Relationships Among Grain Hardness, Pentosan Fractions, and End-Use Quality of Wheat

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ABSTRACT

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Grain texture (hardness) in wheat (*Triticum aestivum* L.) is a major determinant of end-usage. Variation in grain texture can be conceptually assigned to the two major hardness classes that result from the action of one major gene (*Hardness*) or to as-yet undetermined factors contributing to residual variation within hardness classes. Identifying the physicochemical basis of both sources of texture variation could provide a means of better controlling or manipulating this quality trait. Pursuant to this objective, the role of pentosans was examined. Pentosan fractions (membrane-associated, total, and soluble) were isolated from 13 hard and 13 soft wheat samples and their flours. Among the hard wheat samples, pentosans had a minimal role in modifying grain hardness. However, among the soft wheat samples, pentosans appeared to have a significant hardness-modifying effect that carried over into end-use quality. Among the soft wheat samples, pentosan fractions, along with wheat protein, accounted for 53–76% of the variation in grain texture, depending on the method used to quantify texture. Membrane-associated pentosans were the most influential single parameter in modeling grain texture for the soft wheat samples. Membrane-associated pentosans were most influential in accounting for variation (69%) in alkaline water retention capacity. Total pentosans, together with flour protein, accounted for 87% of the variation in cookie diameter for soft wheat samples, with the total pentosan fraction being the more influential.

The texture of wheat (*Triticum aestivum* L.) grain, more frequently referred to as hardness or softness, has a profound influence on milling, baking, and end-use functionality The genetic basis for the two major texture classes (hard and soft) is well established as a single major gene (Symes 1965) whose allelic expression is associated with mutations in the proteins puroindoline a and b (Giroux and Morris 1997, 1998; Lillemo and Morris, *in press*). Loss of puroindoline a (null mutation) or point mutations in puroindoline b confer hard phenotype (*Hardness* locus where *ha* = hard allele and *Ha* = soft allele). Puroindolines are members of a broader family of lipid-binding proteins and are associated with polar lipids and endosperm membranes (Marion et al 1994, Greenblatt et al 1995, Dubreil et al 1998). Even though the effect of *Hardness* is relatively large, substantial variation in texture exists within each of the two major classes. Consequently, other factors or components probably modify or add to the contribution of puroindolines to endosperm texture and the concomitant effects of endosperm texture on end-use quality. Although this model is supported by extensive data, a major issue remains unresolved: what is the basis of the texture variation resulting from the action of the *Hardness* gene, that is, the two major texture classes and the texture variation within the major classes?

Glenn and Saunders (1990) demonstrated that intracellular space exists around the starch granules of soft, but not of hard wheat, forming a discontinuity in the starch-protein matrix. This physical discontinuity provides a natural path for shearing forces during kernel disruption, leading to softer material that is more easily reduced in particle size.

Simmonds et al (1973) indicated that the degree of adhesion between starch granules and the protein matrix surrounding the starch granules within the wheat endosperm is the most likely explanation for variation in grain texture. This model suggests a physical description of texture variation without providing a chemical basis for the starch-protein adhesion. Barlow et al (1973) reached essentially the same conclusion: that the degree of adhesion between starch granules and endosperm protein matrix affects texture. The two studies further concluded that water-soluble material acts as cement between starch granules and storage protein, across the amyloplast membrane interface.

Further studies of components at the starch-protein matrix interface have demonstrated that considerable differences exist in the lipids associated with water-washed wheat starch granules isolated from hard and soft wheat (Greenblatt et al 1995). About tenfold more polar lipids (glyco- and phospho-lipids) are associated with the surface of starch granules isolated from soft wheat endosperm compared with starch isolated from hard wheat endosperm. Furthermore, these lipids are associated with puroindolines and are necessary for puroindoline-starch interaction. These polar lipids are similar in composition to those of intracellular membranes and are probably the remnants of the amyloplast membrane. The interaction between starch granules and amyloplast membranes is different between hard and soft wheats and this difference may be part of the physical manifestation of the *Hardness* gene (Barlow et al 1973). Modification of physical or chemical properties of the amyloplast membrane by puroindolines or other compounds could also modify endosperm texture.

One such class of compounds that have the potential to interact with amyloplast membranes, the nonstarch carbohydrates, known collectively as pentosans (primarily arabinoxylans or arabinogalactans), has been studied over the years as contributors to both end-use functionality in the form of water-binding constituents of flour (Jelaca and Hlynka 1971) and in relation to endosperm texture (Hong et al 1989, Kavitha and Chandrashekar 1992). Total pentosans are usually divided into water-soluble or water-insoluble fractions. Water-insoluble pentosans are typically associated with cell walls, whereas water-soluble pentosans are found interior to endosperm cell walls, probably in association with membranes (Mares and Stone 1973). This association of pentosans with amyloplast membranes may serve to modify starch-protein matrix adhesion and therefore have an effect on texture.

Hong et al (1989) found a positive correlation (r = 0.58) between the amount of water-soluble pentosans and endosperm texture (near-infrared reflectance [NIR] hardness). The correlation was reduced when hard and soft wheats were examined as individual classes instead of being pooled. There were indications, however, that intraclass texture variation could be partly attributed to water-soluble pentosans. This research examines the relationship among three pentosans fractions (membrane-associated, total, and water-soluble), grain texture, hydration effects as measured by alkaline water retention capacity (AWRC), and end-use quality, as reflected by cookie baking quality.

