Distribution of Total, Water-Unextractable, and Water-Extractable Arabinoxylans in Wheat Flour Mill Streams

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ABSTRCT

Arabinoxylans are a minor but important constituent in wheat that affects bread quality, foam stability, batter viscosity, and sugar snap cookie diameter. Therefore, it is important to determine the distribution of arabinoxylans in flour mill streams to better formulate flour blends. Thirty-one genetically pure grain lots representing six wheat classifications common to the western U.S. were milled on a Miag Multomat pilot mill, and 10 flour mill streams were collected from each. A two-way ANOVA indicated that mill streams were a greater source of variation compared to grain lots for total arabinoxylans (TAX), water-unextractable arabinoxylans (WUAX), and water-extractable arabinoxylans (WEAX). TAX and WUAX were highly correlated with ash at $r = 0.94$ and $r = 0.94$, respectively; while the correlation for WEAX and ash decreased in magnitude at $r = 0.60$. However, the 5th middlings mill streams exhibited disparity between TAX and ash content as well as between WUAX and ash content. This may indicate that TAX and WUAX in mill streams are not always the result of bran contamination. Cumulative extraction curves for TAX, WUAX and WEAX revealed increasing gradients of arabinoxylans parallel to extraction rate. Therefore, arabinoxylans may be an indicator of flour refinement.

The biochemical constituents distributed within the mature endosperm and other tissues of wheat (*Triticum aestivum* L.) grain can be determined indirectly by examining their distribution in mill streams. Determining the distribution of beneficial and deleterious constituents in mill streams is important for aspects such as stream blending and assessing the milling quality of wheat. Hinton (1959) determined the distribution of ash in the wheat kernel and found that at <0.6% ash, the flour was composed of mainly pure endosperm. Ash content at >0.6% was the result of bran contamination (Hinton 1959). The effect of bran contamination in mill streams has been well established and negatively correlated with end-use quality. Wheat that mills to low ash and high extraction rates is considered to possess good milling quality; conversely, high ash and low extraction rates are characteristics of wheat with poor milling quality (Robbins 1943; Posner and Hibbs 1997). The distribution of other constituents such as protein (Nelson and McDonald 1977; Sutton and Simmons 2006), enzymes (Kruger 1981; Marsh and Galliard 1986; McCallum and Walker 1990; Every et al 2002; Dornez et al 2006; Every et al 2006a,b), enzyme inhibitors (Gebruers et al 2002), lipids (Morrison and Hargin 1981; Prabhasankar et al 2000), antioxidants (Morrison et al 1982; Engelsen and Hansen 2009), and toxins (Seitz et al 1985) in mill streams has also been established. However, less information is available on the distribution of nonstarch polysaccharides, specifically, arabinoxylans.

The concentration of arabinoxylans varies among wheat cultivars (e.g., Finnie et al 2006; Gebruers et al 2008; Li et al 2009; Gebruers et al 2010), and wheat kernel tissues. Arabinoxylans compose 20–27% of the aleurone (Pomeranz 1988), 23–32% of the bran (Hashimoto et al 1987; Wang et al 2006), and 2–4% of the endosperm (Pomeranz 1988; Stone 2006). Structurally, arabinoxylans consist of a β-1,4 xylose backbone, with arabinose variously substituted at the 2- or 3-carbon position, which results in a random coil conformation with varying degrees of flexibility (Dervilly et al 2000; Courtin and Delcour 2002). Ferulic acid moieties are esterfied at the C (O)-5 positions on some arabinose residues. The substitution pattern of arabinose on the xylose backbone determines, through flexibility and helix formation, the water-extractability of the carbohydrate. Therefore, arabinoxylans can be separated into water-extractable (WE) and water-unextractable (WU) fractions. These fractions are empirically defined and dependent on specific extraction conditions. Nevertheless, they have functional utility and are widely used in the study of arabinoxylans. Generally, WEAX are aqueously extracted from flour or whole grain meal using excess water and gentle mechanical force (stirring) at room temperature (≈20°C).

Water-unextractable arabinoxylans (WUAX) and water-extractable arabinoxylans (WEAX) have different physicochemical properties. WUAX affects the molecular mobility of water (Izydorczyk and Biliaderis 1992; Biliaderis et al 1995), and negatively affects bread quality by binding large amounts of water which prevents proper starch and gluten hydration (Courtin and Delcour 2002). WUAX also physically disrupts the formation of gas cells in bread dough (Courtin and Delcour 2002). Within the WEAX fraction, ferulic acid residues are available for free-radical induced oxidative cross-linking and are partially responsible for variation in batter viscosity and sugar snap cookie diameter (Bettge and Morris 2007).

Therefore, it is of interest to determine the distribution of arabinoxylans in flour streams to better utilize flour stream blending on a product-by-product basis to obtain more functional flour without using additives or further treatments. Previous studies on arabinoxylan distribution in mill streams are incomplete or limited in scope (Loska and Shellenberger 1949; D'Appolonia and MacArthur 1975; MacArthur and D'Appolonia 1977; Ciacco and D'Appolonia 1982; 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). Often, these studies also tended to be more focused on the structural characteristics of arabinoxylans from different mill streams and less on the mechanical fractionation of the wheat kernel during milling, the distribution of arbinoxylans in flour streams, and the relationship of arabinoxylans to other grain constituents.

Currently, there is limited information on the distribution of TAX, WUAX, and WEAX in soft and hard wheat mill streams. Studies have not previously specifically considered larger collections of red and white, winter and spring, hard and soft, and club wheat types provided by genetically pure grain lots. Considering the mill itself, commercial mills, or pilot mills that emulate commercial mills are highly desirable for such studies. Therefore, the

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purpose of this research was to determine the distribution of TAX, WUAX and WEAX in ten Miag Multomat pilot mill flour streams from 31 genetically pure grain lots representing six major wheat types currently bred and grown in the western U.S.

