WHEAT GRAIN ARABINOXYLAN QUANTIFICATION, CHARACTERIZATION, AND FATE DURING BAKING

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Abstract

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The study of wheat (Triticum aestivum L.) as it relates to end-use quality is highly diverse. This dissertation examines end-use quality across the U.S. and how the non-starch polysaccharide arabinoxylan (AX) influences wheat quality. Arabinoxylans occur in water-extractable (WEAX) and water-unextractable fractions, and have been shown to variably influence end-use quality. First and foremost, an accurate, precise, and consistent method of quantifying AX was critical to ascertain. Gas chromatography-flame ionization detection was preferred over a colorimetric phloroglucinol assay for consistency and accuracy. A survey of soft and hard wheat grown in distinct nurseries across the U.S. was undertaken to further understand genetic and environmental influences on wheat quality across the U.S. High levels of variation were observed both across and within each growing region. Specific grain, milling, and baking quality traits were determined to have potential predictive power in determining overall end-use quality. The soft wheat varieties with very high and very poor quality were assayed for AX content. In two of the four nurseries studied, AX negatively influenced cookie diameter.
The total AX content was heavily influenced by genetics, whereas WEAX content was variably influenced by genetics and environment. Two studies were undertaken to further understand the role total AX and WEAX play throughout the baking process. In wholemeal pancakes, total AX exhibited a strong negative influence on pancake quality, whereas in refined flour, WEAX was the greatest contributor to decreases in pancake quality. The AX molecules differed in their availability for quantification throughout the baking process, suggesting that those molecules unavailable for quantification were involved in intermolecular interactions. In bread, total AX availability differed throughout the baking process, suggesting complex intermolecular interactions. The molecular substitution of the AX molecules influenced the intermolecular relationships occurring throughout the baking process, and eventually impacting final quality of the bread loaf. In particular, the substitution pattern of WEAX was the most critical factor in determining the extent to which WEAX molecules enhanced loaf volume. These studies clearly indicate that there are more complex molecular interactions occurring than have previously been elucidated, necessitating further studies on how AX molecules influence final end-use quality.
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Dedication

This dissertation is dedicated to my family, whose support and belief in me never wavered
CHAPTER ONE
INTRODUCTION

Introduction to Wheat

The United States produced an average of 2712 kg/ha on 26.2 million ha of wheat (*Triticum aestivum* L.) between 1990 and 2010 (Figure 1A; National Agricultural Statistics Service; NASS 2010). The state of Washington, comparatively, averaged 3950 kg/ha on 1.03 million ha of wheat for this 20-year period (Figure 1B; NASS 2010). Over the last five years, Washington has produced 6.15% of all of the wheat grown in the United States, ranking fourth-highest in national wheat production (NASS 2010). Wheat is a food staple not only in the United States but also internationally; it is the second-ranked staple crop in the world (Centro Internacional de Mejoramiento de Maíz y Trigo, CIMMYT 2010), with 686 million tons produced world-wide in 2009 (Food and Agriculture Organization, FAOSTAT 2011).

![Figure 1. A. United States wheat production over 20 years. B. Washington wheat production over 20 years.](image)

Wheat belongs to the larger classification of cereals, which also includes maize (*Zea mays* L.), rice (*Oryza* spp.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum* spp.), millet (*Pennisetum glaucum* R.Br.), oats (*Avena sativa* L.), and rye (*Secale cereale* L.). Of the 2.5
billion tons of cereal grains produced in the world in 2009, wheat contributed approximately 
27.5% to this total (FAOSTAT 2011). Cereals used for food provide important nutrients in the 
human diet such as carbohydrates, proteins, fiber, vitamins, and minerals. In industrialized 
countries, cereals contribute approximately half of the carbohydrates and one-third of the protein 
required daily (Orth and Shellenberger 1988).

**Wheat Anatomy and Physiology**

Wheat is made up of five distinct sections (Orth and Shellenberger 1988; Table I).

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<th>Nutrients Present</th>
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<td>Dietary fiber, Phosphorous, K⁺, Mg²⁺, Ca²⁺</td>
</tr>
<tr>
<td><strong>Aleurone layer</strong> Encases the endosperm except between scutellum and endosperm</td>
<td>7</td>
<td>Niacin, minerals, phytic acid</td>
</tr>
<tr>
<td><strong>Endosperm</strong> Stores food</td>
<td>82</td>
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<td>2</td>
<td>B vitamins, Phosphorous</td>
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Although wheat is known primarily to be rich in carbohydrates as a result of the high starch content in the endosperm, wheat also has the highest protein content of the cereal grains. Along with the five main fractions of the wheat kernel listed above, there are additional layers which provide lower levels of nutrients, but play critical roles in structure and development (Orth and Shellenberger 1988).
The outermost layer of the wheat kernel is the pericarp, which is the ripened ovary wall and is dead at harvest ripeness (Evers and Bechtel 1988). The pericarp cells lack cytoplasm and have lignified walls. The pericarp is composed of the outer epidermis, hypodermis, parenchyma, intermediate cells, cross cells, and tube cells. The next section of great importance to the kernel is the seed coat and pigment strand, which together cover the seed completely (Evers and Bechtel 1988). The seed coat and pigment strand control the water relations between the enclosed seed and the surrounding area. The seed coat arises from the integuments, whereas the pigment strand is located between the nucellar projection and the vascular tissues of the crease (Evers and Bechtel 1988).

The next layer is comprised of the nucellar layer and nucellar projection. The nucellar layer consists of crushed, empty epidermal cells located between the endosperm and the seed coat in the mature caryopsis (Evers and Bechtel 1988). The nucellar projection is located on the inner side of the pigment strand, running along the top of the crease. The nucellar tissues surround the endosperm and embryo. The majority of the nucellar layer degenerates during development, with only the epidermis remaining. The epidermis is incorporated into the nucellar projection as a fringe of narrow cells with transverse ridges inside the cell walls (Evers and Bechtel 1988).

Located further into the wheat kernel is the aleurone layer, which is one cell layer thick. The aleurone forms the outermost layer of the endosperm tissue, which surrounds the grain over the endosperm and part of the embryo. This layer is the only endosperm tissue alive at maturity and the cells have large prominent nuclei which, as opposed to the starchy endosperm cells, do not become crushed (Evers and Bechtel 1988).
The endosperm is a layer of a sperm nuclei from the pollen fused with two polar nuclei from the female gametophyte. Because of endosperm cell differences, classifications of cells within the endosperm can be made based on shape, size, and location. This layer is mostly made up of starch and protein, but these proportions vary based on cell location. The grain texture is also influenced by the physiology of the endosperm; endosperm with more air space in the tissue matrix results in a softer wheat texture (Evers and Bechtel 1988) and is referred to as non-vitreous, or yellow berry (Morris 2010).

The embryo is located on the lower dorsal side of the caryopsis and contains two major components: the embryonic axis and scutellum. The embryonic axis has three main regions, the shoot (epicotyl), mesocotyl, and the radicle. The scutellum can otherwise be referred to as a cotyledon and is a storage organ for protein, phytin, and lipid droplets. The scutellum is developed from the ventral and apical portions of the young embryo. Starchy endosperm cells that do not develop normally are crushed and form a layer which separates the scutellar epithelium from the endosperm. The walls of the endosperm cells remain intact, but the cytoplasm is degenerates. The cell walls are then crushed and compressed as the embryo and endosperm expand during development (Evers and Bechtel 1988).

**Wheat Grain Composition**

Wheat is primarily composed of carbohydrates, making up approximately 72% of the kernel by weight. Proteins are considerably less prominent, but still constitute approximately 14% of the kernel (Pomeranz 1988). The kernel is comprised of slightly more than 2% fats. The remaining portions of the kernel consist of moisture and low levels of thiamine, riboflavin, niacin, and pyridoxine (Pomeranz 1988).
The carbohydrates in wheat kernels are highly complex components. Of the carbohydrates, the most basic units are monosaccharides, which can condense to form disaccharides, connected by a glycosidic linkage between the monosaccharide units (Lineback and Rasper 1988). Polysaccharides, also known as carbohydrates, are many units such as this. The structure and characterization of polysaccharides is most commonly performed by identifying the monosaccharide units and their order in the linear or branched polysaccharide (Lineback and Rasper 1988). It is also critical to determine the nature of the anomeric linkage between the monosaccharide units. The main carbohydrates of wheat are starch, cellulose, hemicelluloses, and pentosans.

Starch is the most common carbohydrate found in the wheat kernel. Plastids, which are structures bounded by double membranes, form starch. Two distinct classes emerge in starch: large, type A and small, type B granules (Lineback and Rasper 1988). Typically, the starch content is inversely proportional to the protein content in wheat (Hopkins and Graham 1935). There are two major carbohydrate components to starch, which are both high molecular-weight polymers: amylose and amylopectin (Lineback and Rasper 1988). Amylose is characterized by a linear chain of glucopyranose units connected through α-D-(1-4)-glycosidic linkages. Conversely, amylopectin is a branched polymer with a comparatively high molecular weight in relation to amylose. Amylopectin is also made of glucopyranose units connected by α-D-(1-4)-glycosidic linkages. A branch point occurs approximately every 20 to 25 glucopyranose units. As a result of this extensive branching, amylopectin has greater solubility and solution stability than amylose (Lineback and Rasper 1988).

In addition to starch, there is a class of carbohydrate molecules known as non-starch polysaccharides. These polysaccharides make up the major portion of the cell wall material in
the parenchymatous and lignified tissues in a wheat plant (Lineback and Rasper 1988). Non-starch polysaccharides are also the majority of what is not digested by the endogenous secretions of the human digestive tract, and thus referred to as dietary fiber. These non-starch polysaccharides are made up of cellulose, (1-3)(1-4)-β-glucan, glucomannan, and arabinoxylans (pentosans), making up roughly 75% of the cell wall (Mares and Stone 1973).

Cellulose is considered to be the main polysaccharide in plants, and is a linear polymer made up of several thousand β-(1-4)-linked glucopyranosyl units in each chain. The units in the chain form a twofold helix with two glucosyl units per turn on the helix. There are two hydrogen bonds between the adjacent glucosyl residues which hold the chain in an extended and relatively inflexible conformation (Lineback and Rasper 1988).

In the inner aleurone cell walls, subaleurone endosperm cell walls, and crease area is the polysaccharide (1-3)(1-4)-β-glucan (Lineback and Rasper 1988). This polysaccharide is not as abundant as cellulose and pentosans. It is composed of linear chains of β-D-glucosyl residues and can be covalently associated with arabinosyl and xylosyl residues (Woodward et al 1983). Glucomannans can be found in small proportions in the endosperm cell walls, but can only be extracted with a strong alkali solution (Mares and Stone 1973).

Pentosans, or more specifically, arabinoxylans (AX), make up approximately 85% of all non-starch polysaccharides in the wheat kernel (Mares and Stone 1973). Pentosans are composed of β-1,4 linked D-xylopyranosyl residues as a backbone substituted with monomeric α-L-arabinose units at the second and/or third carbon-positions. The arabinose moiety can possess ferulic acid at the fifth carbon-position (Courtin and Delcour 2002). Factors such as the length of the xylan backbone, the ratio of arabinose to xylose, the substitution pattern on the backbone, and the ferulic acid coupling to other pentosan molecules or the cell wall determine the three-
dimensional structure of pentosans (Courtin and Delcour 2002), which is somewhat-flexible (Dervilly et al 2000). These structural components are common to the total AX structures (TAX) as well as water-extractable (WEAX), and water-unextractable arabinoxylans fractions (WUAX, Courtin and Delcour 2002). The varying substitution patterns influence the molecular weight and physical properties of pentosans (Izydorczyk and Biliaderis 1992). Structurally, WEAX and WUAX are similar, but the molecular weight of WUAX tends to be higher than for WEAX (Philippe et al 2006b).