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MATERIALS AND METHODS

A total of 26 wheat samples representing 20 different commercial cultivars were obtained for use in this study. Samples were derived from field plots grown at Pullman, WA, in crop years 1992–1996. The samples were selected a priori to encompass a range of potential end-use quality with an equal number of hard and soft wheats as summarized in Table I. Environmental effects were not separately addressed in this research. The focus was on relationships among pentosan fractions and end-use parameters in pure cultivar wheat samples representing a broad range of quality. Environmental effects were necessarily reflected indirectly in the overall quality, pentosan content, and texture of the grain.

The wheat grain samples were analyzed for moisture (Approved Method 44-16) (AACC 1995), wheat protein (N × 5.7, Approved Method 46-30), and hardness (NIR) (Approved Method 39-70A) and single kernel characterization system (SKCS 4100, Perten Instruments, Springfield, IL). Grain ground in a sample mill (Cemotec 1090, Tecator, Höganäs, Sweden) was analyzed for membrane-associated pentosans. Grain ground in a cyclone mill (Udy Corp., Boulder, CO) (0.5-mm screen) was analyzed for water-soluble and total pentosan contents.

Grain was tempered (13% for soft and 14.5% for hard, fwb) and milled on modified Quadrumat mills (Jeffers and Rubenthaler 1977). Break flour yield was calculated as the proportion of break flour to total products. The resulting flour was analyzed for moisture (Approved Method 44-16) (AACC 1995), protein (Approved Method 46-30), AWRC (Approved Method 56-10), and starch damage (Starch Damage Kit, Megazyme, Wicklow, Ireland). Flour was analyzed for water-soluble and total pentosans. Flour was baked into sugar snap cookies (Approved Method 10-52) as a measure of end-use quality. Mixograph water absorption was determined using Approved Method 54-40A. All grain and flour analyses, as well as milling and baking, were duplicated. Puroindoline hardness genotype was determined using the methods of Giroux and Morris (1997, 1998).

Wheat grain microsomal membranes were isolated as follows: 3.5 g of grain ground in a sample mill (dwb) was prehydrated for 30 min in 35 mL of 200 m*M* $KHPO_4$, pH 7, 10 m*M* $MgCl_2$, 2 m*M* dithiothreitol, then homogenized with a Polytron mixer (Brinkmann Instruments, Westbury, NY) operated with a 7-mm knife-bladed probe at 25,000 rpm for 15 sec. After extraction, the suspension was centrifuged 10 min at 1,000 × *g*. The supernatant was transferred to a new centrifuge tube and centrifuged at 10,000 × *g* for 20 min. High-speed centrifuge tubes were loaded with 20 mL of the supernatant, representing 1 g (dwb) of ground grain per 10 mL of solution, and centrifuged for 1.5 hr at 100,000 × *g*. The supernatant was discarded, and the pellet washed with water then centrifuged an additional 1 hr at 100,000 × *g*. The resulting pellet was washed with acetone to remove lipids, leaving behind a protein-carbohydrate powder. The powder was analyzed in triplicate for pentosans by the method of Hashimoto et al (1987). Results are expressed as microgram xylose equivalents per gram of original material, dwb.

Wheat grain and flour were analyzed for total pentosans and soluble pentosans with the method of Douglas (1981). For total pentosans, 5 mg of ground grain or flour was used directly for analysis. Soluble pentosans were extracted with water for 30 min from grain (Udy cyclone, 0.5-mm screen) or flour (100 mg, both materials). Samples were centrifuged at 10,000 × *g* for 10 min and the supernatant recovered for analysis. Results are expressed as microgram xylose equivalents per gram of original material, dwb. All pentosan determinations were made in triplicate.

Statistical analyses and model building were made using personal computer software (PC-SAS 6.12, SAS Institue, Cary, NC). Full models (initial models) were constructed using Proc GLM with puroindoline genotype used as a classification variable (all samples categorically classified as hard or soft) (Table I). Reduced models (best models) were constructed to remove parameters with insignificant ($P < 0.10$) contributions to the overall model using Proc Reg with the method = maxr option.

RESULTS AND DISCUSSION

Wheat Grain

The *Hardness* gene has a major influence on many properties of wheat grain and flour (Rogers et al 1993, Bergman et al 1998, Campbell et al 1999, Morris et al 1999). Considering that the samples included in this study were from several crop years and were of different genetic backgrounds and market classes, the common methods of measuring wheat grain hardness, NIR and SKCS, clearly separated the samples into discrete hard and soft classes (Fig. 1). For purposes of genotypic classification of wheat varieties into hard and soft classes, the wheats with wild type