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 the 2009 crop year were obtained from the U.S. Department of Agriculture, Agriculture Research Service, Western Wheat Quality Laboratory in Pullman, WA, and were included in the Pacific Northwest Wheat Quality Council Collaborative Testing Program or the U.S. Wheat Associates Overseas Varietal Analysis Program. The cultivars represented six classifications of wheat: Bitterroot, Brundage96 (two samples), Eltan, ID98-19010A, KWP006, Skiles, Stephens (four samples), ORCF-102, OR2040-726, OR2060395, and Xerpha are soft white winter; Alturus, BZ604-002, BZ6M06-1001, Cataldo, Diva, IDO599, IDO644, and Louise are soft white spring; Cara is a soft white winter club; Darwin, KW7003, Silver, and IDO660 are hard white winter; BC002-2 and Paladin are hard red winter; and SJ908-203W is a hard white spring.

Milling

The 31 samples were milled on a Miag Multomat pilot mill (Fig. 1) (Posner and Hibbs 1997). Milling produced 10 flour streams and four feed streams; only the flour streams were collected and analyzed for this study, resulting in 310 unique flour 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). Straight-grade (straight run) flour from the Miag Multomat is produced by blending all 10 flour streams together. The desired extraction rate to produce straight-grade flour is ≈75–78%. The average extraction rate for all cultivars in this study was 76.1%.

Proximate Analysis

The 310 flour stream samples were analyzed in duplicate for protein and ash. Moisture and ash were measured in a thermogravimetric oven (TGA-601 Leco, St. Joseph, MI). Protein was de-

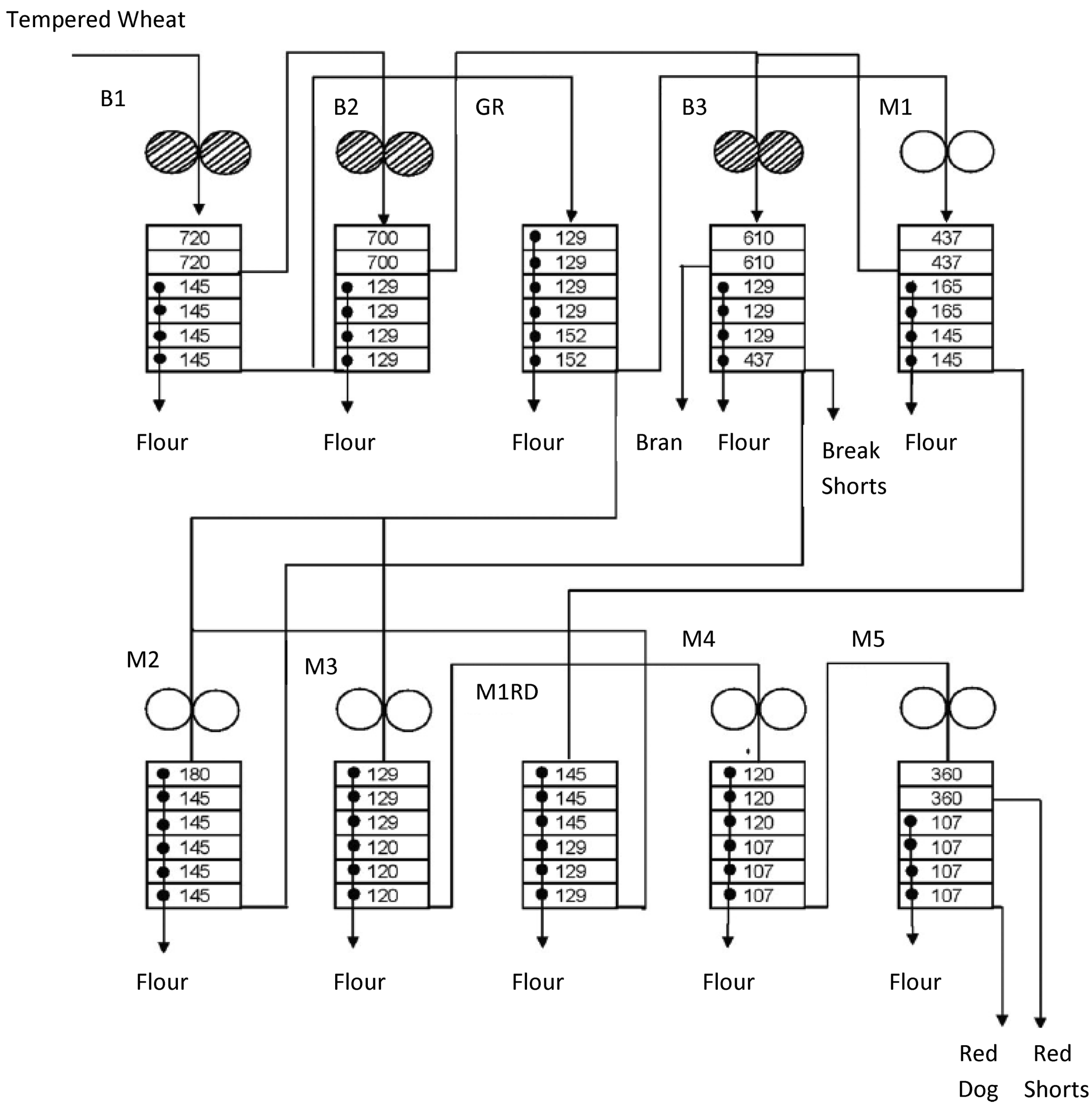

Fig. 1. Flow diagram for the Miag Multomat pilot mill; numbers represent sieve cloth openings in microns. B1, first break; B2, second break; GR, grader; B3, third break; M1, first middlings; M1RD, first middlings redust; M2, second middlings; M3, third middlings; M4, fourth middlings; and M5, fifth middlings.

termined 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 the SKCS 4100 (Perten Instruments, Springfield, IL).