Between one-third and one-half of the pentosans of the wheat kernel are extractable in water. The reason the remaining pentosans are not water extractable is not fully understood, but one proposed hypothesis is that a higher degree of branching in the AX molecule can cause greater physical entanglements, and may result in a lack of solubility in water (Lineback and Rasper 1988).

Water-extractable arabinoxylans are involved in oxidative cross-linking, likely as a result of ferulic acid substitutions. Cross-linking is suggested to be the result of oxidative phenolic coupling of ferulic acid residues of adjacent pentosan molecules. This hypothesis of the mechanism of cross-linking was suggested based on the presence of ferulic and diferulic acids following oxidation, and the absence of diferulic acid prior to oxidation (Geissman and Neukom 1973). The aromatic nucleus of the phenolic acid in the cross-linkage is involved in the gelation mechanism. This cross-linking can also occur with the tyrosine amino acid of proteins (Neukom and Markwalder 1978).

Three key stages of kernel development have been identified: end cellularization, beginning of cell differentiation, and beginning of maturation (Philippe et al 2006a). Arabinoxylan molecules are deposited in the cell wall through vesicles and merge with the
plasma membrane (Philippe et al. 2006b). Arabinoxylans tend to exhibit endogenous micro-
hetereogeneity (Toole et al. 2007). The AX molecules first appear at the cell differentiation stage
and are more highly substituted at the beginning of cell differentiation (Philippe et al. 2006a). The
ferulic acid substitution that occurs in AX molecules increases during the grain-filling stage,
especially in the aleurone layer (Philippe et al. 2006c). Along with substitution differences
throughout development, AX in the central cells are also less highly substituted than those in the
periphery (Philippe et al. 2006a). The AX in the aleurone layer tend to exhibit lower levels of
arabinose substitution, but higher levels of esterification with phenolic acids (Philippe et al
2006c). In both the walls of the prismatic cells and the endosperm, there is a high level of
arabinose substitution of AX molecules, but poor esterification (Philippe et al. 2006c). These
differences in AX structure across different tissue and cell types suggest that the fate of the cells
was genetically programmed prior to development (Philippe et al. 2006c). There are two main
hypotheses for the functional purpose of the substitution variation among the tissue types. The
first is that synthesis of AX may be correlated to fixation of the wall structure at the cell
differentiation stage and may be involved in the strengthening of cell walls with β-(1-3)(1-4)
glucans. The second hypothesis is that strengthening of cell walls may be mediated by
association of AX changes through formation of dehydroferulate bridges (Philippe et al. 2006a).
Desubstitution of AX over the course of kernel development is likely the result of
arabinofuranohydrolases (Philippe et al. 2006a). In barley, which is often used as a model similar
to wheat, AX was detected at a ratio of 4:1 of substituted to unsubstituted AX, but over the
course of coleoptile growth became desubstituted to a ratio of 1:1 (Gibeaut et al. 2005). The
solubility and transport of newly synthesized AX through the endomembrane system can be
attributed to the high degree of arabinosyl substitution (Philippe et al. 2006a). Once the AX
molecules become unsubstituted, they can potentially stiffen cell walls through hydrogen bonding with other cell wall polysaccharides (Philippe et al 2006a).

**Arabinoxylan Influences on Wheat Quality and Nutrition**

Pentosan content has been observed to vary with mill streams (Ramseyer et al 2011). Hard wheat kernels often have a lower rate of water penetration compared to soft wheats. One hypothesis as to the difference between wheat textures was that the lower water penetration into hard wheat was a consequence of increased hygroscopicity of pentosans with lower ratios of xylose to arabinose in hard wheat (Lee and Stenvert 1973). An opposing idea is that the endosperm, not the bran, was responsible for the acid-extractable pentosans, which are then inversely correlated to milling quality (Elder et al 1953).

In addition to milling quality, pentosans also have varying effects on dough development and baking quality. Total arabinoxylan levels were related to changes in dough development time and viscosity (Jelaca and Hlynka 1971), and were associated with soft wheat end-use quality, including variation in cookie diameter (Bettge and Morris 2000). The final product quality may be influenced by the oxidative cross-linking, or gelation that AX molecules participate in. When high levels of both WEAX and WUAX have been present, there has been an increase in the farinograph absorption observed in flour. Pentosans have a high water-absorption capability, observed to absorb up to 10 times their weight in water (Kulp 1968). Whereas the WEAX have been shown to contribute to increased loaf volume (Baker et al 1943, Finney 1943), WUAX impaired loaf volume (Kulp and Bechtel 1963). A definite effect was reported by pentosans on slowing down the firming of starch gels during aging, by slowing the process of retrogradation (Kim and D’Appolonia 1977). Oxidative cross-linking of WEAX can increase batter viscosity
(Bettge and Morris 2007), decrease the diameter of sugar snap cookies (Bettge and Morris 2007), and decrease the volume of Japanese sponge cakes (Ramseyer et al 2011).

Pentosans also play a critical role in human nutrition. Healthy individuals who ate bread product with additional pentosans had a decreased postprandial glucose concentration (Lu et al 2000). In those individuals with Type II diabetes, bread products with pentosan additives had decreased blood glucose and insulin levels 2h after consumption (Lu et al 2004). Along with these benefits, pentosans have also shown effects in humans on lipid metabolism and mineral absorption (Izydorczyk and Biliaderis 2007) and potential antioxidant activity (Noaman et al 2008).

**End-Use Quality**

End-use quality in wheat is comprised of genetic and environmental factors, which work independently and in combination to influence the resulting flour and subsequent products. These factors contribute to variation in milling and baking quality in soft red winter wheats grown in the southeastern U.S. (Baenziger et al 1985; Kiszonas et al *in press*). A similar influence of genetics, environment, and the interaction between them similarly affected hard red winter wheat quality (Peterson et al 1992, 1998). In a study involving two soft wheat cultivars, environment had a greater influence on flour quality variation, though genetic differences did contribute to a small part of the variation (Guttieri et al 2002).

Primarily, comparisons among varieties are performed on sets of varieties grown in specific regions with similar climates and environmental pressures. Wheat quality assessed in only a small environmental area tends to highlight the varying influence on environment based on the specific varieties grown and evaluated for quality, but can potentially bias the
environmental influence as a result of the small growing region (Morris et al 1997). It is critical for fair evaluation to grown varieties in the environment to which they are best adapted.

Grain, milling, and baking are the primary subdivisions of wheat quality assessment (Wrigley and Morris 1996). It is imperative to consider the end-use functionality of the grain and flour when assessing individual traits or characteristics (Morris and Rose 1996). Along with studying each category of wheat quality separately, evaluating them in combination provides more information about correlations and relationships among all traits assessed.

One of the primary characteristics with end-use quality importance is kernel hardness (Morris and Rose 1996). Kernel texture is a product of genetic control and is relatively unrelated to protein content (Simmonds 1974). Soft wheat fractures through cell walls during milling, whereas hard wheat fractures at cell walls (Hoseney et al 1988). The fracture patterns in soft wheat contribute to finer particles and less starch damage; however, milling sieves can become clogged more frequently when milling soft wheat (Hoseney et al 1988). Hard wheat is better suited to bread making, whereas soft wheat is generally used for cookies, pancakes, cakes, and other similar products (Huebner et al 1999).

Wheat protein has a similarly influential role in the end-use quality of wheat. The higher protein levels in hard wheat varieties contribute to the gluten-formation necessary in hard wheat products, whereas gluten formation is generally discouraged in soft wheat products and therefore requires lower protein contents (Gaines 2004). Wheat protein content is primarily influenced by environmental factors, while protein composition is genetically determined.

The water relationships in wheat are influential in different ways for hard and soft wheat. Water absorption is primarily influenced by damaged starch, soluble starch, pentosans, and flour particle size. In soft wheat, damaged and soluble starch can increase dough viscosity and
ultimately decrease cookie diameter (Miller and Hoseney 1997). There is a strong connection between damaged starch and flour particle size; large particle size often correlates to higher levels of damaged starch (Yamamoto et al 1996), which can be detrimental for soft wheat products and contributes to the necessity of using flour with finer particles for soft wheat products.

The water relationships in soft wheat are often evaluated also using the solvent retention capacity (SRC) system. The SRC evaluations can aid in understanding the overall end-use quality, particularly in soft wheat flour (Gaines et al 2000; Guttieri et al 2001; Guttieri and Souza 2003; Ram and Singh 2004; Moiraghi et al 2011; Kweon et al 2011, 2012; Souza et al 2012). The SRC Water parameter gives a general measurement of overall flour quality, SRC sodium carbonate (Carbonate) assesses starch damage, SRC Sucrose indicates pentosan and gliadin contributions, and SRC Lactic acid evaluates the characteristics of glutenin (Gaines et al 2000, Gaines 2004). The SRC Water evaluation does not confer specific information about flour quality, but it helps to identify an overall optimum quality flour profile when taken in combination with the other SRC parameters (Kweon et al 2012).

Cookie diameter is generally considered the best assessment of soft wheat flour quality. Dough spread time and set time work in combination to produce the measured parameter of cookie diameter (Miller and Hoseney 1997). Depending on genotype, Guttieri et al. (2002) proposed that the spread of cookie dough during baking can be influenced by several different factors.

Optimum bread making is primarily controlled by gluten strength. The cohesive, viscoelastic dough and the ability of this dough to retain gas during fermentation is attributed to the gluten network (Hoseney 1998). The loaf volume is primarily a product of the gluten strength
and the variation in gas retention (Hoseney 1998). Several methods are used to estimate the gluten strength of bread dough, most commonly flour SDS sedimentation volume and Mixograph parameters.

The three main parameters in Mixograph evaluation are utilized to predict overall bread making performance: Mixograph water absorption, mixing time, and the calculated bake water absorption. Mixograph absorption evaluates the optimum flour water absorption and is a function of protein content, variety, flour moisture, and environmental influence (Finney and Shogren 1972). Mixograph mixing time assesses the time required to mix the bread dough components to the optimum condition for bread baking (Finney and Barmore 1945). The estimate of water required to create a dough of proper consistency for bread baking is evaluated using the bake water absorption (Finney 1945). Both bake water absorption and mixing time are influenced by flour protein content, and these three parameters in combination tend to predict loaf volume (Ohm et al 1998). The best predictive models for loaf volume incorporate grain or flour protein and the bake water absorption and bake mixing time (Dowell et al 2008).

Although some is already known about the role AX plays in milling, baking, and nutrition, this field of study is open to much more knowledge. Milling and baking in hard and soft wheat is a complex area of study in itself, and incorporating an understanding of the role of AX in these systems can aid the overall evaluation process of wheat for its target end-use quality. It is imperative to continue studying the influence of AX on end-use quality and their interactions with other molecules. This knowledge has the potential to increase milling and baking efficiency and further our understanding of AX benefits to human nutrition.
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CHAPTER TWO

A critical assessment of the quantification of wheat grain arabinoxylans using a phloroglucinol colorimetric assay

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of other that may also be suitable.