TABLE I
Sample Cultivar, Crop Year, *Hardness* Genotype, and Market Class

Cultivar	Year	*Hardness* Genotype[a]	Class[b]
ID377s	1993, 1995	*Pina-D1b/Pinb-D1a*	HWS
Westbred 926R	1994, 1996	*Pina-D1b/Pinb-D1a*	HRS
Butte 86	1994, 1996	*Pina-D1b/Pinb-D1a*	HRS
Hatton	1993	*Pina-D1a/Pinb-D1b*	HRW
Klasic	1994–1996	*Pina-D1a/Pinb-D1b*	HWS
McKay	1996	*Pina-D1a/Pinb-D1b*	HRS
Spillman	1996	*Pina-D1a/Pinb-D1b*	HRS
Wanser	1996	*Pina-D1a/Pinb-D1b*	HRW
Alpowa	1994	*Pina-D1a/Pinb-D1a*	SWS
Centennial	1996	*Pina-D1a/Pinb-D1a*	SWS
Eltan	1996	*Pina-D1a/Pinb-D1a*	SWW
Fielder	1996	*Pina-D1a/Pinb-D1a*	SWS
Hill 81	1993, 1995	*Pina-D1a/Pinb-D1a*	SWW
Lewjain	1996	*Pina-D1a/Pinb-D1a*	SWW
Paha	1993	*Pina-D1a/Pinb-D1a*	Club
Penawawa	1992	*Pina-D1a/Pinb-D1a*	SWS
Stephens	1996	*Pina-D1a/Pinb-D1a*	SWW
Wadual	1996	*Pina-D1a/Pinb-D1a*	SWS
Wawawai	1995, 1996	*Pina-D1a/Pinb-D1a*	SWS

[a] *Pina-D1b/Pinb-D1a* = Puroindoline a-null hard genotype; *Pina-D1a/Pinb-D1b* = Puroindoline b glycine and serine mutation hard genotype; *Pina-D1a/Pinb-D1a* = wild type soft genotype.

[b] HWS = hard white spring; HRS = hard red spring; HRW = hard red winter; SWS = soft white spring; SWW = soft white winter.

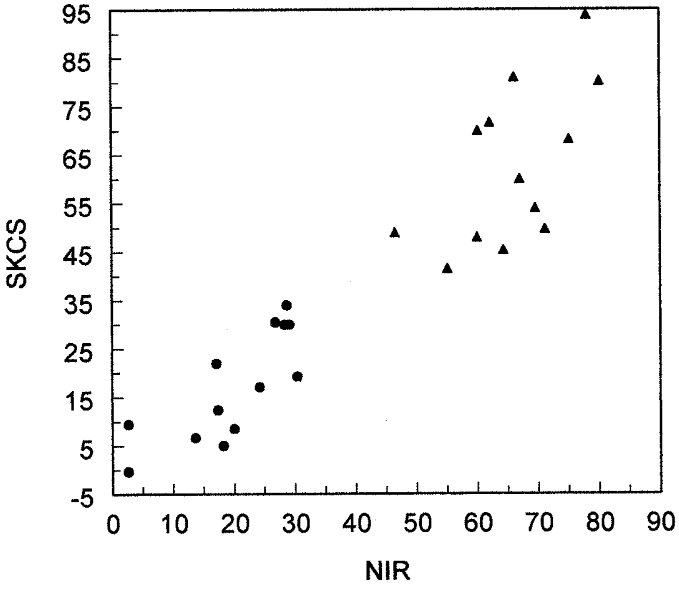

Fig. 1. Near-infrared reflectance (NIR) hardness values vs. single kernel characterization system (SKCS) hardness values for 13 genotypically soft (●) and hard (▲) wheats.

puroindolines a and b were classified as soft wheat, and wheats with a mutation in puroindoline b or an absence of puroindoline a were classified as hard wheats (Table I). Never was the puroindoline genotype inconsistent with the grain texture class (Fig. 1). The mean and standard deviation for NIR and SKCS grain texture and other wheat and flour parameters for the hard and soft wheat samples are summarized in Table II. Gaps between the texture classes occurred at 30–46 for NIR and 34–41 for SKCS. However, classes themselves had a range of 3–30 and 46–80 for soft and hard wheat samples for NIR, respectively, and 0–34 and 41–94 for soft and hard wheat samples for SKCS, respectively. Although these scales are arbitrary, the graphical presentation highlights both the profound effect of the *Hardness* gene and also the considerable variation within texture class (Fig. 1). Among the hard wheat samples, the samples of Klasic were consistently the softest, whereas the Butte 86 sample was the hardest (data not shown). Among the soft wheats, the sample of Penawawa was notably the softest. These rankings relative to other cultivars have been commonly observed (data not shown). Analysis of variance (ANOVA) verified the significant difference ($P < 0.000l$) between puroindoline-based genotype texture classes for the grain texture measures of NIR hardness (model F-value = 171), SKCS hardness (model F-value = 67), break flour yield (model F-value = 107), and flour starch damage (model F-value = 62). A similar comparison for the three pentosan fractions, membrane-associated, total, and water-soluble, also revealed significant differences ($P < 0.01$) between the puroindoline texture genotype classes as well. Model F-values were 16 for membrane-associated pentosans, 9 for soluble wheat pentosans, and 6 for total wheat pentosans.

In addition to separating samples into qualitative hard and soft classes, NIR and SKCS grain hardness, flour starch damage and break flour yield provided effective means of quantifying the effects of both the *Hardness* gene and the secondary, as yet unknown, effects on wheat grain texture. Although these tests are empirical in their relationship to grain texture, they are generally highly intercorrelated (Morris et al 1999), suggesting that they similarly capture the underlying causes of texture variation. In the present study, NIR and SKCS grain hardness, break flour yield, and flour starch damage were intercorrelated at $r = 0.88–0.93$ (absolute values, Table III). Correlations among these parameters within texture class were lower.