Arabinoxylan Determination

Flour streams were analyzed for arabinoxylan content. A colorimetric method described by Douglas (1981) that measures pentose sugar content in wheat flour after hydrolysis was modified to measure both TAX and WEAX from wheat flour (Finnie et al 2006). Replicate flour samples (125 mg) were weighed into 50-mL conical bottom screw-cap polypropylene tubes (Fischer Scientific, Pittsburgh, PA or equivalent). The samples were hydrated with 25 mL of water and suspended by vortexing. Duplicate 1-mL aliquots from each replicate (each aliquot contained 5 mg of suspended flour) were removed immediately after vortexing and transferred into a 15-mL screw cap reaction tube (Pyrex tube, 16 × 100 mm; cap with PTFE liner). The total volume in each tube was brought to 2 mL with water. These aliquots were used to determine TAX content. The original sample suspension was then placed on a laboratory rocker (model AR-100, PGC Scientific, Gaithersburg, MD) for 30 min at room temperature (21°C) and then centrifuged at 2,500 × *g* for 10 min. Duplicate 1-mL supernatant aliquots from each replicate were transferred into 15-mL reaction tubes with an additional 1 mL of water. These aliquots were used to determine WEAX content. Reaction reagent (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, and returned to room temperature. Absorbance of the samples was read at 552 and 510 nm. The absorbance reading at 510 nm was subtracted from 552 nm to mathematically remove the influence of hexose sugars. A standard curve was produced using a stock solution of 100 mg of D-(+)-xylose (X-1500, Sigma) in 1 L of water. Triplicate standard samples were prepared for each standard representing 0.0, 0.05, 0.10, 0.15, and 0.20 mg of xylose, respectively, and subjected to the same procedures as the flour samples. TAX and WEAX content were calculated as mg xylose equivalents after conversion using the xylose standard curve and expressed as mg xylose equivalents (Douglas 1981). WUAX was calculated as the difference of TAX and WEAX.

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 calculating Pearson correlation coefficients. Figures and graphs were produced using SlideWrite Plus (v 7.0, Advanced Graphics Software, Encinitas, CA). A linear curve fit was used for correlation coefficients. The spline curve fit was utilized for the cumulative extraction curves.

RESULTS

Analysis of Variance

Sources of variation for total arabinoxylans (TAX), water-unextractable arabinoxylans (WUAX), water-extractable arabinoxylans (WEAX), protein and ash content are summarized in Table I. The two-way ANOVA models were robust for each response variable in that 98–99% of the total variation (R^2) was explained. Both main effects (flour stream and cultivar) were significant sources of variation ($P < 0.0001$) for TAX, WUAX, WEAX, protein, and ash. Interactions between the main effects were also significant, but the contributions of the interaction to the overall ANOVA models were minor compared to the main effects. For arabinoxylan fractions, the interaction *F*-values were 64–103 times smaller than that of the most significant main effect (Table I), and for protein and ash, 66- and 193-fold smaller, respectively. 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 by far the greatest source of variation for all five flour constituents (TAX, WUAX, WEAX, protein and ash) although differences among cultivars (grain lots) were also important (Table I). Among the arabinoxylan fractions, TAX and WUAX were more highly influenced by mill stream compared to WEAX. The cultivar effects were similar among TAX, WUAX, and WEAX. A similar pattern was observed for protein and ash: flour streams contributed by far the most variation, followed by a significant contribution from cultivars.

Correlations among Mill Stream Arabinoxylan Content and Kernel Hardness

Because hard and soft wheat cultivars possess different milling characteristics due to endosperm texture, correlations were calculated among all five flour constituents and the kernel texture (SKCS) of the parent grain lots for each of the flour fractions (i.e., across cultivars). TAX was significantly negatively correlated ($P < 0.05$) with kernel hardness in mill streams M3 ($r = –0.43$), M4 ($r = –0.49$), and M5 ($r = –0.47$) indicating that in these three flour streams, hard wheats tended to have lower TAX contents compared to soft. WUAX and kernel hardness were significantly correlated ($P < 0.05$) in mill streams B1 ($r = 0.40$), M3 ($r = –0.60$), M4 ($r = –0.51$), and M5 ($r = –0.48$). Hard wheat cultivars tended to have higher WUAX in the first flour producing stream (B1), and less in the 3rd, 4th, and 5th Middlings which are near the tail end of the mill. WEAX was not significantly correlated with kernel hardness in any of the mill streams at $P < 0.05$, suggesting independence for both texture per se and market class characteristics.

Although the parent grain lots of the hard wheat cultivars had on average higher protein content (11.0–14.9%) than soft wheats (8.4–12.1%), flour stream was a much greater source of variation for flour stream protein content than was cultivar (Table I). Examination of the results based on kernel texture showed that hard wheat cultivars tended to have a higher concentration of protein in mill streams B1, B2, GR, B3, M1, M1RD, and M2 (earlier in the break and reduction process). The correlations between protein and SKCS hardness were significant for mill streams B1 ($r = 0.85$), B2 ($r = 0.82$), GR ($r = 0.82$), B3 ($r = 0.76$), M1 ($r = 0.53$), M1RD ($r = 0.66$), and M2 ($r = 0.65$). Mill streams M3 and M4 had nonsignificant correlations between kernel texture and protein content at $P < 0.05$. Some hard wheat cultivars had a lower protein content in flour stream M5 than did the soft cultivars. Indeed, flour stream M5 had a negative correlation between kernel texture and protein content ($r = –0.44$).