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ABSTRACT

Arabinoxylans (AX) of wheat (Triticum aestivum L.) play a critical role in processing, end-use quality, and human health and nutrition. Consequently, an efficient, accurate method of AX quantification is desirable. The objective of this work was to evaluate a standard phloroglucinol colorimetric method for quantification of wheat AX. The method is based on the formation and spectrophotometric quantification of a phloroglucide product, which results from the reaction of furfural produced during the condensation of pentose sugars with phloroglucinol. Method parameters, including reaction reagents and reaction times were varied to identify areas for improved accuracy and consistency. Phloroglucide formation at three xylose concentrations was examined over time. The optimal reaction reagents and reaction times were determined based upon improved consistency in xylose quantification. The optimized method was used on xylose and arabinose standards, and whole meal wheat samples for total and water-extractable AX content. Glucose was shown to be unnecessary in the reaction and was eliminated. A second order polynomial equation provided a slightly better fit to the nearly linear standard xylose curve. A reduced concentration of phloroglucinol of 10% was found to give equivalent results to the standard 20%. Optimum reaction time was 25 min, and required the inclusion of all reagents. The phloroglucide product decreased in absorbance over time such that within the range of xylose concentration examined, about 40-50% of the colored product was lost over 100 min; however, the rate of loss was linear over time. Four operators performed the optimized method on whole wheat meal samples for total and water-extractable arabinoxylans. Inter- and intra-operator variation were identified as an area requiring further study and improvement. However, all operators tended to rank the samples in a consistent manner. Compared to a gas
chromatography-flame ionization detection (GC-FID) method, the phloroglucinol method underestimated total AX by about 2.3% and water extractable AX by about 0.08%.
INTRODUCTION

The non-starch carbohydrates of cereal grains are important for food processing, end-product quality, and human health and nutrition. In wheat (*Triticum aestivum* L.) grain, non-starch carbohydrates are primarily associated with cell walls. Cell walls are comprised of up to 75% non-starch carbohydrates, which are mostly (85%) substituted pentose polymers referred to as arabinoxylans (Mares and Stone 1973). Other non-starch carbohydrates include cellulose, lignin, glucomannans, and β-glucans (Lineback and Rasper 1988).

Arabinoxylans (AX) are composed of a β-1, 4 linked D-xylopyranosyl backbone with substituted monomeric α-L-arabinofuranoside at the second and/or third carbon-positions. The arabinose moiety can possess ferulic acid at the fifth carbon-position (Courtin and Delcour 2002). The three-dimensional structure of AX is mainly determined by the length of the xylan backbone, the ratio of arabinose to xylose, the substitution pattern on the backbone, and the ferulic acid coupling to other AX molecules or the cell wall (Courtin and Delcour 2002). Arabinoxylans have a somewhat flexible structure (Dervilly et al 2000). These structural characteristics and large molecular weight (65,000 Da) (Andrewartha et al 1979) contribute to empirically-derived sub-fractions based on extractability (generally in water at room temperature), vis-à-vis, water-extractable (WEAX) and water-unextractable arabinoxylans (WUAX) (Courtin and Delcour 2002; Izydorczyk and Biliaderis 1992). Arabinoxylans also have the capacity to form oxidative cross-links and gel networks (Izydorczyk and Biliaderis 1992) and affect end-product quality (Bettge and Morris 2007; Ramseyer et al 2011a,b), thus increasing their overall molecular weight and exerting different effects on food processing and human physiology.
Arabinoxylans exhibit nutritive properties as well as influencing end-use quality (Lu et al 2000). The postprandial glucose concentration was decreased after healthy individuals ate bread products with AX as an additive (Lu et al 2000). Arabinoxylans added to bread products also lowered the glucose and insulin levels of the blood 2 h after consumption in individuals with Type II diabetes (Lu et al 2004).

There are several techniques to quantify AX, including colorimetric assays (Wheeler and Tollens 1889; Dische and Borenfreund 1957; Douglas 1981; Ford 1981; Bell 1985), and gas chromatography-flame ionization detection (GC-FID) (Gebruers et al 2010). Some structural characteristics of AX can be determined by GC-FID (Gebruers et al 2010), enzyme mapping (Saulnier and Quemener 2010), molecular weight distribution (Andersson et al 2010), FT-IR (Toole et al 2010), Raman spectroscopy (Toole et al 2010), fluorescence microscopy (Toole et al 2010), and carbohydrate-binding molecules (Toole et al 2010). For quantification, phloroglucinol has been the most commonly used colorimetric assay since its first description in 1889 (Wheeler and Tollens 1889) as it can measure five-carbon monosaccharides. The method was later extended to quantify hydrolyzed pentosans (Krober 1901, 1902). This general method was used with phloroglucinol as the reactant and a distillation step to capture furfural until 1957 (Dische and Borenfreund 1957), when the distillation was eliminated, and the reagent concentrations and timing evolved into the widely-used Douglas (1981) method. This method is among the quickest and most efficient methods for the quantification of total arabinoxylans (TAX) and water extractable arabinoxylans (WEAX). The biggest disadvantage observed in this method, however, was the loss of colored product over time, which decreased the accuracy of the test by roughly 20% in 60 min (Douglas 1981). Nevertheless, the method of Douglas (1981) has been particularly popular among cereal chemists. Based on information provided by ISI Web of
Science (Thomson Reuters, New York, NY), this method has been cited approximately 110 times. Rouau and Surgent (1994) developed a semi-automated version of the assay.

The phloroglucinol method of Douglas (1981) has been used to determine the arabinoxylan content of wheat flour (Hashimoto et al 1987; Biliaderis et al 1995; Izydorczyk et al 1991; Finnie et al 2006; Bettge and Morris 2000, 2007; Li et al 2009; Ramseyer et al 2011a), and barley flour (Izydorczyk and Dexter 2008; Izydorczyk et al 2003). Using this method, AX levels were shown to vary among wheat varieties (Finnie et al 2006; Li et al 2009) and flour mill streams (Ramseyer et al 2011a), and were influenced by growing environment (Finnie et al 2006; Li et al 2009). Arabinoxylan levels were related to changes in dough development time and viscosity (Jelaca and Hlynka 1971), and were associated with soft wheat end-use quality, including variation in cookie diameter (Bettge and Morris 2000).

The objective of this research was to evaluate the Douglas (1981) phloroglucinol method and determine ways in which it might be improved by varying components of the assay. Experiments were carried out to define the optimal reagents and timing of each step of the reaction to achieve more consistent and accurate results as well as evaluate the repeatability of the assay. The method was applied to whole wheat meal samples and results were compared with a standardized GC-FID method.

MATERIALS AND METHODS

Basic Method

The basic method of Douglas (1981) was conducted using the following procedure. Triplicate 2.0-mL aliquots each of a dilution series of xylose (Sigma-Aldrich, St. Louis, MO) were prepared to 0.0, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mg/mL, and were added to 12-mL
stopped reaction tubes (Pyrex tube, 16 X 100 mm, screw cap with PTFE liner). The assay can also be performed using arabinose as a standard with an identical absorbance response curve (see below). Water was added to each tube to bring the total volume to 2.0 mL; then 10.0 mL of the reaction reagent (110 mL glacial acetic acid, 2 mL hydrochloric acid, 5 mL 20% w/v phloroglucinol in ethanol, and 1 mL of 1.75% w/v glucose in water) was added to each tube. The tubes were then placed in a boiling water bath for 25 min, after which time they were removed, cooled in an ice bath, and moved to a room-temperature (ca. 22ºC) bath. The reaction is presented schematically in Figure 1. The tubes were removed, laid horizontal and covered with aluminum foil. Absorbance of the samples was read at 558 and 505 nm using an autosampler (1.0 mL) attached to a BioSpec-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). The absorbance reading at 505 nm was subtracted from 558 nm to remove the influence of hexose sugars (Douglas 1981). Absorbance readings of all 21 tubes required about 15 min. Values of unknown AX content can be interpolated using the second-order polynomial equation derived from the standard xylose curve. This procedure does not include a correction factor for molecules of water released during AX hydrolysis.

**Inclusion of Glucose**

The basic method was performed but without glucose in the reaction reagent. The reaction reagent components of acetic acid, hydrochloric acid, and phloroglucinol were held constant; the two different treatments were to add 1.0 mL of glucose solution (1.75% w/v aqueous) or 1.0 mL H₂O. Three replications of each xylose concentration were used.

**Absorbance Response vs. Xylose Concentration**
The basic method was carried out as previously described and with water replacing glucose. The standard curve spanning 0 to 0.30 mg xylose/mL was assayed and absorbance response was modeled. Least squares regression was used to calculate the fit of each line for absorbance-concentration response.

**Phloroglucinol Concentration**

The basic method was followed wherein the proportion of reaction reagents acetic acid, hydrochloric acid, and water (substituted for glucose) were held constant, but the concentration of phloroglucinol in ethanol was varied at 10%, 20%, and 40% (w/v). Henceforth, all concentrations will be referred to by percentage of phloroglucinol. The reaction procedure was carried out as described for the basic method.

**Variation in Reaction Time**

The basic method was used, but the phloroglucinol concentration was reduced to 10%. Once the reaction reagent was added to the standard xylose solutions, four different (reaction) times in the water bath were used: 15, 25, 35, and 45 min. The experiment was then continued as described for the basic method.

A second experiment was performed to determine the effect of varying reaction times prior to the addition of phloroglucinol. As described in the basic method, reaction reagent containing acetic acid, hydrochloric acid, and water was added to the xylose standards. The tubes were placed in a boiling water bath for 15, 25 and 45 min. The test tubes were removed from the boiling water and immediately placed in an ice bath. Once cool, phloroglucinol was added to each tube and the tubes were returned to a boiling water bath for 25 min. The tubes were then
removed, cooled in an ice bath, and brought to room temperature in a room temperature water bath. The colorimetric analysis was then carried out as previously described.

**Reaction Product Absorbance Loss Over Time**

Eleven tubes each of 0.10, 0.20, and 0.30 mg xylose/mL, and three tubes of a zero xylose standard were prepared. The experiment was carried out as previously described in the basic method, with water in place of glucose, and the 20% w/v concentration of phloroglucinol in ethanol, with a reaction time of 25 min in a boiling water bath. Immediately after removing the tubes from the room temperature water bath, the three tubes of the zero xylose standard were measured. At time zero (< 2 min following removal from the water bath) the absorbance of one of each of the xylose concentrations (0.10, 0.20, and 0.30 mg) was measured. Every 10 min following this, the absorbance of one tube each of the three concentrations was again measured until 100 min.

**Comparison of Operators – Whole Wheat Meals**

The whole wheat-meal sample preparation and sampling was performed as described by Finnie et al (2006). Twelve commercially grown, varietal wheat samples were provided by Dr. Laura Hansen (General Mills Inc.). Samples included two soft white spring, seven soft white winter, two club, and one hard red winter wheat varieties. With the exception of the hard red winter, all the varieties were developed in and adapted to the Pacific Northwest states of Idaho, Oregon and Washington. Sample origin was south-central Idaho. Samples were assayed in duplicate, by adding 125 mg meal to a 50-mL conical screw-cap polypropylene tube (Fischer Scientific, Pittsburgh, PA or equivalent). To each tube, 25.0 mL water was added and the
mixture was suspended by vortexing (ca. 7 s). After mixing, duplicate 1.0-mL aliquots of the slurry were rapidly withdrawn (before settling) with a modified large-aperture (~3 mm) pipette and added to 12-mL stoppered reaction tubes. These samples were used to quantify the TAX content of the meals. The conical tubes containing hydrated meals were then placed on a laboratory rocker (model AR-100, PGC Scientific, Gaithersburg, MD) for 30 min and then centrifuged for 10 min at 2500 X g. Duplicate 1.0-mL aliquots of the supernatant were added to stoppered reaction tubes, representing the WEAX content of the samples. These samples were then assayed using the optimized procedure (no glucose and 10% w/v phloroglucinol in ethanol in the reaction reagent). The assay was performed by four operators who were trained identically and were assessed to perform the assay proficiently. Each operator had at least five months of experience in performing the assay prior to undertaking the TAX and WEAX quantification with these 12 “standard” whole wheat meal samples.