Pentosans, intrinsic biochemical components of the wheat kernel, were evaluated as potential contributors to texture variation by testing their linear relationships to grain texture correlation (Table III) and GLM (Table IV). The three pentosan fractions were correlated with the four grain texture measures at $r \approx 0.5$ (absolute values) (Table III). Although most are statistically significant, the correlation values do not reflect a high degree of linear association. Inspection of graphical plots suggested that these correlations largely resulted from the major and overriding effect of texture class. Consequently, each texture class was examined independently.

When hard and soft wheat samples were examined as separate classes, ostensibly removing the effect of the *Hardness* gene, the ranges of grain texture measures and pentosan contents were markedly reduced (data not shown). For the hard wheat samples, correlations among NIR and SKCS grain hardness and break flour yield were reduced to $r \approx 0.5$ (absolute values); and these three with flour starch damage to $r \approx 0.7$ (absolute values). None of these texture measures were significantly correlated with any of the three pentosan fractions (data not shown).

For the soft wheat samples, NIR and SKCS grain hardness measurements were correlated with membrane-associated pentosans of whole ground grain at $r \approx -0.75$ ($P < 0.005$). These two negative correlations contrast the generally positive correlations among grain hardness measures and pentosan fractions when hard and soft texture classes were analyzed together (Table III). A slightly reduced hardness level was correlated with increasing membrane-associated pentosans, but this relationship did not carry over to flour and end-use results. The inverse relationship between membrane-associated pentosans and texture measurements may indicate that there are differences in amyloplast membranes. These differences may include composition of membrane lipids (Greenblatt et al 1995) or membrane-associated pentosans between hard and soft wheats, as well as differences in the total amount present or extractable. Total and soluble pentosans were not significantly correlated with measurements of texture, in keeping with previously reported results (Hong et al 1989).

The various relationships among grain texture measurements, grain pentosan fractions, and grain protein were next evaluated using a GLM approach to ANOVA (Table IV). When texture class, the three pentosan fractions, and grain protein content were included in the initial model (initial models included all terms regardless of level of significance), texture class was by far the major term, with F-values of 50–144 ($P < 0.0001$). Pentosan fractions ranged from nonsignificant F-values to 9.0 ($P < 0.01$). The best single relationships in the initial models were between membrane-associated pentosans and NIR and SKCS grain hardness, total grain pentosans, and break flour yield, and soluble grain pentosans and flour starch damage (Table IV).

Best models (models that eliminated nonsignificant [$P > 0.10$] parameters) showed little change in model R^2 values, but an increase in model F-values, relative to the initial models (Table IV). Membrane-associated and total grain pentosan fractions were significant contributing parameters, after texture class, to the overall

TABLE II
Mean and Standard Deviation (SD) Values for Grain Texture Measures, Pentosan Fractions,[a] and End-Use Properties for Hard ($n = 13$) and Soft Wheat Samples ($n = 13$)

	Hard Wheat Samples		Soft Wheat Samples	
	Mean	SD	Mean	SD
NIR grain hardness	66	9.3	21	7.9
SKCS grain hardness	63	16.3	17	11.3
Break flour yield (%)	36.9	2.89	47.7	2.43
Flour starch damage (%)	3.9	0.77	1.8	0.59
Membrane pentosans	165	23.5	124	28.4
Grain total pentosans	215	15.2	203	12.3
Grain soluble pentosans	28	4.4	24	3.7
Grain protein (%)	14.3	1.79	12.6	1.55
Flour total pentosans	79	8.6	73	8.5
Flour soluble pentosans	33	8.7	27	5.5
Flour protein (%)	12.2	1.70	10.2	1.29
AWRC[b]	69.9	4.77	58.9	3.63
Mixograph absorption (%)	61.9	3.55	61.7	4.91
Cookie diameter (cm)	8.36	0.28	9.18	0.29

[a] Pentosans are expressed as µg of xylose equivalents/g of wheat or flour.

[b] Alkaline water retention capacity.

best model for NIR and SKCS grain hardness. Membrane-associated and total grain pentosan fractions, with grain protein, were significant parameters in the best model for break flour yield. For flour starch damage, total and soluble grain pentosan fractions were significant contributors to the overall best model.

When each texture class was considered independently, the GLM analysis suggested conclusions similar to those drawn from the correlation analysis (Table III). None of the grain pentosan fractions or grain protein explained much of the texture variation among the hard wheat samples (total model R^2 = 0.07–0.35 for NIR and SKCS grain hardness, break flour yield, and flour starch damage) (data not shown).

However, among the soft wheat samples, membrane-associated pentosans were a significant and usually leading parameter explaining texture variation in both the initial and best models (Table IV). Differing from models incorporating all samples, the models for soft wheat samples alone showed a decrease in model R^2 values between initial and best models, but with greater model F-values. A notable exception was for the NIR grain hardness model that showed no decrease in model R^2 but an increase in model F-value. For the best model of NIR grain hardness, membrane-associated pentosans, plus grain-soluble pentosans explained 76% of the variation in texture (model $P < 0.0001$; F-value 16). Other best models for SKCS grain hardness, break flour yield, and flour starch damage used membrane-associated pentosans as a parameter, plus total grain pentosans (for break flour yield), or soluble-grain pentosans (for flour starch damage). These best models only explained ≈55% of the variation in the texture parameter (Table IV) within the soft wheat samples.