TABLE I
Sources of Variation for Total Arabinoxylans, Water-Unextractable Arabinoxylans, Water-Extractable Arabinoxylans, Protein and Ash Among Mill Streams from 31 Varietal Grain Lots[a]

	df[b]	TAX[c]	WUAX[d]	WEAX[e]	Protein	Ash
Whole Model R^2	309	0.99	0.99	0.98	0.99	0.99
Flour Stream *F*-Value	9	4751	4181	898	9294	7755
Variety *F*-Value	30	140	130	171	1265	90
Interaction *F*-Value	270	46	44	14	140	40

[a] All *F*-values are significant at $P < 0.0001$.
[b] Degrees of freedom.
[c] Total arabinoxylans.
[d] Water-unextractable arabinoxylans.
[e] Water-extractable arabinoxylans.

Ash content of mill streams and cultivars followed a similar trend to protein content (Table I). The cultivar effect for ash was much less than the stream effect. Kernel hardness was positively correlated with ash content in streams B1 (r = 0.79), B2 (r = 0.64), GR (r = 0.68), B3 (r = 0.43), M1 (r = 0.46), M1RD (r = 0.51), and M2 (r = 0.49) (i.e., harder texture, higher ash). Conversely, in mill streams M3, M4, and M5, hard wheat cultivars had lower ash concentration than soft wheat (M3, r = –0.69; M4, r = –0.61; and M5, r = –0.51).

Distribution of Constituents in Mill Streams

The distribution of TAX, WUAX, WEAX, protein, and ash in flour streams (across varietal grain lots) is summarized in Table II. The mill streams are arranged in the order in which they were collected off the mill (Fig. 1). Protein and ash range in mill streams was similar to those previously reported and not the focus of further discussion (Nelson and McDonald 1997; Wang et al 2006).

Among the different mill streams, mean TAX, WUAX, and WEAX contents were 0.99–4.47%, 0.54–3.70%, and 0.43–0.77% (14% moisture basis), respectively, across cultivars. The range for TAX and WEAX were similar to those previously reported (Wang et al 2006).

The variation in TAX and WUAX content increased in the reduction streams, especially M4 (TAX mean 3.07%, range 1.68–5.80%; WUAX mean 2.39%, range 1.15–5.05%), and M5 (TAX mean 4.47%, range 2.39–7.21%; WUAX mean 3.70, range 1.77–6.86%) (Table II). The order of decreasing TAX content in streams was M5 (highest concentration), M4, M3, B3, M2, B2, B1, GR, M1RD, and M1 (lowest concentration). Flour streams B1, B2, and GR were not significantly different from each other at $P < 0.05$. GR and M1RD were also not significantly different at $P < 0.05$. WUAX content deceased in the order M5 (highest concentration), M4, M3, B3, B2, M2, B1, GR, M1RD, and M1 (lowest concentration). Flour streams B1, GR, and M2 were not significantly different from each other. Streams M1 and M1RD also were not significantly different ($P < 0.05$).

The M3, M4, and M5 flour mill streams, which are all at the tail end of the mill, contained the highest content for TAX, WUAX, and WEAX and followed in the same rank order. For the remaining flour streams, TAX and WUAX contents ranked the streams in essentially the same order. WEAX content, however, did not follow this pattern (Table II). The order for mill streams ranked by WEAX content was M5 (highest concentration), M4, M3, M2, M1RD, M1, GR, B1, B2, and B3 (lowest concentration). Most noticeably, mill streams B2 and B3 were in the upper half of mill streams based on TAX and WUAX contents, but in terms of WEAX content, these two break streams contained the least amount. M1 and M1RD contained the lowest concentrations of TAX and WUAX but had considerably higher concentrations of WEAX compared to the other mill streams. Flour streams GR and M1 were not significantly different from each other for WEAX content. B1 and GR also were not significantly different for WEAX content ($P < 0.05$) (Table II).

Correlations Among Arabinoxylan Fractions, Ash, and Protein Content

Across all flour streams and cultivars (n = 310), TAX content was highly correlated with ash content (r = 0.94) (Fig. 2A and Table III). TAX content was also highly correlated with WUAX content (r = 0.99). TAX content was less highly correlated with WEAX (r = 0.70) and protein (r = 0.68). WUAX also had a positive correlation with ash content (r = 0.94) (Fig. 2B and Table III). These results are consistent with the high correlation between TAX and WUAX (r = 0.99). Consistent with this, WUAX content was less highly correlated with WEAX (r = 0.62) and protein (r = 0.68). WEAX was considerably less well correlated with ash (r = 0.60) (Fig. 2C and Table III) and was only moderately correlated with protein content (r = 0.45). Clearly, TAX and WUAX exhibited similar relationships with other flour stream constituents. The distribution of data points for Fig. 2A and B showing TAX and WUAX versus ash exhibited similar trends. A large proportion of the samples are tightly clustered at <0.6% ash. At >0.6% ash, the samples are less tightly clustered, forming a defuse scatter and are primarily from mill streams B3, M3, M4, and M5. The relationship between TAX and ash as well as WUAX and ash was less homogenous at >0.6% ash. The increasing variation of TAX and WUAX as ash content increased is different from the WEAX-ash relationship (Fig. 2C).

TABLE III
Correlation Coefficients for Total Arabinoxylans, Water-Unextractable Arabinoxylans, Water-Extractable Arabinoxylans, Protein, and Ash Among 10 Mill Streams from Each of 31 Varietal Grain Lots[a]

	WUAX[b]	WEAX[c]	Ash	Protein
TAX[d]	0.99	0.70	0.94	0.68
WUAX		0.62	0.94	0.68
WEAX			0.60	0.45
Ash				0.75

[a] All correlations are significant at $P < 0.0001$.
[b] Water-unextractable arabinoxylans.
[c] Water-extractable arabinoxylans.
[d] Total arabinoxylans.