**Analysis of Non-Cellulosic Sugar Content and Composition Using Gas Chromatography**

Whole wheat meal samples were derivatized and analyzed by the method described by Englyst and Cummings (1984) with some slight modifications (Courtin et al 2000). Through this method, both TAX and WEAX were quantified. The samples were first hydrolyzed and subsequently derivatized to alditol acetates before injection into a capillary column with a split-splitless injector. The samples then entered the flame ionization detector. The column used in the GC was a Supelco SP-2380 polar column (Supelco, Bellefonte, PA, USA) in an Agilent 6890 Series Chromatograph (Wilmington, DE) with an autosampler and splitter injection port (injection volume: 1.0 µL; split ratio 1:20). The flame ionization detector used helium as the
carrier gas and separation at 225 °C, while injection and detection were at 270 °C. This procedure was performed at the Laboratory of Food Chemistry and Biochemistry, University of Leuven.

Data Analysis

All data were analyzed using SAS 9.2 (SAS Institute, Cary, NC). The general analyses were done using PROC GLM. Three replicates of standard xylose (or arabinose) were used, and duplicates as well as replicates were used for whole wheat meal samples—resulting in four samples representing each whole wheat meal variety.

RESULTS

Inclusion of Glucose

The objective of this portion of the study was to determine the necessity of glucose as a reagent. Douglas (1981) provided no justification or explanation as to its inclusion in his method. We were unable to identify why glucose would be necessary for the reaction itself, for the development or stability of the colored product or for the spectral analysis. Consequently, we performed the standard assay with and without glucose using a range of xylose concentration from zero to 0.30 mg/mL.

The ANOVA of the results indicated that 98% of the variation could be explained among replicates, xylose concentration, and presence/absence of glucose (whole model $R^2$). Comparing the two treatments, viz. reaction with or without glucose, indicated no significant effect on absorbance (therefore ability to measure xylose content) (Table I). This result provided evidence that glucose was unnecessary in the reaction reagent and that the development of colored product (and absorbance) was the same when water was substituted. The concentration range of xylose
was chosen to encompass a wide range of quantity/absorbance, commensurate with levels encountered in wheat samples. The ANOVA interaction observed between xylose concentration and presence/absence of glucose indicated some non-parallelism of response slopes. Upon closer examination it was seen that the absorbance of the solutions containing water was greater than the absorbance of the solutions containing glucose at both the 0.25 and 0.30 mg xylose concentrations by 11 and 10%, respectively (Fig. 2).

**Absorbance Response vs. Xylose Concentration**

The curve-fit portion of the experiment was conducted because it was observed that the standard curves consistently deviated from linear at the highest xylose concentrations. The fit of the standard curve is of utmost importance when interpolating data. Linear and second-order polynomial curves were fit to the xylose concentration series (Fig. 3). The linear equation was $y = 2.23x + 0.053$ with an $R^2$ value of 0.984. The polynomial curve fit had an equation of $y = 3.25x - 3.39x^2 + 0.011$ with an $R^2$ value of 0.992. While both equations described the standard curve quite well, the polynomial curve fit slightly better. It has been consistently observed that as the xylose concentration increases beyond 0.15 up to 0.30 mg/mL, the absorbance drops off slightly, whereas at zero and 0.05, 0.10, and 0.15 mg/mL the relationship is fairly linear (Fig. 3 and data not shown). The present data are consistent with these past observations. A subsequent experiment compared the concentration – absorbance response of arabinose to xylose. The results (data not shown) indicated the two monosaccharides produced identical absorbance response curves such that either could be used as the standard.

**Variation in Phloroglucinol Concentration**
The phloroglucinol concentration was varied to determine whether it was a limiting reagent, especially at the higher concentrations of xylose. As shown in Figure 4, three concentrations of phloroglucinol, each a two-fold change from the standard 20%, were used in the reaction reagent and compared using five xylose concentrations. Over the entire range of xylose, phloroglucinol concentrations of 10% and 20% were observed to produce similar absorbances, whereas the absorbance at 40% phloroglucinol was markedly lower. Based on these results, it can be concluded that from 0.0 to 0.30 mg/mL xylose, a phloroglucinol concentration as low as 10% can be used without limiting the reaction, yielding similar results as the traditionally used concentration of 20% phloroglucinol (Douglas 1981). In addition, 40% phloroglucinol was clearly disadvantageous and significantly hindered the efficacy of the method (i.e. concentration response – color reaction).

Variation in Reaction Time

The objective of the reaction time variation experiment was to determine the optimal time that the reaction tubes should spend in the water bath; i.e., sufficient time for the reaction to occur while limiting the time for the reaction products to degrade. The reaction occurs in two parts: hydrolysis of the arabininoxylan to pentose and then into furfural, and condensation reaction of the furfural with phloroglucinol to form the pink-red phloroglucide precipitate (Leach 1905) (Fig. 1).

Figure 5 depicts the four reaction times across a range of seven xylose concentrations. Overall the absorbance values were consistently greatest at 25 min, but decreased at 15 and 35 min, and were lowest at 45 min. Based on these results, the optimal water bath time was selected as 25 min for the concentrations of xylose used, consistent with the method of Douglas (1981).
The reaction times were also varied at 15, 25 and 45 min prior to the addition of phloroglucinol. A standard curve was generated; however, the variation of this experiment was great, and no clear pattern was observed (data not shown). The coefficient of variation of the absorbance response observed throughout this portion of the experiment was 17%, while the coefficient of variation for the previous water bath experiment was only 4%, showing more than a four-fold difference in variation between experiments. We concluded that the phloroglucinol must be present and available for reaction with the generated furfural during the condensation reaction step.

**Reaction Product Absorbance Loss Over Time**

Douglas (1981) observed that there was a 20% decrease in absorbance over 60 min (loss of colored product). Because this method may be performed with a varying number of reaction tubes (often a greater number of assays is desired), it is important to determine the rate at which the colored reaction product decreases in absorbance over time.

When three concentrations of xylose (0.10, 0.20, 0.30 mg/mL) were evaluated for loss of absorbance over 100 min, all three showed a significant decrease (Fig. 6). A linear rate function was fit to each xylose concentration over time and adequately modeled the phenomenon. At 0.30 mg xylose the equation was \( y = 0.613 - 0.00251x \), where \( y \) is the absorbance and \( x \) is the time in min. At 0.20 mg xylose, the color loss rate was \( y = 0.447 - 0.00197x \), and at 0.10 mg xylose the rate was \( y = 0.1924 - 0.000914x \). These data show the general pattern for decrease in absorption over time as well as highlighting the differences in rate of absorption loss, which is dependent on xylose (phloroglucide) concentration. In this regard, the highest concentration of xylose had the greatest absolute rate of color loss (negative slope), and conversely, the lowest xylose
concentration lost color at the slowest absolute rate. When expressed as a proportionality, all xylose concentrations lost on the order of about 40-50% of their colored product over 100 min (48%, 44% and 38%, 0.1, 0.2 and 0.3 mg xylose/mL, respectively) (Fig. 6).

An equation can be developed to describe this decrease in absorbance. Figure 7 illustrates the combined rate loss function derived from the three xylose concentrations shown in Figure 6. By adding a 0,0 data point, the linear least squares fit produced the following equation: $y = 0.00859x + 0.000061$, wherein $y$ = the rate of absorbance loss per minute per mg xylose, and $x$ is the original xylose concentration.

**Comparison of Operators**

Many experimental procedures are performed by more than one person and the objective of this portion of the experiment was to determine how consistent this procedure was when comparing a set of samples across different operators using the exact same analytical procedure. As shown in Table II, there were four experienced laboratory technicians who measured the TAX and WEAX content of 12 whole wheat meal samples in the same laboratory and using all the same equipment and reagents. All were provided some training to follow the same procedure. The amount of variation attributable to the samples for each of the operators varied for the TAX measurement, as quantified by calculating the $R^2$ values, which ranged from 0.62 to 0.86. This result indicates that 14-38% of the variation could not be accounted for by the model (error variance). The within operator range of TAX concentration for the 12 whole wheat meal samples (highest sample mean minus lowest sample mean) varied from 1.11% (Operator 4) to 2.42% (Operator 1). Operators 1 and 4 obtained a level of precision that declared different wheat samples to be significantly different ($P$-values <0.003). The respective least significant
differences \((P = 0.05)\) were 0.82\% and 0.40\% TAX, respectively. Operator 2 was near the limit
for declaring the highest and lowest means different, whereas the results of Operator 3 returned
no significant differences among the wheat samples.

The WEAX measurements also varied among operators, ranging from 0.33\% to 0.50\%
(means across all 12 samples). Two of the four operators (Operators 2 and 4) showed clear mean
separation between the varieties \((P<0.0004)\). The least significant difference for both was 0.04\%
WEAX. Operators 1 and 3, however, were near the threshold of significance with \(P\)-values of
0.053 and 0.049, respectively. An analysis of variance was carried out, combining all four
operators and all sample results, to determine the effects of operator, sample, and any interaction
between sample and operator. When the operator and sample were analyzed for an interaction,
the \(P\)-value for TAX was 0.20 and for WEAX 0.48. Overall, the rank order among samples was
similar for all four operators.

**Comparison of Phloroglucinol vs. GC-FID Methods**

The AX content of the 12 whole wheat meal samples was also measured via gas
chromatography-flame ionization detection. The purpose of this portion of the experiment was to
compare the two fundamentally different methods to determine how closely the measured values
agreed. The percentage of TAX observed from the GC-FID method (6.48\%) was markedly
greater than that detected in the colorimetric phloroglucinol method (4.20\%) (means across 12
samples and across four operators) (Table II and *data not shown*). The percentage of WEAX
observed in the GC-FID and phloroglucinol colorimetric methods also varied considerably with
the GC-FID having an overall average of 0.48\% as compared to 0.40\% from the phloroglucinol
method. The TAX content was also evaluated on a GC-FID at the Western Wheat Quality Lab
using the same laboratory procedures and instrument settings as at the Laboratory of Food Chemistry and Biochemistry. Results were consistent between the two GC-FIDs with means across the 12 samples differing by 0.12% TAX (data not shown). It should be noted that the GC-FID procedure extracts WEAX at 7°C, whereas the phloroglucinol method extracts at room temperature. A side experiment using low (0.42%) and high (0.55%) WEAX whole wheat meal varietal samples indicated that room temperature extraction increased WEAX by about 8-9 percentage points (data not shown).

In the TAX quantification, the difference between the GC-FID and phloroglucinol methods was twice (in magnitude) that of the differences between operators in the phloroglucinol method. In the WEAX quantification, the difference between the GC-FID and phloroglucinol methods was slightly over half of the difference between operators in the phloroglucinol method.