The alleles of the *Hardness* gene do not confer pronounced, quantitative differences in the amount of membrane-associated, total, or soluble pentosans in grain, although each may play some secondary textural role. Among hard wheats, it appears that residual (within class) variation in texture is not related to pentosan content to any significant extent and that other factors are involved. Among soft wheats, membrane-associated pentosans were the primary factor influencing texture measures (Table IV). For SKCS grain hardness, break flour yield, and flour starch damage, membrane-associated pentosans were best at explaining variation in texture ($R^2 \approx$ 0.5–0.6). For NIR grain hardness, the membrane-associated pentosans, with soluble-grain pentosans, were most influential in explaining variation (model R^2 = 0.76).

Flour

Flour traits were highly influenced by the *Hardness* gene, and reflect the breeding objectives of hard and soft wheat cultivars. For example, Bergman et al (1998) showed a high correlation (r = –0.96) for kernel texture and AWRC in a set of genetically defined recombinant inbred lines derived from a hard by soft cross. Similar to the analysis for grain, ANOVA for flour demonstrated signi-

TABLE III
Correlation Coefficients and P-Values for Wheat Grain Texture Measures, Pentosan Fractions, and Grain Protein for Hard and Soft Wheat Grain (n = 26)

	SKCS Grain Hardness	Break Flour Yield	Flour Starch Damage	Grain Membrane Pentosans	Total Grain Pentosans	Soluble Grain Pentosans	Grain Protein
NIR grain hardness	0.93	–0.92	0.92	0.49	0.46	0.46	0.43
	<0.0001	<0.0001	<0.0001	<0.01	<0.05	<0.05	<0.05
SKCS grain hardness		–0.89	0.89	0.45	0.47	0.48	0.45
		<0.0001	<0.0001	<0.05	<0.01	<0.05	<0.05
Break flour yield			–0.88	–0.56	–0.42	–0.53	–0.53
			<0.0001	<0.005	<0.001	<0.005	<0.005
Flour starch damage				0.42	0.44	0.21	0.39
				<0.05	<0.05	ns[a]	<0.10
Grain membrane pentosans					0.56	0.69	0.59
					<0.005	<0.0001	<0.005
Total grain pentosans						0.45	0.42
						<0.05	<0.05
Soluble grain pentosans							0.61
							<0.001

[a] Not significant ($P > 0.10$).

TABLE IV
General Linear Models (Initial and Best) for F-Values for Grain Texture Measures Using Texture Class,[a] Pentosan Fractions, and Wheat Protein for Hard and Soft Wheat Grain Samples (n = 26) and for Soft Wheat Grain Samples Alone (n = 13)

	NIR Grain Hardness		SKCS Grain Hardness		Break Flour Yield		Flour Starch Damage	
	Initial	Best	Initial	Best	Initial	Best	Initial	Best
Hard and soft wheat samples								
Texture class	126.4	143.5	44.2	49.9	74.2	78.4	59.9	72.6
Grain membrane pentosan	5.5	6.0	4.1	2.9	3.8	3.7	0.7	ns[b]
Total grain pentosan	2.5	3.0	2.3	2.9	9.0	9.5	3.5	2.8
Soluble grain pentosan	0.2	ns	0.6	ns	0.3	ns	3.1	9.8
Grain protein	0.1	ns	0.3	ns	1.5	2.3	0.2	ns
Model R^2	0.91	0.91	0.79	0.78	0.89	0.89	0.83	0.82
Model F-value	39.2	70.3	15.3	25.8	31.9	41.3	18.4	31.7
Soft wheat samples only								
Grain membrane pentosan	23.7	30.5	17.7	12.3	9.6	8.3	3.4	4.0
Total grain pentosan	0.1	ns	3.3	ns	3.9	8.7	1.8	ns
Soluble grain pentosan	1.7	6.8	3.5	ns	0.1	ns	1.0	5.0
Grain protein	0.1	ns	1.6	ns	0.4	ns	0.8	ns
Model R^2	0.76	0.76	0.75	0.53	0.65	0.58	0.66	0.58
Model F-value	6.4	15.5	5.9	12.3	3.7	6.9	3.8	6.8

[a] Classification variable, hard or soft, as determined by puroindoline genotype.

[b] Not significant ($P > 0.10$).

ficant differences between the hard and soft texture classes. In addition to flour starch damage, AWRC was significantly higher for the hard wheat class of samples (class means in Table II). Flour protein content was also significantly higher in the hard wheat class compared with the soft (Table II and data not shown). Cookie diameters were typical of the results generally seen with samples such as these. Hard wheat flours produced smaller (8.36 ± 0.28 cm), whereas soft wheat flours produced larger (9.18 ± 0.29 cm) cookies.

Correlation analysis across all samples indicated that end-use properties, especially cookie diameter, were significantly correlated with some of the physical and chemical constituents of flour that were measured here. Once again, when hard wheat flours were analyzed separately, variation in cookie diameter and AWRC could be modeled reasonably well. The best model for cookie diameter incorporated total flour pentosans ($F = 9.0$) and flour protein ($F = 17.0$) for a model $R^2 = 0.76$, and a model $F = 14.3$ ($P < 0.005$).