TABLE II
Distribution of Total Arabinoxylans, Water-Unextractable Arabinoxylans, Water-Extractable Arabinoxylans, Protein, and Ash in Flour Mill Streams[a]

Mill	TAX (%)[c]		WUAX (%)[d]		WEAX (%)[e]		Protein (%)		Ash (%)	
Streams[b]	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
B1	1.11f	0.84–1.67	0.68f	0.38–1.21	0.43g	0.28–0.78	8.21i	5.01–14.55	0.35g	0.24–0.52
B2	1.13f	0.85–1.76	0.74e	0.56–1.12	0.39h	0.25–0.72	10.35e	7.02–16.77	0.39f	0.29–0.54
GR	1.10fg	0.87–1.64	0.66f	0.43–0.99	0.44fg	0.29–0.75	9.88f	6.69–14.37	0.42e	0.28–0.61
B3	1.28d	0.58–1.74	0.91d	0.19–1.23	0.37i	0.25–0.62	11.68c	7.52–18.79	0.68d	0.53–0.96
M1	0.99h	0.73–1.67	0.54g	0.31–0.95	0.44f	0.26–0.89	8.41h	6.46–12.31	0.33h	0.25–0.43
M1RD	1.05g	0.73–1.54	0.57g	0.27–0.90	0.48e	0.32–0.87	9.31g	6.62–15.44	0.36g	0.31–0.46
M2	1.21e	0.72–1.89	0.69f	0.34–1.05	0.51d	0.35–0.91	9.35g	6.94–12.17	0.36g	0.29–0.48
M3	1.71c	1.22–2.61	1.16c	0.68–1.84	0.55c	0.37–1.08	10.49d	7.22–13.07	0.72c	0.46–1.17
M4	3.07b	1.68–5.80	2.39b	1.15–5.05	0.68b	0.45–1.08	13.45b	9.76–18.66	1.62b	0.70–2.71
M5	4.47a	2.39–7.21	3.70a	1.77–6.86	0.77a	0.53–1.19	15.52a	6.04–19.11	2.50a	1.17–3.41

[a] Mean values followed by the same letter in the columns are not significantly different at $P < 0.05$. Range corresponds to the two most extreme varietal grain lots for that mill stream.
[b] 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, and M5, fifth middlings.
[c] Total arabinoxylans.
[d] Water-unextractable arabinoxylans.
[e] Water-extractable arabinoxylans.

Examination of Fig. 2C revealed that there was considerable scatter about the least squares fitted line for WEAX and ash at any given ash content; there was a considerable range in WEAX. Also, by the nature of the milling process, there were a large number of individual samples clustered at <0.6% ash. However, there was large variation, almost a threefold difference, in WEAX content within mill streams B1, B2, GR, M1, M1RD, and M2 at <0.6%. This result is different than that for TAX and WUAX content where at <0.6% ash there was considerably less variation among mill streams. The higher ash mill streams (B3, M3, M4, and M5) also had substantial scatter around the least squares fitted line in relationship to WEAX content. The greater variation for WEAX content resulted in a reduced correlation coefficient (Table III).

TAX and WUAX in 5th Middlings

It was apparent that mill streams M4 and M5 were more variable in terms of TAX, WUAX and WEAX content when compared to ash content. In 5th middlings flour stream (M5), wheat cultivars separated into two distinct groups when comparing TAX and ash (Fig. 3A) as well as WUAX and ash (Fig. 3B). One group clustered at ≈3% TAX and spanned 1–3% ash (Fig. 3A). The other group centered at ≈3% ash but spanned ≈5–8% TAX. One soft wheat cultivar (BZ6M06-1001) did not associate with either grouping. Six of the seven hard wheat cultivars were associated with the grouping at ≈3% TAX (Paladin was outside the group). Similar groupings were present for WUAX. One grouping was at 2–3%WUAX and 1–3% ash (Fig. 3B). The other group was at ≈3% ash and 4–7% WUAX. Six of the seven hard wheat cultivars were associated with the groupings at ≈2–3% WUAX (again Paladin was outside the group). These results clearly show that ash and arabinoxylan contents are, at times, largely independent phenomena in these streams.