DISCUSSION

Analytical procedures are founded on accuracy and precision. In the present series of studies, we evaluated individual parameters of a popular phloroglucinol colorimetric assay (Douglas 1981) for pentose sugars with application to the measurement of arabinoxylans in wheat and other cereals. The first parameter examined, glucose, was included in the original assay described by Douglas (1981) with no stated justification. Based on our results (Table I), glucose had no significant effect on the measurement of xylose. Consequently, we concluded that Douglas’ objective was to demonstrate that the subtraction of absorbance at 505 nm from 558 nm effectively removed any interference of hexoses, thereby verifying that the phloroglucinol method could accurately quantify pentoses in the presence of hydrolyzed starch. For wheat in particular, any method that hydrolyzes all grain or flour carbohydrates must
accommodate large quantities of glucose (arising from starch). Rouau and Surget (1994) included glucose in their semi-automated phloroglucinol ‘Douglas’ system and also showed that it had no effect on pentose measurements, but made no suggestion to eliminate it from future analyses. In the present study, an equivalent volume of water was used in place of glucose solution so as to maintain consistent volumes and concentrations of the other reagents used in the reaction mixture.

The second feature of the phloroglucinol method involved the relationship between analyte concentration and product concentration (in this case absorbance of phloroglucide). When absorbance was plotted against xylose concentration (Fig. 3), there was a slight but consistent deviation from linearity at the higher concentrations. Proportionally, there was a slightly lower absorbance response per unit of xylose at the highest concentrations. Douglas (1981) and Rouau and Surget (1994) also observed a drop in absorbance at higher pentose concentrations. As expected, a second-order polynomial, when fitted to our data, captured this slight curve with a small, but appreciable increase in $R^2$ as compared to a linear fit.

The phloroglucinol concentration in the original assay (Douglas 1981) appeared to be excessive from a molar-based, theoretical basis (assuming two phloroglucinol molecules per furfural as the chemical formulation and reaction would indicate). At the lowest concentration (10%), phloroglucinol was easily dissolved in ethanol, which was not the case at the higher concentrations. The reduced 10% concentration did not cause an absorbance response difference as compared to the original phloroglucinol concentration of 20% (Fig. 4). Reducing the phloroglucinol concentration by half saves cost, and is easier to prepare. The highest phloroglucinol concentration examined (40%) was clearly detrimental to the assay, and the reagent was difficult to prepare.
Reaction time is important as the arabinoxylan must be reduced to constituent sugars, and the pyranose ring of xylose and arabinose converted to furfural. The furfural molecule then donates electrons to two molecules of phloroglucinol, which condense to form phloroglucide. This phloroglucide precipitate is pink/red in color, and is the molecule analyzed spectrophotometrically. As seen in Figure 5, the reaction time appeared to be optimum at 25 min. However, it was imperative that the hydrolysis and phloroglucinol-phloroglucide reaction occur together in the same reaction vessel, as opposed to hydrolyzing the sugars (or potentially the AX polymer) first, and subsequently reacting the resulting furfural with phloroglucinol. The complete reaction appears to proceed in a concerted chain, and it is imperative that there are no interruptions in any of the steps. We conclude that the phloroglucinol must be available to react with the furfural as it is generated to produce the phloroglucide product. Rouau and Surget (1994), on the other hand, hydrolyzed the TAX in a separate pre-hydrolysis step with sulfuric acid, and indicated that it was the phloroglucinol mixed with acid that was unstable. In our preliminary studies, inclusion of this pre-hydrolysis step produced dramatically lower TAX levels. Conversely, Rouau and Surget (1994) stated that using the Douglas (1981) acid reagent, “underestimated pentosan values”. The type of acid(s) and hydrolysis may deserve further study.

The loss of absorbance over time (Fig. 6) illustrates one of the primary limitations of the phloroglucinol method. Consequently, time and the number of samples must be considered when planning AX quantification using phloroglucinol. The colored product apparently breaks down fairly rapidly, but linearly and in predictable fashion over time. This result suggests that one approach may be to limit the reaction to a smaller number of samples, or introduce a correction factor based on the rate of absorbance loss. The percentage (proportionality) of absorbance loss decreased slightly with increasing xylose concentrations, as is seen in Figure 6. Of interest, when
this color rate loss was plotted, it produced a reasonably linear function (Fig. 7), suggesting that absorbance loss could potentially be accounted for.

With a standard laboratory assay, the need for consistency between operators is often crucial. Inter- and intra-operator consistency appears to be a significant issue with the Douglas (1981) method. Across all 12 whole wheat meal samples, mean TAX varied from 3.60 to 4.66%, with error variance ranging up to 38% (100 - model $R^2$). Variety rankings, however, were quite consistent across operators. Among Operators 1-3, operator means varied by no more than 0.06% TAX. WEAX measurements overall showed greater whole model $R^2$ values and more similar results between operators. This result may indicate that the soluble fraction of AX is more consistently and accurately captured by the phloroglucinol method. In the analysis of the WEAX results, the four operators tended to have greater levels of mean separation among varieties, but no consistent operator-dependent trend was evident (TAX vs. WEAX). As noted above, in the combined analysis there was no significant interaction between operator and sample. It should be noted that WEAX concentrations are on the order of tens times less than TAX concentrations. Given the ANOVA and statistical power to separate means, the results suggest that WEAX is inherently much more reliably measured.

One feature of TAX that may reduce its consistent measurement is the highly heterogeneous and particulate nature of ground whole wheat meals which must be sampled from aqueous suspension. The samples were vigorously vortexed and an aliquot was withdrawn as quickly as possible, always at the same height within the tube. In addition, the pipette tip was cut off to effect a larger orifice in order to improve speed. The error surrounding the TAX measurement is nevertheless seen as a notable weakness of the assay. While there were efforts made to attain a homogeneous mixture in the reaction vessel, the TAX solution was, in fact, still
a slurry (i.e., suspended particles). This is hypothesized to be the source of some portion of the underestimation of TAX content. However, considering the variation of the WEAX data as well, sampling does not appear to be the entire issue. As mentioned previously, a pre-hydrolysis step with sulphuric acid (Bell, 1985) was performed, but resulted in an even greater underestimation of TAX levels.

The quantification of pentoses and AX can be performed in multiple ways. When quantified using GC-FID, absolute values were greater than those obtained through the spectrophotometric assay. This suggests that cross-comparison between assays may be an important consideration for interpreting published values and for planning future research. Differences between the two methods may be due, in part, to the chemical reactions that are taking place in the two assays. The sample preparation and manner in which the samples are analyzed are markedly different, and this could contribute to differences in the quantification of AX. Whereas the phloroglucinol method depends upon a chain-like series of chemical reactions and indirect measurement, the GC-FID analysis is a more direct analysis of a product that more closely resembles the starting chemical structure. Other possible sources of underestimating AX by phloroglucinol may include incomplete hydrolysis of the AX molecule into pentose sugar constituents to complete the reaction, as well as the immediate degradation of the colored phloroglucinol product upon completion of hydrolysis. There are conceptually several different issues to consider: the hydrolysis of liberated arabinose and xylose and their reaction with phloroglucinol, the liberation of bound AX from seed tissue, and the particle size, tissue composition and general “accessibility” of AX for reaction. Suffice to say that the procedure is highly empirical, but we have identified areas of problems and improvement in the basic
Douglas method. However, the GC-FID analysis is more demanding in terms of time and instrument cost.

**CONCLUSION**

The commonly used AX colorimetric quantification method of Douglas (1981) was successfully modified to increase accuracy by determining a closer relationship between xylose concentration and absorbance, and becoming more aware of sample product decomposition over time (loss of color). The original method (Douglas 1981) was validated in the hydrolysis time, stressing the importance of the specific time outlined. The assay was simplified by eliminating glucose, as well as by decreasing the phloroglucinol concentration, and thus, allowing the solution to solubilize with greater ease. Instability of the colored phloroglucide product was confirmed as a significant source of variation. Operator-to-operator variation was observed to be a significant issue that must be considered at the outset before performing the assay. Apparently there are as-yet unidentified sources of technique variability that limit the absolute value and repeatability of AX measurement. Multiple operators did tend to rank wheat samples similarly, however. Therefore within a study or lab, rankings and differences amongst samples are likely reliable. Increasing replication should provide greater statistical robustness. The phloroglucinol method apparently underestimates total and water extractable arabinoxylan contents compared to the GC-FID method.
ACKNOWLEDGMENTS

We would like to acknowledge the technical staff of the USDA-ARS Western Wheat Quality Laboratory. This project was supported by the Agriculture and Food Research Initiative Grant 2009-02347 from the USDA National Institute of Food and Agriculture.
LITERATURE CITED


Izydorczyk, M. S., Jacobs, M., and Dexter, J. E. 2003. Distribution and structural variation of


**TABLE I**

ANOVA of a phloroglucinol colorimetric assay for pentoses using xylose at seven concentrations, and with or without glucose in the reaction (treatment)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole model</td>
<td>13</td>
<td>0.1382</td>
<td>179.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Xylose concentration</td>
<td>6</td>
<td>0.2977</td>
<td>386.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.0013</td>
<td>1.69</td>
<td>0.201</td>
</tr>
<tr>
<td>Concentration x Treatment</td>
<td>6</td>
<td>0.0023</td>
<td>2.94</td>
<td>0.018</td>
</tr>
</tbody>
</table>

<sup>a</sup>Degrees of freedom
TABLE II

Comparison of four operators measuring the total arabinoxylan content and water-extractable arabinoxylan content of 12 whole wheat meal samples using an optimized phloroglucinol colorimetric method for pentoses

<table>
<thead>
<tr>
<th>Variety</th>
<th>Total Arabinoxylan</th>
<th>Water-Extractable Arabinoxylan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operator 1</td>
<td>2</td>
</tr>
<tr>
<td>Alpowa</td>
<td>5.92</td>
<td>4.54</td>
</tr>
<tr>
<td>ID0587</td>
<td>5.02</td>
<td>3.71</td>
</tr>
<tr>
<td>Madsen</td>
<td>4.97</td>
<td>4.96</td>
</tr>
<tr>
<td>Finch</td>
<td>4.68</td>
<td>4.44</td>
</tr>
<tr>
<td>Stephens</td>
<td>4.52</td>
<td>4.22</td>
</tr>
<tr>
<td>Alturas</td>
<td>4.51</td>
<td>3.85</td>
</tr>
<tr>
<td>Brundage</td>
<td>4.47</td>
<td>4.41</td>
</tr>
<tr>
<td>Jagger</td>
<td>4.40</td>
<td>4.29</td>
</tr>
<tr>
<td>Simon</td>
<td>4.04</td>
<td>3.49</td>
</tr>
<tr>
<td>Hiller</td>
<td>3.95</td>
<td>3.81</td>
</tr>
<tr>
<td>Coda</td>
<td>3.52</td>
<td>3.49</td>
</tr>
<tr>
<td>Brundage 96</td>
<td>3.49</td>
<td>3.88</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.86</td>
<td>0.72</td>
</tr>
<tr>
<td>Overall mean</td>
<td>4.46</td>
<td>4.09</td>
</tr>
<tr>
<td>$F$-value</td>
<td>6.48</td>
<td>2.74</td>
</tr>
<tr>
<td>$P$-value</td>
<td>0.0016</td>
<td>0.0485</td>
</tr>
</tbody>
</table>
Figures

**Chemical Reaction**

![Chemical Reaction Diagram](image)

**Fig. 1.** Reaction scheme of the conversion of a monosaccharide (xylose) into furfural, and subsequent reaction with phloroglucinol to yield the colored precipitate phloroglucide.