The influence of water and its plasticizing and hydration effects on ingredients that together contribute to cookie diameter (rate and duration of spread) (Miller et al 1997) was reflected by the degree of correlation, among all samples, with AWRC ($r = -0.83$) (Table V). AWRC may be influenced by pentosans, particularly the membrane-associated or total flour pentosan fraction. These correlations suggest that pentosans might influence cookie spread through modification of water relationships during mixing and baking. Previous research has indicated that water-controlling compounds such as damaged starch or some pentosan fractions were not well correlated with cookie spread (Miller et al 1997). However, the influence of pentosan subfractions, especially membrane-associated pentosans were not examined in previously published work (Miller et al 1997). The high correlation ($r = -0.85$) between flour protein and cookie diameter was examined in greater detail using GLM. The 2% difference in flour protein between the two texture classes (Table II) was significant ($P < 0.005$). When flour protein was used to help model cookie diameter across the two texture classes, the significant difference in flour protein content between hard and soft flour samples, coupled with the significant difference in cookie diameter found between hard wheat and soft flour samples, led to this high correlation.

No significant correlations were noted for mixograph water absorption among the pentosan fractions or for flour protein when all flour samples were considered. Neither were significant correlations noted when the samples were separated into hard or soft groups. The greatest correlation observed between mixograph water absorption and the pentosan fractions, when all samples were considered, was for membrane-associated pentosans ($r = 0.28$) ($P = 0.18$). When correlations for hard wheat flours were examined separately, the highest correlation was $r = 0.38$; $P = 0.21$ for mixograph water absorption and soluble flour pentosans. No significant models incorporating penotsan fractions and flour protein were obtained for mixograph absorption (data not shown). No particularly noteworthy correlations were observed for cookie diameter within the class of hard wheat (data not shown). As noted above, hard wheat flours do not generally produce much spread, such that diameters are restricted. For this reason, cookie baking is generally of limited use for assessing quality of hard wheat flours.

Determining the biochemical basis for variation in end-use quality among soft wheat flours has been generally problematic. AWRC was moderately correlated to flour starch damage, flour protein, and total flour pentosans to a similar extent ($r \approx 0.5–0.6$, absolute values) but more highly correlated to membrane-associated pentosans

TABLE V
Correlation Coefficients and *P*-Values for Pentosan Fractions, Flour Protein, and Starch Damage, and End-Use Parameters for Hard and Soft Wheat Flour Samples ($n = 26$)

	Total Flour Pentosans	Soluble Flour Pentosans	Flour Starch Damage	Flour Protein	AWRC[a]	Cookie Diameter
Grain membrane pentosans	0.52 <0.01	0.58 <0.005	0.42 <0.05	0.67 <0.0005	0.63 <0.0005	–0.76 <0.0001
Total flour pentosans		0.42 <0.05	0.20 ns[b]	0.43 <0.05	0.55 <0.005	–0.64 <0.0005
Soluble flour pentosans			0.24 ns	0.56 <0.005	0.23 ns	–0.57 <0.005
Starch damage				0.42 <0.05	0.60 <0.05	–0.62 <0.001
Flour protein					0.63 <0.0005	–0.85 <0.0001
AWRC						–0.83 <0.0001

[a] Alkaline water retention capacity
[b] Not significant ($P > 0.10$).

TABLE VI
Correlation Coefficients and *P*-Values for Pentosan Fractions, Flour Protein, and Starch Damage, and End-Use Parameters for Soft Wheat Flour Samples Only ($n = 13$)

	Total Flour Pentosans	Soluble Flour Pentosans	Starch Damage	Flour Protein	AWRC[a]	Cookie Diameter
Grain membrane pentosans	0.58 <0.05	0.09 ns[b]	–0.60 <0.05	0.58 <0.05	0.83 <0.0005	–0.56 <0.05
Total flour pentosans		0.56 <0.05	–0.50 <0.10	0.76 <0.005	0.58 <0.05	–0.90 <0.0001
Soluble flour pentosans			–0.49 <0.10	0.77 <0.005	0.25 ns	–0.71 <0.01
Starch damage				–0.66 <0.05	–0.52 <0.10	0.55 <0.01
Flour protein					0.63 <0.0005	–0.85 <0.0005
AWRC						–0.56 <0.05

[a] Alkaline water retention capacity
[b] Not significant ($P > 0.10$).

($r = 0.83$) (Table VI). Traditionally, AWRC has been used as a predictor of cookie baking quality (Yamazaki 1955). Among this set of soft wheat flours, flour starch damage and membrane pentosans were similar to AWRC in their level of correlation with cookie diameter ($r \approx 0.5$–0.6, absolute values). Of increasingly higher correlation with cookie diameter were soluble flour pentosans ($r = –0.71$), flour protein ($r = –0.85$), and total flour pentosans ($r = –0.90$). Flour protein has a major influence on cookie quality (diameter) (Gaines 1985). Water sequestration or control of hydration and therefore rate of cookie spread due to modification of dough viscosity has been proposed as the mechanism that controls cookie diameter (Miller et al 1997). Furthermore, the glass transition of gluten proteins may play a role in this process (Miller et al 1997). However, Yamazaki (1955) indicated that the starch tailing fraction, rich in insoluble pentosans that would be reflected in total pentosan content, had a major role in controlling water and therefore dough viscosity and cookie spread. Overall, final cookie diameter has been attributed to control of water in dough. The biological components contributing to water control and sequestration have not been well elucidated, however.