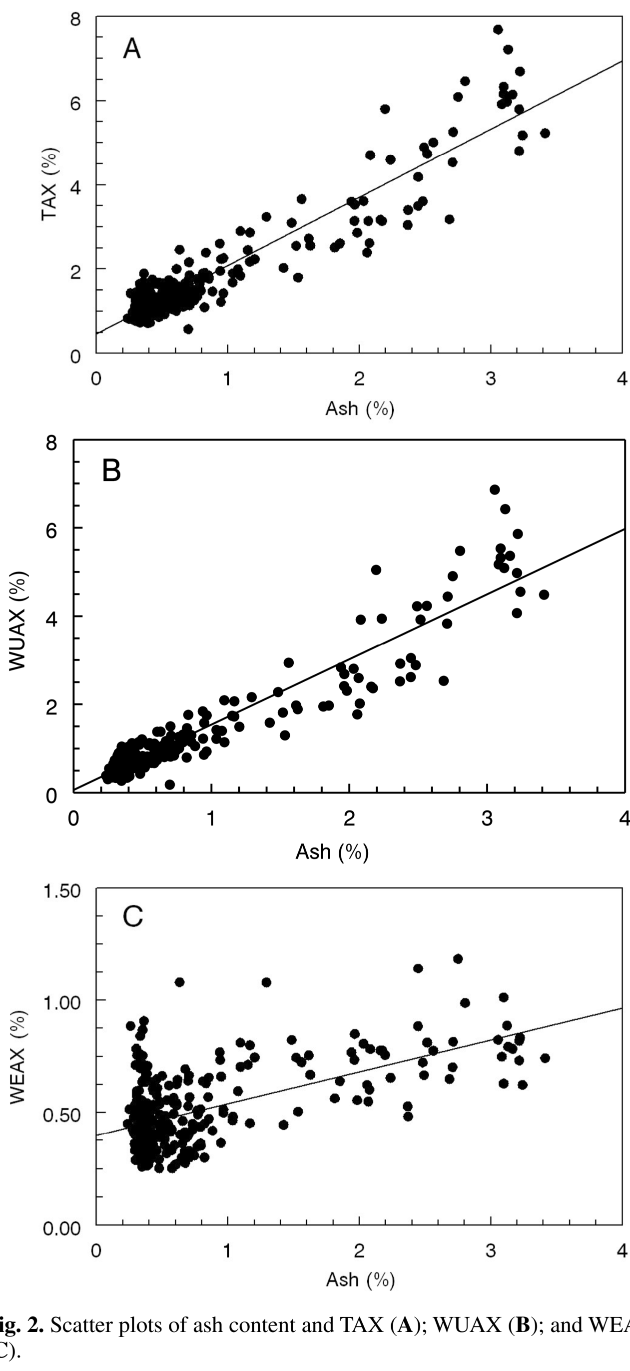

Fig. 2. Scatter plots of ash content and TAX (**A**); WUAX (**B**); and WEAX (**C**).

Cumulative Extraction Curves

Correlation analysis was conducted to examine further the possible relationship between TAX and WUAX contents in each M5 grouping (Fig. 3A and B) with other flour and grain constituents in an attempt to understand or identify causes of these groupings. The grain and flour constituents were M5 protein and ash contents; grain (parent grain lot) kernel hardness, protein, TAX, WUAX, WEAX, and ash; straight-grade flour protein, ash, TAX, WUAX, and WEAX. This analysis produced only one significant correlation. Among the samples at <3% WUAX grouping (Fig. 3B), there was a positive correlation between M5 WUAX and WEAX at $r = 0.50$ ($P < 0.05$). The other groupings in Fig. 3A and B produced no significant correlations with any of the preceding variables ($P > 0.10$).

Cumulative flour extraction (yield) curves were constructed to better understand the distribution of TAX, WUAX, WEAX, and

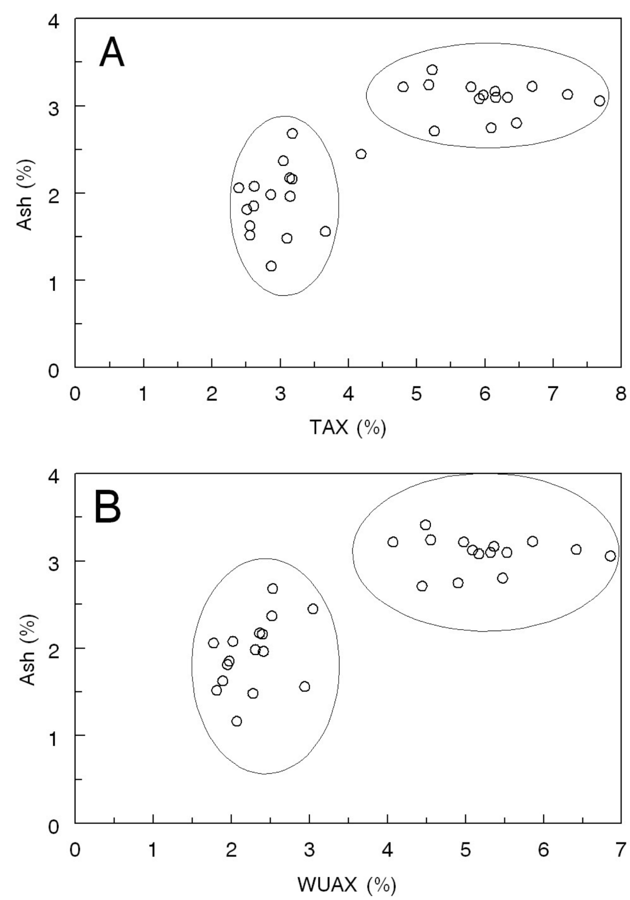

Fig. 3. Scatter plots of flour mill stream M5 groupings for TAX and ash (**A**); and WUAX and ash (**B**).

ash among flour streams, and their relationship to the milling process. Cumulative ash curves are a common means of evaluating milling performance (Hinton 1959; Posner and Hibbs 1997). Cumulative extraction curves for TAX, WUAX, WEAX, and ash were constructed by ordering all 10 flour streams according to the constituent of interest. For example, for TAX (Fig. 4A), flour streams were ordered from lowest TAX content (M1) to highest (M5). The abscissa represents the cumulative flour extraction as a percentage of total products, reaching 76.1% (grand mean over all 31 varietal grain lots). The ordinate represents the actual calculated TAX content at any given point along the cumulative curve, for example the last point at 76.1% flour yield contains ≈1.26% TAX.

The ash curve was typical of what is observed using the Miag pilot mill (Fig. 4D). First Middlings and 1st Break are often the lowest ash flour streams followed by 1st Middlings redust, 2nd Middlings, and 2nd Break. The line rises at some moderate slope reflective of increasing ash, generally interpreted as coinciding with increasing bran contamination. Nearly all cumulative ash curves exhibit an inflection point where the line curves dramatically upward. This inflection point is generally construed as the point at which the recovery of more pure starchy endosperm (and at low ash content) shifts to increasing fragmentation of bran, hence greater inclusion of bran in the flour streams and marked increase in ash content. These high ash streams are commonly the 3rd Break and the 3rd, 4th, and 5th Middlings. In the final stream (M5), very little additional flour is produced (0.8% yield), whereas the overall ash content increased 0.2%. Naturally this is due to the fact that the M5 stream alone has an ash content of 2.50%. For comparison, the reduction shorts feed stream contained an average of 3.65% ash and is nearly devoid of endosperm (data not shown).

The curve for TAX (Fig. 4A) followed a very similar trend to that of ash (Fig. 4D). First Middlings was lowest in TAX as well as ash. The last four streams ranked for increasing ash (B3, M3, M4, and M5) were also the highest TAX streams. However, some disparity in rank order was apparent. The low yielding stream Grader (GR) exhibited the largest shift in rank appearing at the inflection point for ash but having the third lowest TAX content.