![Absorbance vs Xylose](image)

**Fig. 2.** Comparison of a phloroglucinol colorimetric reaction for pentose sugars with or without the inclusion of glucose. Glucose inclusion is represented by a solid line, and substitution with water is represented by a dotted line.
Fig. 3. Comparison of linear and polynomial fits to the standard xylose curve. The linear equation was $y = 2.23x + 0.053$, with an $R^2$ value of 0.984. The polynomial equation fit to the line was $y = 3.25x - 3.39x^2 + 0.011$ with an $R^2$ value of 0.992.

Fig. 4. Comparison of phloroglucinol concentrations. The concentration previously used (Douglas 1981) was 20% phloroglucinol (w/v). Three concentrations were compared: 10% (■), 20% (▼), and 40% (●) w/v.
Fig. 5. Comparison of four water bath (hydrolysis) times at seven xylose concentrations. The times used were 15 (▼), 25 (■), 35 (♦), and 45 min (●).

Fig. 6. Loss of colored phloroglucide product over time at three xylose concentrations, 0.1 (●), 0.2 (▼), and 0.3 mg (■), each sample was an independent experimental unit, absorbance was measured every 10 min for 100 min.
Fig. 7. Relationship of colored phloroglucide product loss over time as a function of beginning xylose concentration. Abscissa values are derived from the decay slopes of Figure 5, a 0,0 data point was added. The linear equation is \( y = 0.00859x + 0.000061 \), where \( y \) is the rate of color loss as absorbance units per min per mg beginning xylose, and \( x \) is the beginning xylose amount in mg per reaction.
CHAPTER THREE

A Comprehensive Survey of Soft Wheat Grain Quality in United States Germplasm

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of other that may also be suitable.

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ABSTRACT

Wheat (*Triticum aestivum* L.) quality is dependent upon both genetic and environmental factors, which work in concert to produce specific grain, milling, flour and baking characteristics. This study surveyed all of the 132 soft wheat varieties (cultivars and advanced breeding lines) grown in the U.S. Regional Nursery system, which encompassed the three main soft wheat producing regions of the United States (eastern and southern soft red winter and western soft white). The quality parameters included test weight, kernel hardness, weight and diameter, wheat and flour protein, polyphenol oxidase, break flour yield, flour yield, flour ash, milling score, Flour Swelling Volume, flour SDS sedimentation volume, solvent retention capacity (SRC) water, carbonate, sucrose and lactic acid, Rapid ViscoAnalyzer peak-pasting viscosity, and cookie diameter. High levels of variation were observed among varieties, regions and specific environments, with environment being in general a much greater source of variation than varieties. Variety was observed to have a relatively stronger influence on wheat quality in the western nurseries, compared to the eastern and southern regions where location effects had a stronger impact on overall wheat quality. The greater influence of variety was particularly notable for kernel hardness in the western nurseries. Kernel hardness also varied considerable due to environment. For the two soft red winter wheat nurseries, the western U.S. environment produced substantially harder kernels (37-40) compared to the same varieties grown in eastern U.S. locations (15-20). Inter-trait quality relationships were observed to be unique to the specific nursery and germplasm in which they were studied and these relationships were not consistent across nurseries. Nevertheless, on average, soft wheat quality was fairly similar across the U.S. indicating that breeding and testing models have been successful in achieving a relatively uniform target for quality. However, many traits showed high levels of variability among
varieties suggesting that a greater level of selection for end-use quality would benefit end-users by increasing consistency/reducing variability. The often large role of environment (location) in quality indicates that end-users must be assiduous in their origination and grain procurement. Clearly, ‘nursery mean’ quality does not reflect the potential that can be obtained as reflected by a few exceptional soft wheat varieties.
INTRODUCTION

Since the advent of agriculture, humankind has strived to improve crops. For wheat (Triticum aestivum L.), this activity accelerated with the adoption of cross breeding near the end of the 19th century (Bonjean and Angus 2001). Today, wheat breeders begin efforts towards end-use quality improvement with parent selection, and then evaluate progeny in any generation from the F2 onwards. In practice, most end-use quality testing and selection begin in earnest in the F5 or F6 generation, and then continues for 4-6 years through the cultivar release process. In the United States, the most advanced-generation testing employs regional cooperative nurseries. These nurseries are market class and region specific, and are amalgamations of advanced lines from all cooperating breeders. Nurseries are grown at multiple locations and grain from one or more may be evaluated for end-use quality, historically at regional quality laboratories of the U.S. Dept. of Agriculture.

AACC International has long been involved in the standardization and advancement of end-use quality testing methods and procedures. Even so, universal standardization of all wheat quality testing is not fully attainable, especially as regards milling and baking evaluations. Consequently, to be directly comparable, data must derive from tests performed in a single laboratory.

Wheat quality as perceived by the miller or baker is a product of both genetic (G) and environmental (E) influences. Baenziger et al. (1985) found that genotype, environment and their interaction were all highly significant sources of variation on milling and baking quality of soft red winter wheats grown in the southeastern U.S. However, variety performance for any given environment was highly correlated with variety performance across all environments, suggesting that rank order remained similar. Peterson et al. (1992, 1998) similarly found significant effects
of G, E, and GxE on hard red winter wheat quality. Although varieties differed in their “response to environment,” no indication of rank change was presented. Guttieri et al. (2002) found that in two soft wheat cultivars, environment played a larger role in flour quality variation than did genotype, although both environment and variety had strong, independent influences on flour quality. Environmental influences had a varying impact on specific quality traits, as seen in a study performed in multiple environments with many varieties in Kazakhstan and Siberia (Gómez-Becerra et al 2010). In a study in Austria, some genotypes showed end-use quality stability for one particular trait, but instability for another, showing that the interplay between genetics and environment varied between measured traits (Grausgruber et al 2000). A similar phenomenon was evident in Mediterranean-grown wheat (Rharrabti et al 2003).

Comparisons between varieties are most often performed on sets of varieties grown in specific regions or areas of similar climatic and edaphic conditions. Morris et al. (1997) highlighted the potential consequences of confining wheat quality studies to a small environmental area. This study also called attention to the variable impacts of environment depending on the specific varieties being evaluated. In most studies, environmental variation was evaluated within a region of general varietal adaptation. Morris et al. (2004, 2005) examined U.S. soft wheat quality by growing both western and eastern U.S. soft wheat cultivars at two western (Washington State) locations. Consequently, the analysis provided primarily genetic comparisons and indicated varietal differences within and between regional gene pools. However, in these studies the quality of eastern varieties grown in their general area of adaption was not determined.

Wheat quality may be sub-divided into three main categories: grain, milling, and flour/baking quality. Individual traits, however, must be considered in the context of the desired
processing and end-use functionality of the grain and flour (Morris and Rose 1996). Wheat varieties have been well-documented to exhibit genetic differences in physical, biochemical, and end-use quality (Ram and Singh 2004; Morris et al 2004, 2005; Peterson et al 1992, 1998; Baenziger et al 1985). While these three main categories of wheat quality may be assessed separately, there are opportunities for predictive relationships and correlations between the components of each.

Kernel hardness is among the most fundamental factors that determine how wheat will perform in various end-use applications (Morris and Rose 1996). Kernel texture is genetically controlled with limited direct relationship to protein content (Simmonds 1974; Morris 2002; Bhave and Morris 2008). During milling, hard and soft wheats perform vastly differently: hard wheats tend to fracture at cell walls, whereas soft wheats fracture through the cell (Hoseney et al 1988). These differences in fracture patterns lead to smaller particle size distribution in soft wheat flour with less starch damage (Hoseney et al 1988). Hard wheats are favored for breads, while soft wheats are used for cookies, cakes, pancakes, and other similar products (Huebner et al 1999). Within the soft wheat class, a significant amount of kernel texture variation was observed (Gaines et al 2000; Morris et al 2004, 2005). Other grain characteristics such as kernel weight, size and morphology can influence end-use quality, especially milling performance (Morris and Rose 1996).

Wheat grain protein content plays a major role in determining functionality of flour. Hard wheat grain generally has higher protein levels which contribute to gluten formation and optimum bread-baking. Most soft wheat products do not need as extensive gluten-protein formation, and thus, high protein levels are undesirable in most soft wheat products (Gaines 2004). A lower protein level is particularly critical for optimum cookie set time with soft wheat
flour (Miller and Hoseney 1997). Along with protein content, protein composition is highly influential on soft wheat product quality (Huebner et al 1999). Whereas protein content is largely environmentally controlled (including soil fertility), protein composition is essentially genetically controlled.

Another important category of flour quality relates to water absorption/water relations. Water absorption may be controlled by many factors, including damaged starch, soluble starch, pentosan levels, flour particle size, and protein content and composition. Damaged starch releases soluble dextrins when water is added to flour. Along with damaged starch, this soluble starch increases dough viscosity, and consequently decreases cookie diameter (Miller and Hoseney 1997). A strong relationship has been observed between damaged starch and flour particle size, i.e. flours with larger particle size tend to have higher levels of damaged starch (Yamamoto et al 1996). In this way, soft wheats for cookies, cakes and pastries would ideally have finer particles, also indicating less damaged starch.

Another approach to examining flour water relations while estimating some underlying physical-chemical traits, is to use the “solvent retention capacity” (SRC) system. SRC tests are effective predictors of end-use quality, primarily applied to soft wheat flour (Gaines et al 2000; Guttieri et al 2001; Guttieri and Souza 2003; Ram and Singh 2004; Moiraghi et al 2011; Kweon et al 2011, 2012; Souza et al 2012). SRC Water is a general measurement of overall flour quality, SRC sodium carbonate (SRC Carbonate) indicates starch damage, SRC Sucrose reflects contributions from pentosans and gliadins, and SRC Lactic acid relates to the characteristics of glutenin (Gaines et al 2000, Gaines 2004). Whereas SRC Water does not appear to convey as much specific detail about flour quality as do the other three SRCs, it tends to aid in identifying an overall optimum-quality flour “profile” when combined with the other SRC parameters.
A low SRC Sucrose response in addition to indicating low pentosans, may also indirectly aid in selection of varieties with high flour yield (Souza et al 2012). Guttieri et al. (2002) found that flour protein was well-correlated with SRC Sucrose and SRC Lactic acid measurements, and within a cultivar, an increase in flour protein resulted in an increase in SRC Lactic acid. An important caveat to this finding was that the correlations between flour extraction and SRC measurements within a variety across environments may be specific to the variety being studied (Guttieri et al 2002). However, in their study there was only a small genotype-by-environment interaction observed, and this effect had considerably less impact than did genotype-by-growing year interactions (Guttieri and Souza 2003). Recent findings suggest that while each individual SRC measurement is useful for quality assessment, the combination of all four parameters may provide a better predictive model for end-use quality characteristics (Kweon et al 2011; Ram and Singh 2004).

Cookie diameter is often considered the best overall assessment of soft wheat flour quality. Cookie diameter is a function of dough spread time and set time (Miller and Hoseney 1997). Guttieri et al. (2002) suggested that the spread of cookie dough during baking is controlled by different factors depending on genotype.