ANOVA, through a GLM approach, was conducted to examine the contribution of various factors to end-use quality. For AWRC, the initial model included texture class, total and soluble flour pentosans, and flour protein as the leading parameters (Table VII). In a best (reduced) model, the major effect was texture class, followed by total and soluble flour pentosans and flour protein, which were all approximately equal contributors to the model. These model parameters explained 80% of the variation in AWRC. However, hard wheat flours are not well suited for cookie baking and the range of cookie diameters among the hard wheat flours was only 7.92–8.88 cm, whereas the range for soft wheat flours was 8.42–9.52 cm. Consequently, modeling the variation among hard wheat flours was considered of limited importance. A traditional and more important quality trait for hard wheat flours is dough water absorption as determined by the mixograph. But as noted above, the best correlation with mixograph water absorption was obtained with soluble pentosans where only 14% of the total variation was explained. When all samples, hard and soft, were analyzed in the initial model, texture class (hard vs. soft), total pentosan content, and flour protein were the major contributors to cookie diameter (Table VII). Within the best (reduced) model, texture class, total pentosans and flour protein were significant contributors in addition to texture class and protein. When these factors were used, most of the variation for cookie diameter across hardness classes was accounted for ($R^2 = 0.95$, $P < 0.0001$).

When hard wheat flours were analyzed separately, variation in cookie diameter and AWRC could be modeled reasonably well, though this is not necessarily useful as hard wheats are not traditionally used to make cookies. The best model for AWRC using hard wheat flours incorporated total flour pentosans ($F = 2.4$; $P < 0.15$), soluble flour pentosans ($F = 5.5$; $P < 0.05$), and flour protein ($F = 3.4$; $P < 0.10$) for a model $R^2 = 0.53$ and with a model $F = 3.0$ ($P < 0.10$). The best model for cookie diameter incorporated soluble flour pentosans ($F = 9.0$; $P < 0.05$) and flour protein ($F = 17.0$; $P < 0.005$) for a model $R^2 = 0.76$ and a model $F = 14.3$ ($P < 0.005$). However, because hard wheat flours are not well suited for cookie baking, these results were considered of limited practical importance. A traditional and more important quality trait for hard wheat flours is dough water absorption commonly determined by the mixograph. But as noted above, the best correlation with mixograph water absorption was obtained with soluble pentosans where only 14% of the total variation was explained.

Within the class of soft wheats, membrane-associated pentosans were more important than other factors in the ANOVA (GLM) for AWRC (Table VII). AWRC among soft wheat flours seemed to be substantially due to the membrane-associated pentosan fraction. Over two-thirds of the variation in AWRC ($R^2 = 0.69$, $P < 0.0005$) was explained by a model using membrane-associated pentosans. The high correlation between membrane-associated pentosans and AWRC implies that end use and processing quality in soft wheat such as moisture bake-out time and temperature may be substantially influenced by variation in the amount of membrane-associated pentosans. Soluble and membrane-associated pentosans might play a larger role in explaining hydration effects and water retention if oxidative gelation occurred and therefore led to additional water sequestration effects (Neukom and Markwalder 1978).

Within the class of soft wheats, contributions to the reduction of model error sums of squares for cookie diameter came primarily from total pentosans and flour protein (Table VII). The reason flour protein appears to have decreased in importance to the cookie diameter modeling ANOVA, as opposed to simple correlation or the model including both hard and soft flour samples, is

TABLE VII
General Linear Models (Initial and Best) for *F*-Values for End-Use Measures (AWRC[a] and Cookie Diameter) Using Texture Class,[b] Pentosan Fractions, and Flour Protein and Starch Damage for Hard and Soft Wheat Samples ($n = 26$) and for Soft Wheat Samples Alone ($n = 13$)

	AWRC		Cookie Diameter	
	Initial	Best	Initial	Best
Hard and soft wheat samples				
Texture class	5.2	28.7	15.3	61.6
Grain membrane pentosan	0.6	ns[c]	0.2	ns
Total flour pentosan	3.4	7.3	17.7	22.7
Soluble flour pentosan	9.2	8.2	0.0	ns
Flour protein	3.5	5.7	16.2	63.3
Flour starch damage	0.2	ns	0.5	ns
Model R^2	0.82	0.80	0.96	0.95
Model F-value	13.7	21.7	64.6	144.7
Soft wheat samples only				
Grain membrane pentosan	9.9	24.3	1.0	ns
Total flour pentosan	0.1	ns	7.6	11.7
Soluble flour pentosan	2.3	ns	1.9	ns
Flour protein	1.5	ns	0.0	5.0
Starch damage	0.3	ns	0.3	ns
Model R^2	0.78	0.69	0.90	0.87
Model F-value	4.9	24.3	12.3	33.5

[a] Alkaline water retention capacity
[b] Classification variable, hard or soft, as determined by puroindoline genotype.
[c] Not significant ($P > 0.10$).

that ANOVA examines the cumulative interactions among factors instead of each factor individually. For the best model within the class of soft wheat flours, total flour pentosans plus flour protein provided an $R^2 = 0.87$ ($P < 0.0001$). The use of these factors explains a high proportion of the within-class variation of soft flour end-use quality as defined by cookie diameter.

CONCLUSIONS

The *Hardness* gene, as defined by puroindoline allele, is the major effector of hard or soft wheat grain texture (Giroux and Morris 1997, 1998). Secondary hardness variation among hard wheats seems to have little relationship to pentosan content. Apparently among hard wheats, biochemical factors other than pentosans play the major texture-modifying role. Among soft wheats, however, pentosans appear to have a grain texture-modifying role as well as being major contributors to absorption and baking quality in conjunction with other flour components such as protein.