WUAX (Fig. 4B) followed a similar trend as ash and TAX. A notable feature of this curve was the considerable increase in WUAX between the two lowest streams (M1 and M1RD) and 2nd Middlings. The WUAX rank order was most similar to TAX; the difference being that mill streams M2 and B2 switched places in the rank order. WUAX did not exhibit the distinctive two-part curve characteristic of the ash curve. Indeed, if M4 and M5 were not included, a linear fit for the remaining WUAX curve was $r = 0.98$, $P < 0.05$.

The WEAX (Fig. 4C) cumulative curve differed the most compared to ash, and was distinctly different from the cumulative TAX and WUAX curves. The WEAX curve was the most linear with little indication of an inflection point. With the exception of M3, M4, and M5 at the upper limit of the curve, the other six flour streams (all ash contents <0.68%) showed little rank relationship to ash. In this regard, 3rd Break was the most notable in that it had distinctly the lowest WEAX content but was third highest in ash and fourth highest in TAX. Conversely, 2nd Middlings was a high yielding flour stream of relatively low ash, but was near the inflection point for TAX and was near the highest stream in terms of WEAX.

DISCUSSION

Effective flour milling requires a detailed understanding of wheat kernel morphology, histology, and composition. In this regard, understanding the distribution of different types of arabinoxylans (TAX, WUAX, and WEAX) and protein and ash in flour mill streams is important for flour blending and assessment of flour refinement. TAX, WUAX, WEAX, protein, and ash content all varied dramatically among flour mill streams, more so than indi-

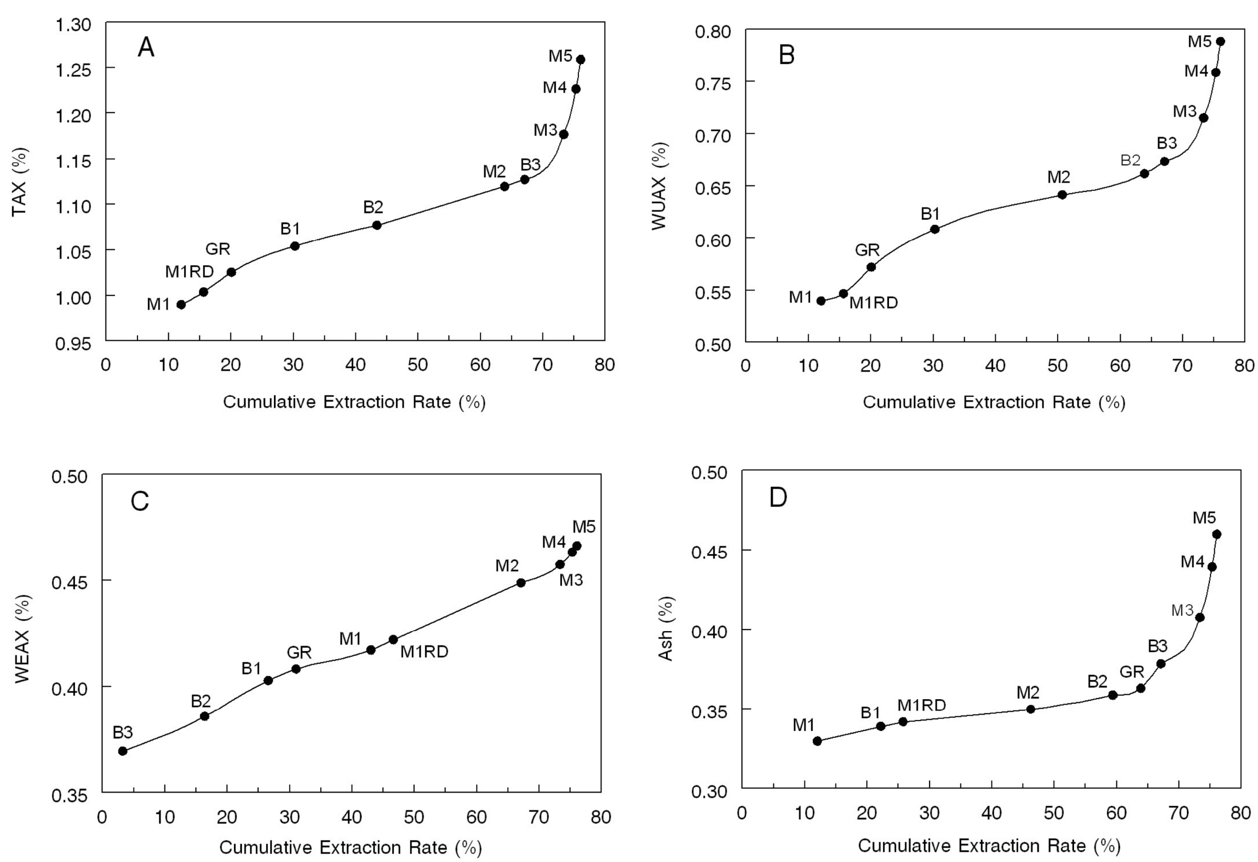

Fig. 4. Cumulative extraction rate and TAX (**A**); WUAX (**B**); WEAX (**C**); and ash (**D**).

vidual cultivars (Tables I and II, Figs. 2–4). This result is notable in that our study included a relatively large number of pure varietal samples and encompassed hard and soft, white and red, spring, winter, and club wheat types. Our results included only flour streams (Fig. 1). Including the high bran content feed streams would have produced even greater compositional differences (Hinton 1959; Posner and Hibbs 1997). For this reason, traditionally, roller milling has been viewed as a mechanical process that aims to separate as cleanly as possible the starchy endosperm from the bran and germ (Posner and Hibbs 1997). To evaluate the success of the milling process, ash content of mill streams has been used as a proxy for bran contamination. The basis for this stems from the distinctly high concentration of ash in tissues with bran (Hinton 1959). However, it is widely accepted that ash, which is simply noncombustible mineral content, likely has a trivial direct effect on flour quality and baking performance. Therefore, understanding wheat grain composition from a functional standpoint is desirable. At times, arabinoxylans exert strong influences on flour and baking performance. In the present study, we observed that ash content and TAX content were well correlated and likely both reflect the content of bran in flour streams in general. WEAX, on the other hand, which can exert a significant functional effect on flour and baking performance (Jelaca and Hlynca 1971; Bettge and Morris 2000; Courtin and Delcour 2002; Bettge and Morris 2007), showed a much more limited relationship to ash.