There were three objectives for this research. The first was to survey soft wheat end-use quality across the contiguous United States and analyze the regional and varietal differences in grain, milling, flour and baking quality. The second objective was to examine potential relationships between different traits across a wide range of varieties and environments. The third objective was to identify predictive factors for end-use quality based on observed relationships between grain, milling, flour and baking quality traits. In the present report, we document the quality of soft wheat germplasm from the United States drawn from regional
nurseries harvested in 2009. Prior to this study, no such comprehensive survey has been undertaken.

**MATERIALS AND METHODS**

**Grain Samples**

A total of 347 soft wheat samples representing 132 different varieties were surveyed for this study. (In this study, both commercial cultivars and elite unreleased breeding lines are included; both are termed ‘varieties’). These samples were derived from nine discrete or composited growing locations (= ‘environments’) (Table I), spanning the contiguous United States, and harvested in 2009. Varieties were developed for specific regions with unique edaphic, climatic, agronomic and pest considerations. For cooperative regional evaluations, varieties were grouped and grown in four separate nurseries coordinated by the U.S. Dept. of Agriculture as follows: Uniform Eastern Soft Red Winter (SRW) Wheat Nursery, Uniform Southern SRW Wheat Nursery, Western Regional Soft White Winter (SWW) Wheat Nursery, and Western Regional Soft White Spring (SWS) Wheat Nursery. Uniform Eastern and Southern SRW Wheat Nursery reports may be accessed at: [http://www.ars.usda.gov/Main/docs.htm?docid=21894](http://www.ars.usda.gov/Main/docs.htm?docid=21894); Western Regional Nursery data may be accessed at [http://www.ars.usda.gov/Services/docs.htm?docid=3712](http://www.ars.usda.gov/Services/docs.htm?docid=3712). Samples of the Eastern SRW wheat nursery from Wooster, OH were supplemented with grain from Owensville, IN, and Urbana, IL, in approximate proportion of 80:10:10, respectively, and are termed “composite” (Table I). The Southern SRW wheat nursery included two separate composites, an “interior” composite derived from equal amounts of grain from Warsaw, VA, Battle Ground, IN, and Belle Mina, AL, and a “coastal” composite derived from equal amounts of grain from Blacksburg, VA, Plains, GA,
Greenville, MS, and Winnsboro, LA. This compositing scheme is the routine protocol for quality testing of varieties in the eastern U.S., commonly performed at the U.S.D.A. Soft Wheat Quality Laboratory, Wooster, OH. The Western Regional Nurseries are historically evaluated at the Western Wheat Quality Laboratory (WWQL), Pullman, WA.

Quality Measurements

The analyses performed on these grain and flour samples were classified into three categories: grain, milling, and flour/baking quality. The individual quality parameters were assessed using AACC International Approved Methods (2010). Table II presents a list of these parameters along with their units of measure for each and the specific AACC International Approved Method used. The grain quality parameters studied were: test weight, Single Kernel Characterization System (SKCS) 4100 parameters of kernel hardness, weight and diameter; wheat grain protein content, and polyphenol oxidase (PPO) content (L-DOPA substrate). The milling quality characteristics measured were: break flour yield, straight-grade flour yield, flour ash and milling score. The milling procedure followed the modified Quadrumat Sr. method of Jeffers and Rubenthaler (1977). Milling Score was calculated according to Morris et al. (2011). The flour and baking quality parameters studied were: flour protein, Flour Swelling Volume, peak-pasting viscosity using the Rapid ViscoAnalyzer, micro-flour SDS sedimentation volume, solvent retention capacity (SRC) parameters water, sodium carbonate, sucrose and lactic acid, and cookie diameter.

Statistical Analysis
The statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was conducted using PROC GLM. Each regional nursery was analyzed separately with varieties and locations as fixed effects. Due to the lack of in-lab analysis of field replication, no interaction term could be included in the model. Type III mean squares were used to calculate variety:location (“G:E”) ratios. The model consisted of determining the variation due to varietal and location differences within each nursery. The 95% distribution limits were calculated by adding and subtracting two standard deviations from the nursery mean (in a normal distribution, about 95% of the observations fall within ±2 standard deviations).

RESULTS

Grain Quality Characteristics – Eastern and Southern Soft Red Winter

Variation in grain quality characteristics was well modeled in the ANOVA using ‘variety’ (= ‘genotype’) and ‘location’ (≈ ‘environment’) as model main effects (whole model $R^2$s of 0.84-0.96) (Table III). All models were significant, as were the variety and location model components for both nurseries and all grain quality traits. The high model $R^2$’s indicated that GxE interaction was likely a minor source of variation, although it could not be accurately estimated due to lack of in-lab analysis of field plot replicates. The absence of analyzing more than one field plot replicate is a near universal limitation in end-use quality analysis of wheat breeding programs, as a simple two- vs. one-replicate system would necessarily double the number of analyses.

To examine the relative contribution of genotype vs. environment, ratios of the respective mean squares were calculated and are presented in Table III. Naturally, a ratio of 1 indicates an
equal contribution from each source of variation. For all grain quality traits with the exception of SKCS weight, location was the greater source of variation. SKCS kernel weight for the Eastern SRW nursery had a ratio of 3.35. This value indicated that there may have been some direct biotic or abiotic stress that adversely affected kernel weight, on a variety-specific basis, or that the three environments (Table I) were highly consistent.

Test weights were fairly similar between the two SRW nurseries, but individual grain lots varied widely (Table III and data not shown). The greatest range was observed in the Southern SRW nursery with 95% distribution limits of 70.4 and 84.0 kg/hL (5% of values were even more extreme, i.e. greater than two standard deviations from the mean, data not shown). For millers and other end-users, the mean and 95% distribution limits provide a good estimate of the range of quality available in the market place, with the caveats that these are pure varieties derived from a limited, albeit, representative sampling of environments.

Mean SKCS hardness values were similar between the two nurseries (23.6 and 23.3), as were the 95% distribution limits (Table III). Of interest, negative SKCS hardness values were commonly encountered in both nurseries, as were values considered to be ‘hard’ or nearly so, viz. greater than 45. SKCS kernel weight and diameter were slightly larger on average for the Eastern compared to Southern SRW nursery. The lower mean kernel diameter for the Southern SRW nursery was reflected in the lower range of the 95% distribution limits for size (2.61 mm vs. 2.50, Eastern and Southern, respectively).

Grain protein averaged 10.3% and 11.6% for the two SRW nurseries, but individual samples varied widely. The 95% distribution limits ranged from a low of 6.9% to a high of 14.1%. As seen above, the G:E ratios were less than 0.02, location having a much greater influence on protein content than inter-varietal differences. PPO activity exhibited, on average,
moderate levels. However, the 95% distribution limits indicated that there were some relatively low PPO samples \((A_{475} < 0.30)\) and some fairly high \((A_{475} > 1.00)\).

**Grain Quality Characteristics – Western Soft White Winter and Spring**

With one exception, the Western SWW and SWS wheat nurseries also returned high ANOVA model \(R^2\)s \((0.86-0.98)\) (Table IV); the \(R^2\) for grain protein for the western SWW nursery was 0.52 (model not significant). These results indicated that there were similar variance structures overall between the Eastern, Southern and Western nurseries. The Western nurseries, however, exhibited by-and-large greater G:E ratios than the SRW nurseries. These ranged from 0.079 for Western SWW test weight up to more than 1.5 for SKCS hardness and diameter for the SWW nursery. These large ratios indicated that there was a greater amount of genetic variation among varieties (less consistency), or that the western environments contributed much less variation (i.e., they were very similar). Indeed, ‘location’ was not significant for these two parameters. Location was also not significant for SKCS kernel hardness for the Western SWS wheat nursery.

Mean test weight for the Western SWW nursery was not dis-similar to that of the SRW nurseries, averaging 77.7 kg/hL (Table IV). The mean test weight of the SWS nursery, however, was notably higher at 82.0 kg/hL. Whereas the 95% distribution limits for the SWW nursery were similar to those of the SRW, the Western SWS lower limit of 79.5 kg/hL exceeded or nearly so the mean value of the other three nurseries.

The mean SKCS hardness of the Western SWW nursery (34.8) was notably the highest of the four nurseries (Tables III and IV), whereas the SWS nursery mean of 27.0 was slightly higher than the SRW nurseries. In all four nurseries, the upper 95% distribution limit indicated the
The marked difference in SKCS kernel hardness of the Western wheats compared to the Eastern and Southern SRW was seen in the lower limit of the 95% distribution, wherein no negative values were observed in the Western nurseries (lower limits of 16.6 and 11.1, SWW and SWS, respectively). Mean SKCS kernel weight of the Western Regional nurseries was similar to that of the Eastern and Southern SRW nurseries (all ranging from 34.4 to 36.0 mg) (Tables III and IV). The 95% distribution limits were similar among the four nurseries with the exception that the Southern SRW nursery tended to have a greater proportion of smaller kernels (lower limit of 25.1 mg). SKCS kernel diameters were also similar with means of the Western nurseries (2.78 and 2.82 mg) similar to those of the SRW nurseries (Table III).

Mean wheat grain proteins of the Western nurseries, 10.8% and 11.3%, SWW and SWS, respectively, (Table IV) were similar to those of the SRW nurseries. Historically, the range of protein content of soft white spring wheat commercial production is often higher than that of soft white winter. The upper 95% limit of the SWS nursery (13.2%) is notably higher than that of the SWW nursery at 11.8%. The surprising comparison is the upper limits of the SRW nursery samples, 13.8% and 14.1% (Table III). These high levels for soft wheats would not be consistent with traditional views of soft wheat quality.

Mean PPO levels were similar to slightly higher for the Western nursery samples. However, the SWS wheats notably exhibited the greatest 95% distribution range and the lowest and highest limits. The upper limit of $A_{475}$ 1.30 for PPO would be considered very high and generally predictive of undesirable end-product color. Conversely, the lower limit of $A_{475}$ 0.24 would be among the lowest PPO activity levels encountered in our routine testing of breeding samples.
Milling Quality Characteristics – Eastern and Southern Soft Red Winter

ANOVA of the milling quality of the Eastern and Southern SRW wheat samples indicated that break flour yield was well modeled by the genotype and environment model ($R^2 = 0.92$ and $0.95$), and in both nurseries, the environmental contribution to variation was 67 times that of variety ($1/(G:E)$) (Table V). Break flour yield is a good assessment of kernel softness. The nursery means were both in the range of 48-50% yield. However, of much greater importance was the markedly wide range encountered. Although mean break flour yields for the two nurseries were fairly similar, the Eastern nursery had the wider 95% range, 38.1-61.1%. This result is remarkable in the sense that it represents a 1.6 fold difference.

Straight grade flour yield variation was somewhat different between the two SRW nurseries. The Eastern SRW nursery had a slightly higher model $R^2$ (0.93 vs. 0.88) with a much greater effect of environment compared to genotype (G:E ratio = 0.009), whereas in the Southern SRW nursery, the G:E ratio was 0.15 (Table V). Overall, the Eastern SRW wheat samples produced higher flour yields by 3.7%. On the WWQL modified Quadrumat milling system, this difference is considerable. By examining the 95% distribution limits, it appeared that this result was essentially due to some exceptionally good milling samples in the Eastern SRW nursery (upper limit at 80.0% flour yield) compared to the same statistic for the Southern nursery (upper limit at 72.5% flour yield). The lower limits were similar for both nurseries.