LITERATURE CITED

Al Saleh, A., Marion, D., and Gallant, D. J. 1986. Microstructure of mealy and vitreous wheat endosperms (*Triticum durum* L.) with special emphasis on location and polymorphic behaviour of lipids. Food Microstructure 5:131-140.

American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9th ed. The Association: St. Paul, MN.

Barlow, K. K., Buttrose, M. S., Simmonds, D. H., and Vest, M. 1973. The nature of the starch-protein interface in wheat endosperm. Cereal Chem. 50:443-454.

Bergman, C. J., Gualberto, D. G., Campbell, K. G., Sorrells, M. E., and Finney, P. L. 1998. Genotype and environmental effects on wheat quality traits in a population derived from a soft by hard cross. Cereal Chem. 75:729-737.

Campbell, K. G., Bergman, C. J., Gualberto, D. G., Anderson, J. A., Giroux, M. J., Hareland, G., Fulcher, R. G., Sorrells, M. E., and Finney, P. L. 1999. Quantitative trait loci associated with kernel traits in a soft × hard wheat cross. Crop Sci. 39:1184-1195.

Douglas, S. G. 1981. A rapid method for the determination of pentosans in wheat flour. Food Chem. 7:139-145.

Dubreil, L., Gaborit, T., Bouchet, B., Gallant, D. J., Broekaert, W. F., Quillien, L., and Marion, D. 1998. Spatial and temporal distribution of the major isoforms of puroindolines (puroindoline-a and puroindoline-b) and nonspecific lipid transfer protein (ns-LTP1e$_1$) of *Triticum aestivum* seeds. Relationships with their *in vitro* antifungal properties. Plant Sci. 138:121-135.

Gaines, C. S. 1985. Associations among soft wheat flour particle size, protein content, chlorine response, kernel hardness, milling quality, white layer cake volume, and sugar-snap cookie spread. Cereal Chem. 62:290-292.

Giroux, M. J., and Morris, C. F. 1997. A glycine to serine change in puroindoline b is associated with wheat grain hardness and low levels of starch-surface friabilin. Theor. Appl. Genet. 95:857-864.

Giroux, M. J., and Morris, C. F. 1998. Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. Proc. Natl. Acad. Sci. USA 95:6262-6266.

Glenn, G. M., and Saunders, R. M. 1990. Physical and structural properties of wheat endosperm associated with grain texture. Cereal Chem. 67:176-182.

Greenblatt, G. A., Bettge, A. D., and Morris, C. F. 1995. Relationship between endosperm texture and occurrence of friabilin and bound polar lipids on wheat starch. Cereal Chem. 72:172-176.

Hashimoto, S., Shogren, M. D., and Pomeranz, Y. 1987. Cereal pentosans: Their estimation and significance. I. Pentosans in wheat and milled wheat products. Cereal Chem. 64:30-34.

Hong, B. H., Rubenthaler, G. L., and Allan, R. E. 1989. Wheat pentosans. I. Cultivar variation and relationship to kernel hardness. Cereal Chem. 66:369-373.

Jeffers, H. C., and Rubenthaler, G. L. 1977. Effect of roll temperature on flour yield with the Brabender Quadrumat experimental mills. Cereal Chem. 54:1018-1025.

Jelaca, S. L., and Hlynka, I. 1971. Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. Cereal Chem. 48:211-222.

Kavitha, R., and Chandrashekar, A. 1992. Content and composition of nonstarchy polysaccharides in endosperms of sorghums varying in hardness. Cereal Chem. 69:440-443.

Lillemo, M., and Morris, C. F. *In press*. A leucine to proline mutation in puroindoline b is frequently present in hard wheats from Northern Europe. Theor. Appl. Genet.

Mares, D. J., and Stone, B. A. 1973. Studies on wheat endosperm. III. Galactose-rich polysaccharides. Aust. J. Biol. Sci. 26:1005-1007.

Marion, D., Gautier, M.-F., Jourdrier, P., Ptak, M., Pézolet, M., Forest, E., Clark, D. C., and Broekaert, W. 1994. Structure and function of wheat lipid binding proteins. Pages 175-180 in: Wheat Kernel Proteins, Molecular and Functional Aspects. Università Degli Studi Della Tuscia, S. Martino al Chimino: Viterbo, Italy.

Miller, R. A., Hoseney, R. C., and Morris, C. F. 1997. Effect of formula water content on the spread of sugar-snap cookies. Cereal Chem. 74:669-671.

Morris, C. F., DeMacon, V. L., and Giroux, M. J. 1999. Wheat grain hardness among chromosome 5D homozygous recombinant substitution lines using different methods of measurement. Cereal Chem. 76:249-254.

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.

Rogers, D. E., Hoseney, R. C., Lookhart, G. L., Curran, S. P., Lin, W. D. A., and Sears, R. G. 1993. Milling and cookie baking quality of near-isogenic lines of wheat differing in kernel hardness. Cereal Chem. 70:183-187.

Simmonds, D. H., Barlow, K. K., and Wrigley, C. W. 1973. The biochemical basis of grain hardness in wheat. Cereal Chem. 50:553-562.

Symes, K. J. 1965. The inheritance of grain hardness in wheat as measured by the particle size index. Aust. J. Agric. Res. 16:113-123.

Yamazaki, W. T. 1955. The concentration of a factor in soft wheat flours affecting cookie quality. Cereal Chem. 32:26-37.

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