minor compared with the main effects (as judged by *F*-values). Therefore interactions were concluded to be of low practical significance and were not investigated further. A plot of the spring wheat flour WS-AX means (environment means vs. cultivar means) showed essentially parallel slopes with no cross-over; the exception being Alpowa, which exhibited a higher relative WS-AX content at the higher WS-AX environments (steeper slope).

Based on ANOVA *F*-values for these soft white spring and winter wheat samples (Tables I and II), cultivar had a greater influence on WS-AX content variation in flour and meal than did environment. For WI-AX content, cultivar exhibited the greater contribution for variation among flours, whereas for meals the environment was relatively greater. This observation was relatively more pronounced for the spring wheat samples compared with the winter wheat samples, where *F*-values indicated a more nearly equal contribution. For TO-AX content, the situation was similar to the WI-AX. For the spring wheat sample set, cultivar was a greater relative source of variation for flour, with environment a greater source of variation for meal. For the winter wheat sample set, the relative contribution of cultivar and environment was, again, more nearly similar.

Based on simple descriptive statistics and univariate ANOVA, it can be stated in very general terms that the soft white spring and winter wheat sample sets behaved more similarly than differently, i.e., no marked differences between the samples sets based on growth habit or the unique environments in which each was grown were observed. Because this result indicated that the two sample sets behaved similarly in their response to cultivar and environmental sources of variation, and that different arabinoxylan fractions differed in their relative influence due to cultivar or environment, we next sought to substantiate and explore further the variation in arabinoxylan fractions using multivariate analysis (MANOVA).

MANOVA with canonical analysis was used to further explore the influence of cultivar and environment on the variation of WS-AX and WI-AX fractions in soft wheat. TO-AX content was not analyzed in MANOVA due to multicollinearity because TO-AX content is a summation of WS-AX and WI-AX contents. The MANOVA for flour and meal samples were evaluated separately due to multicollinearity as well because flour samples are a physical subfraction of the grain.

For the soft white spring wheat flours, cultivar was again a significant source of variation for the arabinoxylan fractions (Wilks's lambda *F*-value of 50.1) (Table III). Nearly all the variation (99%) in WS-AX and WI-AX contents associated with cultivar was represented by the first eigenvalue (22.37). Cultivar had a greater influence on the WS-AX fraction than on the WI-AX fraction, as evidenced by the standardized canonical variables 1 for eigenvalue 1 (WS-AX 3.98 and WI-AX 0.92). Environment was also a significant source of variation among arabinoxylan contents of the spring wheat flour samples (*F*-value 11.6) (however, Wilks's lambda test indicated a smaller *F*-value for environment than for cultivar). The overall variation in WS-AX and WI-AX contents associated with environment was represented in both eigenvalues; the first eigenvalue contributed 63.6% of the total variation, and the second eigenvalue contributed 36.4%. Both arabinoxylan fractions were influenced by environment. WS-AX had a greater standardized canonical 1 variable for eigenvalue 1 (1.96) but WI-AX had a greater standardized canonical 2 variable (1.32) for eigenvalue 2.

Soft spring wheat meal samples provided results similar to the soft spring wheat flour samples from the MANOVA and canonical variable analyses (Table III). Cultivar was a significant source of variation for the arabinoxylan fractions (*F*-value 43.3). A substantial proportion (95.9%) of total variation in WS-AX and WI-AX contents associated with cultivar was represented by eigenvalue 1 (12.70). Cultivar had a greater influence on WS-AX than on WI-AX, as evidenced by standardized canonical 1 variables for eigenvalue 1 (WS-AX 3.67 and WI-AX 0.07). Environment was again a significant source of variation for arabinoxylan contents among the soft

TABLE III
Multivariate Analysis of Variance (MANOVA)[a] with Canonical Analysis of Cultivar and Environment Sources of Variation[b]

Sample	Source	Wilks's λ *F*-Value	Eigen Value 1	% of Total Variation	Eigen Value 2	% of Total Variation	Standardized Canonical Variable 1		Standardized Canonical Variable 2	
							WS-AX	WI-AX	WS-AX	WI-AX
Flour	Cultivar	50.1	22.37	99.0	0.23	1.0	3.98	0.92	–0.90	1.41
Flour	Environment	11.6	2.58	63.6	1.48	36.4	1.96	0.74	–0.07	1.32
Meal	Cultivar	43.3	12.70	95.9	0.66	4.9	3.67	0.07	0.001	2.16
Meal	Environment	21.0	8.60	88.0	1.17	12.0	0.96	1.60	–1.59	1.02

[a] All Wilks's λ *F*-values are significant at $P = 0.0001$.
[b] Water-soluble and water-insoluble arabinoxylan contents of flour and meal from seven soft white spring wheat cultivars grown in 10 environments: WS-AX, water-soluble arabinoxylan; WI-AX, water-insoluble arabinoxylan.