Bran is, depending on the definition, all of the tissues lying outside the starchy endosperm and exclusive of the germ. These tissues are by no means uniform in their structure or composition, and include the aleurone, nucellus, testa, tube cells, cross cells, thin walled cells, hypodermis, and outer epidermis (Hinton 1959; Lersten 1987). The increased concentration of TAX and WUAX in flour mill streams M3, M4, and M5 is likely a reflection of the milling process and represents increased content of bran. More specifically, the higher content of TAX and WUAX in these streams may be partially attributed to an increased amount of arabinoxylan-rich aleurone (Hashimoto et al 1987; Wang et al 2006). Similarly, aleurone contains a large proportion of the total kernel ash (Hinton 1959; Pomeranz 1988). Due to these compositional relationships, it was not surprising to find that TAX and WUAX were both highly correlated with ash (Table III). The correlation coefficient for WEAX and ash was considerably less than that for TAX-ash and WUAX-ash. Therefore, this disparity relative to arabinoxylan fraction must reflect a fundamental difference in the occurrence and distribution of WEAX in the wheat kernel. Although no histology was conducted in our study, WEAX are not covalently bound to cell walls like WUAX and instead are linked weakly to the surface of cell walls (Mares and Stone 1973a). Nevertheless, our results clearly indicate that flour streams that have higher concentrations of ash (and bran) are generally not the same streams that have higher concentrations of WEAX (e.g., streams M1 and B3) (Table II, Fig. 4).

Delcour et al (1999) reported a general positive correlation between TAX and ash, and WEAX and ash for one European cultivar. Their dataset included the entire mill output of 19 individual streams, including the feed streams. Nine streams had >0.6% ash (Delcour et al 1999). However, for both arabinoxylan fractions, there was no significant correlation among the 10 streams with <0.6% ash. In our study, which included only flour streams (Fig. 1), we observed a highly significant correlation between TAX and ash, as well as WUAX and ash (Fig. 2A and B, Table III). This correlation appeared to hold true for TAX and WUAX throughout the entire set of flour streams (Fig. 2A and B, 4A and B, Table III). Factors that may relate to this observation include 1) WEAX are associated with membranes in the endosperm (Mares and Stone 1973a; Bettge and Morris 2000) and 2) even pure starchy endosperm contains ≈20–26% of the total proportion of kernel ash (Hinton 1959). While it is undoubtedly generally true that bran contamination in flour mill streams is correlated with ash content (Kim and Flores 1999) and that ash content is also correlated with TAX and WUAX content (Delcour et al 1999; Wang et al 2006), this study indicates that in some higher ash flour streams (e.g., 5th Middlings) TAX content and WUAX content are not directly associated with the conventional view of bran contamination. Based on the nature of the milling process, reduction stream M5 would be assumed to contain the highest amount of bran contamination of the flour streams included here. M5 certainly contained the highest concentrations of TAX, WUAX, and ash. However, the unique result presented in Fig. 3 indicates that the pure varietal samples included here sorted into two very distinct groups, and this supports a conclusion that the composition of this stream bore little connection between arabinoxylan and ash, and therefore by extension, bran. However, we would hasten to acknowledge, as mentioned above, that bran is not a uniform material (in this regard, neither is endosperm).

The cumulative extraction rate curves (Fig. 4A, B, and C) supported further the conclusion that WUAX and WEAX derive from different tissues of the wheat kernel. With the exception of the highest ash B3, M3, M4, and M5 streams, the ordering of streams based on ash and WUAX versus WEAX were notably different, as was the shape of the curves. These results are not immediately reconcilable with those of Delcour et al (1999) and Nyman et al (1984). Results of Delcour et al (1999) (their Fig. 5) indicated that when streams were plotted based on cumulative flour extraction rate, that ash, total pentosan, and WEAX followed a similar order ≤70–80% extraction. The scaling of the data may obscure more subtle features. Nyman et al (1984) found that soluble fiber content was independent of extraction rate for a commercial hard winter wheat from Denmark, whereas we observed a near linear increasing gradient for WEAX throughout the cumulative curve (Fig. 4C).

The cumulative extraction curves for TAX and WUAX were similar to the cumulative ash curve, each with a distinct inflection point. Therefore, TAX and WUAX, like ash, may be indicators of flour refinement. Wang et al (2006) came to a similar conclusion based on TAX distribution in reduction and feed streams.

In conclusion, dramatic differences exist among the flour mill streams for TAX, WUAX, and WEAX content. Contrary to previous studies, TAX and WUAX contents were highly correlated with ash content in the flour streams. However, the presence of TAX, WUAX and ash may reflect subtle differences in the specific tissues of bran and, as such, may suggest differences in flour functionality. This will require further study. An increasing gradient of TAX, WUAX, and WEAX was observed as flour extraction rate increased, but ordering of flour streams was different for WEAX compared to TAX, WUAX, and ash. Therefore, flour milling and flour stream blending may benefit from a greater understanding of the origin of different arabinoxylan fractions and their relationship to flour refinement. Ultimately, a greater understanding of the role of arabinoxylans in end product quality will aid the milling process.

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