TABLE IV
Multivariate Analysis of Variance (MANOVA)[a] with Canonical Analysis of Cultivar and Environment Sources of Variation[b]

Sample	Source	Wilks's λ *F*-Value	Eigen Value 1	% of Total Variation	Eigen Value 2	% of Total Variation	Standardized Canonical Variable 1		Standardized Canonical Variable 2	
							WS-AX	WI-AX	WS-AX	WI-AX
Flour	Cultivar	67.0	12.27	85.9	2.01	14.1	4.17	0.08	1.07	2.08
Flour	Environment	32.4	2.38	65.2	1.27	34.8	1.33	1.13	–0.07	1.01
Meal	Cultivar	17.3	2.64	82.6	0.56	17.4	1.89	–1.03	1.07	1.34
Meal	Environment	14.5	0.93	60.1	0.62	40.0	–0.35	1.20	1.00	0.03

[a] All Wilks's λ *F*-values are significant at $P = 0.0001$.
[b] Water-soluble and water-insoluble arabinoxylan contents of flour and meal from 20 soft white winter wheat cultivars grown in 12 environments: WS-AX, water-soluble arabinoxylan; WI-AX, water-insoluble arabinoxylan.

spring wheat meal samples (*F*-value 21.0), albeit less than cultivar. The overall variation in arabinoxylan contents contributed by environment was represented by eigenvalue 1, which explained 88.0% of the total variation. Environment had a greater influence on the WI-AX fraction compared with WS-AX, as shown by the standardized canonical 1 variables for eigenvalue 1 (WS-AX 0.96 and WI-AX 1.60) (Table III).

Soft white winter wheat flour and meal MANOVA and canonical variable analysis results were similar to the soft spring wheat flour and meal results (Table IV). For soft winter wheat flour, cultivar was a significant source of variation of the arabinoxylan fractions (*F*-value 67.0). Greater than 85% of the total variation in WS-AX and WI-AX contents was explained by the first eigenvalue. Cultivar had a greater influence on the WS-AX fraction than the WI-AX fraction (standardized canonical 1 variables for eigenvalue 1, WS-AX 4.17 and WI-AX 0.08). Environment was also a significant source of variation among the soft winter samples (*F*-value 32.4). The overall variation in WS-AX and WI-AX contents associated with environment was represented by both eigenvalues, with the first associating with 65.2% of the total variation and the second associating with 34.8%. Both arabinoxylan fractions were influenced by environment; WS-AX had greater standardized canonical 1 variable for eigenvalue 1 (1.33) and WI-AX had greater standardized canonical 2 variable (1.01) for eigenvalue 2 (Table IV).

For the soft winter meal samples, cultivar was a significant source of variation in the WS-AX and WI-AX fractions (*F*-value 17.34). The overall variation in arabinoxylan contents associated with cultivar was represented by both eigenvalues, with the first eigenvalue explaining 82.6% of the total variation, and the second eigenvalue contributing 17.4%. Of the two arabinoxylan fractions, cultivar had a greater influence on the WS-AX fraction (standardized canonical 1 variables for eigenvalue 1 of WS-AX 1.89 and WI-AX –1.03). The standardized canonical 2 variables were similar between arabinoxylan fractions (WS-AX 1.07 and WI-AX 1.34), indicating that within the 17.4% of the variation expressed by the second eigenvalue, both fractions were influenced similarly. Environment was also a significant source of variation for arabinoxylan fractions among soft winter meal samples (*F*-value 14.5). The overall variation in arabinoxylan content contributed by environment was represented by both eigenvalues, with the first eigenvalue explaining 60.1% of the total variation and the second eigenvalue explaining 40.0% of the total variation. Both arabinoxylan fractions were influenced by environment, where WS-AX had a greater standardized canonical 1 variable for eigenvalue 1 (1.20) but WI-AX had a greater standardized canonical 2 variable (1.00) for eigenvalue 2.

Cultivar means for WS-AX, WI-AX, and TO-AX are presented in Tables V and VI. Mean separation was conducted using DMRT with a more stringent α set at $P = 0.001$. In all cases, the LSD was roughly one-tenth the mean. Arabinoxylan contents of cultivars

TABLE V
Water-Soluble, Water-Insoluble, and Total Arabinoxylan Contents[a] of Soft White Spring Wheat Flour and Meal

	Flour			Meal		
Cultivar	**WS-AX**	**WI-AX**	**TO-AX**	**WS-AX**	**WI-AX**	**TO-AX**
Alpowa	5.74a	9.22a	14.97a	6.74a	40.70a–c	47.44a
Fielder	4.53b	8.91ab	13.45b	5.28b	41.86ab	47.14a
Nick	4.09c	7.45c	11.54c	5.18b	39.24bc	44.41ab
Alturas	3.77c	7.69c	11.46c	4.83c	42.50ab	47.32a
Zak	3.39d	7.96bc	11.35cd	4.51cd	40.49a–c	45.00ab
Eden	3.19de	7.24c	10.43de	4.22d	42.95a	47.17a
WA7920	2.99e	7.10c	10.09e	4.40d	37.69c	42.09b
$LSD_{(0.001)}$	0.34	1.03	0.97	0.34	3.27	3.28

[a] WS-AX, water-soluble arabinoxylan; WI-AX, water-insoluble arabinoxylan; TO-AX, total arabinoxylan (mg xylose equivalents/g of sample) with Duncan multiple range groupings (mean values followed by the same letter are not significantly different at $P = 0.001$).

TABLE VI
Water-Soluble, Water-Insoluble, and Total Arabinoxylan Contents[a] of Soft White Winter Wheat Flour and Meal

	Flour			Meal		
Genotype	**WS-AX**	**WI-AX**	**TO-AX**	**WS-AX**	**WI-AX**	**TO-AX**
Rely	5.74a	7.18hi	12.92a–f	5.50a	34.93d–g	40.44c–f
Eltan	5.18b	8.37d–g	13.55ab	5.46a	39.12ab	44.58ab
Bruehl	5.11b	8.18e–g	13.29a–c	5.46a	34.33e–g	39.79d–f
ARS00235	5.11b	7.29hi	12.39e–h	5.29ab	32.92g	38.21ef
Coda	4.84c	6.96hi	11.80g–j	5.13a–c	33.81fg	38.93d–f
ARS00226	4.70cd	7.76gh	12.46d–g	5.19a–c	35.26c–g	40.45c–f
ARS96277	4.67cd	8.57c–g	13.24a–d	5.45a	37.00b–f	42.45a–d
Hiller	4.47de	7.19hi	11.66h–j	5.04b–d	32.19g	37.23f
Edwin	4.31ef	6.92i	11.23j	4.66d–g	36.56b–f	41.22b–e
OR990055	4.29ef	8.11fg	12.39e–h	4.86c–f	39.85ab	44.71ab
ID587	4.17fg	9.41ab	13.58ab	5.01b–d	38.56a–c	43.57a–c
ARS00173	3.96gh	7.14hi	11.10j	4.94b–d	34.72d–g	39.66d–f
Masami	3.91h	9.09b–d	13.00a–e	5.01b–d	34.87d–g	39.88d–f
Dune	3.84hi	8.68b–f	12.52c–g	4.90b–e	37.32b–e	42.22a–d
Madsen	3.82hi	9.08b–d	12.89b–f	4.38g	37.64a–e	42.02a–d
Stephens	3.79h–j	9.37a–c	13.16a–e	4.67d–g	37.08b–f	41.75b–d
Finch	3.73h–j	9.97a	13.70a	4.32g	41.06a	45.38a
Simon	3.62ij	8.94b–e	12.56c–g	4.48fg	37.72a–e	42.20a–d
ORCF101	3.55j	8.63b–f	12.18f–i	4.51e–g	39.27ab	43.79a–c
Brundage96	3.23k	8.32d–g	11.55ij	4.47fg	38.04a–d	42.51a–d
$LSD_{(0.001)}$	0.25	0.75	0.72	0.38	3.10	3.18

[a] WS-AX, water-soluble arabinoxylan; WI-AX, water-insoluble arabinoxylan; TO-AX, total arabinoxylan (mg xylose equivalents/g of sample) with Duncan multiple range groupings (mean values followed by the same letter are not significantly different at $P = 0.001$).

were similar to those of previous reports (Hong et al 1989; Saulnier et al 1995).

For spring wheat flours, the WS-AX content was roughly one-third of TO-AX; whereas for meals, the WS-AX content was ≈10–15% of TO-AX. Among the individual soft white spring wheat cultivars, Alpowa had the significantly highest WS-AX content of both flour and meal, the highest WI-AX content of flour, and the highest TO-AX content of flour and meal. Alpowa has been the leading soft white spring wheat cultivar in Washington State for nine years (1997-2005) and was recently grown on ≈100,000 ha (NASS 2005). In 2005, Oregon and Idaho grew over 50,000 ha of Alpowa. At the low end of the arabinoxylan content range were the experimental line WA7290 and the soft white spring club cultivar Eden. Although not grown on significant areas, they exhibited a reduction of ≈45% in flour WS-AX and 35% reduction in meal WS-AX compared with Alpowa.

For winter wheat flours, the WS-AX content was again roughly one-third of TO-AX with 28–44% of TO-AX. For meals, the WS-AX content was similar to that observed in the spring wheat set, ≈10–15% of TO-AX. Among the individual soft white winter wheat cultivars, there was considerable overlap in mean arabinoxylan content. However, there were also highly significant differences among cultivars. Rely club wheat had the highest WS-AX content of both flour and meal, among the highest WI-AX content of flour, but was fairly average in TO-AX content of meal. Finch exhibited the highest flour and meal TO-AX and WI-AX contents of any soft white winter wheat cultivar but had the lowest meal WS-AX and fourth lowest flour WS-AX of any soft white winter wheat cultivar. Brundage 96 was notable in having the lowest flour WS-AX content. Of particular note, Eltan has been the leading soft white winter wheat cultivar in Washington State for the last five years (2001-05) and last year (2005) was grown on about a quarter-million ha (NASS 2005). Consequently, it represents the singularly greatest genetic impact on Pacific Northwest wheat production. It had a high level (5.18 and 5.46 mg xylose equivalents/g) of WS-AX in flour and meal, respectively. The three soft white winter wheat cultivars Eltan, Stephens, and Madsen were grown on over 620,000 ha in Washington, Oregon, and Idaho in 2004-05.

Although only an analysis of the variation due to environments (not the identification or evaluation of specific environments) was the primary aim of this research, some presentation of environment means is useful. Generally, there was some indication of crop year effect. For example, among the spring wheat environments, there was a complete assortment of WS-AX, WI-AX, and TO-AX environment means based on crop year locations; for all three arabinoxylan fractions, the 2004 locations had the lower values. Among the spring wheat flours, there was considerably more overlap of environment means for the two crop years. Among the winter wheat flour and meal samples, there was no distinct separation of the crop year environments. Further analysis was not merited because the crop years were confounded with geographic location for both sample sets; the spring wheat crop years shared only one location (Dayton), whereas the winter wheat crop years shared three locations in common (Lamont, Lind, and Moses Lake). Based on the analysis available, the two Dayton means (2003 vs. 2004) were significantly different according to DMRT at the more stringent $P = 0.001$ level for all arabinoxylan fractions from both flour and meal. Generally, the same was true for the three winter wheat locations common to both crop years. These results suggest that, for a given geographical location, all arabinoxylan fractions of both flour and meal may differ significantly across crop years.

DISCUSSION

Understanding the sources of variation in WS-AX, WI-AX, and TO-AX contents is useful to wheat breeding programs where the aim is to produce wheat of superior and consistent end-use quality. The results of this study show a strong genetic basis for variation for WS-AX, and a relatively weaker genetic basis for variation in TO-AX and WI-AX (Tables I–VI). These results indicate that considerable intervarietal variation exists, even among commercial cultivars, that could be exploited in breeding, production, and utilization.

The results of this study are generally consistent with previous results on the sources of variation among arabinoxylan content in hexaploid wheat. Hong et al (1989) and Saulnier et al (1995) found that wheat cultivar played an important role in WS-AX content. The results from those studies, however, were limited by lack of adequate environmental assessment. In durum wheat, Lempereur et al (1997) concluded that cultivar and environment were both sources of variability for WS-AX and TO-AX in durum wheat fractions. In the present study, soft wheat WS-AX exhibited a high genetic source of variation and a low environmental source of variation.

For the results of this study to be extended, further research is needed to characterize more fully the effects of WS-AX on end product quality. WS-AX clearly has the potential to influence various quality attributes through interactions with water (Jelaca and Hylnka 1971, 1972; Kim and D'Appolonia 1977; Michniewicz et al 1991; Izydorczk and Biliaderis 1992; Yin and Walker 1992; Biliaderis et al 1995; Wang et al 2003). Despite the considerable amount of research on arabinoxylans, there are contradictions as to the impact of arbinoxylans on various food products such as bread (Jeleca and Hlynka 1972; Kim and D'Appolonia 1977; Biliaderis et al 1995; Courtin and Delcour 2002). One end-use quality attribute currently being researched is on the mechanism of syruping in refrigerated doughs. Results show a connection between the degradation of arbinoxylans by xylanases and the amount of dough syruping present (Gys et al 2004; Courtin et al 2005). Currently, very little research has been conducted on the effects of arabinoxylans on soft wheat products. Bettge and Morris (2000) demonstrated that arbinoxylans have a negative effect on sugar-snap cookie diameter by decreasing the diameter of the cookie. Information on the influence of arbinoxylans on other soft wheat flour products such as cakes, crackers, noodles, and pancakes is needed.

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