ANOVA for flour ash was dis-similar between the two SRW nurseries. Again, the Eastern SRW nursery exhibited a high model $R^2$ and a low G:E ratio (0.014), whereas the Southern nursery model $R^2$ was only 0.62 but the G:E was a relatively low 0.43 (Table V). Overall, the Southern SRW samples had more desirable (lower) mean ash content and 95%
distribution. Milling Score aims to balance flour yield and ash. For example, although high flour yield is desirable, it cannot come (theoretically) at the expense of greater bran shattering and hence higher ash. Conversely, a very low ash flour is similarly highly undesirable if it corresponds to very low yield. The Milling Scores of both nurseries were similar (ca. 83); the distribution limits of the Southern SRW nursery indicated that these samples were more variable than those from the Eastern SRW nursery.

**Milling Quality Characteristics – Western Soft White Winter and Spring**

ANOVA of milling quality of Western wheats indicated robust models with all $R^2$s $\geq 0.87$ (Table VI). In contrast to the SRW results, G:E ratios for break flour yields were very large, 50.3 and 14.8, indicating that this milling parameter was essentially a varietal character. Indeed, in both instances the ‘location’ model component was not significant. Mean break flour yields were similar to those observed in the Eastern and Southern SRW nurseries (cf. Table IV). In both Western nurseries, the 95% distribution limits were notably narrower than in the Eastern and Southern SRW nurseries, indicating less variation among samples. This observation is notable in the sense that there were not-as-poor, but also not-as-good samples in the Western nurseries compared to the SRW. Of note, the higher SKCS kernel hardness observed in the Western SWW nursery (Table IV) was not reflected in a lower mean break flour yield.

Mean straight-grade flour yield of the two Western nurseries (70.7 and 70.4%) (Table VI) fell in between the SRW nursery means (71.7 and 68.0%) (Table V). In addition to the means, the 95% distribution limits were quite similar for the two Western nurseries. And as seen with break flour yield, varieties were less variable. The G:E ratios for flour ash were low (only 0.0023 for the SWW nursery, ‘variety’ not significant). Flour ash of the SWW wheat nursery was on
average nearly identical to that of the Southern SRW nursery, whereas the SWS nursery mean at 0.320% was the lowest of the four nurseries, and substantially below the 0.452% observed with the Eastern SRW samples.

Milling Scores of the Western nurseries were similarly affected by genotype vs. environment; the G:E ratios were about 0.0057, indicating more than 170 fold greater contribution of environment compared to variety (Table VI). Overall, Western nursery Milling Scores exceeded those of the SRW nurseries. The SWS nursery mean (90.3), in particular, was the highest of all four nurseries and was nearly equal to the upper 95% limit of both SRW nurseries (Table V). The lower 95% limit of the SWS samples (85.2) was greater than either SRW nursery mean. In contrast, the SWW Milling Scores were the most variable of all four nurseries, indicating that some varieties were very poor milling (lower 95% limit of 73.1), whereas others were exceptional (upper limit of 100).

Flour and Baking Quality Characteristics – Eastern and Southern Soft Red Winter

The category of “flour and baking quality” included all of our (WWQL) routine flour tests and cookie baking (Table II), excluding rheological tests. For the Eastern SRW nursery, these flour and baking quality tests returned high ANOVA model $R^2$s ranging from 0.82 to 0.90 (Table VII). For the Southern SRW nursery the model $R^2$s were similar-to-somewhat lower, ranging from only 0.54 for cookie diameter to 0.94 for SRC Lactic acid.

The G:E ratios for the Eastern SRW nursery ranged from a low of 0.011 for flour protein and cookie diameter to near unity for RVA peak-pasting viscosity and SRC Lactic acid (Table VII). Ratios for the Southern SRW nursery were in a similar range for most traits, although flour SDS sedimentation volume and SRC Water were notably higher (13.6 and 24.0, respectively).
Mean flour protein of the SRW nurseries (Table VII) was typical for soft wheats, and was 1.6% and 1.8% lower than the nursery average grain protein contents (Table III). This difference (grain vs. flour) is typical of what is often observed with our modified Quadruplet milling system for soft wheats. The wide 95% ranges for flour protein contents (Table VII) reflected the highly variable grain protein (Table III) encountered in these SRW samples.

Two tests are aimed primarily at starch properties, Flour Swelling Volume and RVA peak pasting viscosity (Table VII). Flour Swelling Volume nursery means were in the range of typical values. Examination of the 95% distribution limits, however, indicated that those samples near the lower limit may have been sprouted, whereas those at the upper limit may be partial waxy with reduced starch amylose (Zeng et al. 1997). The RVA peak pasting viscosity results were consistent with this interpretation, the means of 159 and 132 reflect ‘sound’ wheat, the lower limits of 104 and 20 may reflect the presence of sprouting or late-maturity α-amylase (especially values below 100). Values over 200 indicate that the variety may be a mixture of normal and partial waxy genotypes, while values above 240 indicate that the variety is nearly uniform for the partial waxy trait. Closer examination of the Southern SRW samples indicated that the Coastal Composite (Table I) had serious problems. The majority of these samples had RVA peak pasting viscosities less than 100, with the highest value 151 (data not shown).

Flour SDS sedimentation volume is a function of protein content and protein “quality.” The higher mean of the Southern SRW nursery indicated that, on average, these samples were “stronger” in a gluten rheological sense (Table VII). However, in both SRW nurseries, there was a wide range in variation. At the present time, a de-coupling of protein content and genetic “strength” is not possible for these samples, especially considering the very wide range in flour protein contents among these samples. In this regard, the higher mean value of the Southern
SRW nursery may simply be a reflection of the 1.1% higher average flour protein content. At the upper limit of the 95% distributions, the flour protein contents of the two SRW nurseries were similar (~12.2%), whereas the SDS sedimentation volume of the Southern SRW nursery was higher (16.5 vs. 13.8 mL/g Eastern SRW), indicating that there may be some varieties with greater “strength” in the Southern nursery.

Solvent Retention Capacity (SRC) tests have provided a valuable tool to gain insight into the physical-chemical composition of soft wheat flours. SRC Water means were essentially identical for the two SRW nurseries (Table VII), and the variation as described by the 95% distribution limits were also quite similar. This range (~10% SRC value) however, is of practical concern to end-users based on our previous experience. In general, soft wheat users prefer lower SRC Water values as lower values correspond to better flour processing and functionality. SRC Carbonate has been associated with primarily starch damage. Again, the two SRW nursery means were essentially the same. In this case the Southern SRW nursery was more variable with the upper limit (83.0%) indicating less desirable flour qualities. SRC Sucrose captures primarily pentosan effects and some contribution of gliadin proteins. For this measure, the nursery mean of the Southern SRW nursery was 4% higher than that of the Eastern, and although the lower 95% limits were similar between the two nurseries, the upper limit (114%) for the Southern SRW nursery would be considered highly undesirable. SRC Lactic acid reflects protein “quality” in a similar sense as flour SDS sedimentation volume. Here we observed a significantly higher nursery mean for the Southern compared to the Eastern SRW nursery (98.9% vs. 78.8%, respectively). However, as noted above, this higher value cannot be fully decoupled from the higher average protein content of the Southern SRW nursery. Nevertheless, the lower limits are not too dis-similar whereas the upper limit of 136% for the Southern SRW nursery with a
corresponding upper limit for flour protein of 12.3% suggests considerable genetic “strength” may be available in these samples/varieties. In this sense, SDS sedimentation and SRC Lactic acid were in agreement.

Lastly, cookie diameters were nearly the same between the two SRW nurseries. The 95% ranges, too, were nearly the same with some indication that at the upper limit, better samples/varieties were present in the Eastern SRW nursery (9.90 cm). The lower limits pointed towards some very poor performing flours (< 8.9 cm).

**Flour and Baking Quality Characteristics – Western Soft White Winter and Spring**

Flour and baking quality of the Western SWW nursery indicated that most traits were well modeled in ANOVA with $R^2 \geq 0.84$ (Table VIII). Models for flour protein and SRC Carbonate were not significant, however. The Western SWS dataset also returned mostly highly significant ANOVA models ($R^2 > 0.73$) with the exception of SRC Water (Table VIII). Inspection of the G:E ratios revealed a wide range in the relative contribution of variety and location, and differences between nurseries. For example, SRC Sucrose was at unity for the Western SWS nursery samples but at 0.023 for the SWW dataset. This pattern was observed for RVA peak-pasting viscosity, SRC Water, SRC Carbonate and SRC Sucrose, wherein the G:E ratio was much greater for the SWS nursery and the contribution of ‘location’ was often not significant.

Western nursery mean flour protein contents were similar for SWW and SWS, and in the range commonly encountered (Table VIII). In addition, the means of all four nurseries were within approximately 1% (cf. Table VII). In contrast to the Eastern and Southern SRW nurseries, the 95% distribution ranges were much reduced, particularly for the SWW nursery.
Flour Swelling Volume mean and distribution for the SWW nursery was similar to those of the Eastern and Southern SRW nurseries, whereas the Western SWS nursery showed a relatively high mean and the highest 95% distribution limit of the four nurseries. Based on prior experience, there are a number of partial waxy soft white spring wheat varieties and breeding lines in the Western U.S. germplasm. RVA peak-pasting viscosity was consistent with this interpretation. The upper 95% limit of the SWW nursery (163) indicates normal amylose starch types, whereas the upper limit of the SWS nursery (236) is clearly in the range of partial waxy samples. In both nurseries, the lower 95% distribution limits below 100 (80 and 84) indicate either some sprout damage or late-maturity α-amylase may be present.

Of particular interest is the level of gluten strength in the Western germplasm as evidenced by flour SDS sedimentation volume (Table VIII). The SWW nursery mean of 9.4 mL/g is fairly similar to those of the two SRW nurseries (9.3 and 11.6 mL/g). The mean for the SWS nursery (14.7 mL/g), however, illustrates the presence of some very “strong” wheat varieties in this germplasm. Indeed the upper 95% limit for the Western SWS nursery (21.5 mL/g) is markedly higher than all other nurseries (13.8-16.5 mL/g). A second observation relates to the lower 95% distribution limit. That of the SWW nursery (2.5 mL/g) is reflective of the very “weak” club wheats present in the Western soft white winter wheat germplasm. Club wheats are specifically bred and selected to have weak gluten.

SRC Water means of the two Western nurseries were less than or equal to those of the Eastern and Southern SRW nurseries, with the lowest overall that of the SWS (53.7%) (Table VIII). All four nurseries exhibited similar 95% distribution limits indicating similar ranges of variation. The key feature here is that in all nurseries this range encompasses about 10% SRC Water value. SRC Carbonate means were similar among the Eastern, Southern and Western
SWW nurseries. Since lower SRC Carbonate values are considered better for end-users, the lowest mean value (68.9%) for the Western SWS nursery is notable. The 95% distribution limits follow this same trend with the Western SWS lower limit of 61.1% being noteworthy. Of further note is the markedly broad range observed among all four nurseries suggesting that this high level of variability could create problems for processors who usually value consistency of raw materials. SRC Sucrose nursery means were lowest for the Western SWW nursery (90.7%), followed by the SWS nursery. All four nurseries appeared to be similarly variable with the most notable aspects of the 95% distributions being the lower limit of the Western SWW (79.5%) (Table VIII) and the upper limit of the Southern SRW nursery (83.0%) (Table VII). SRC Lactic acid again identified the Western SWS nursery as having considerable gluten strength. The mean value of 116% with an upper 95% limit of 155% clearly highlighted an important feature of this germplasm. As seen with flour SDS sedimentation volume, the SWW nursery possesses some very weak gluten wheats (nursery lower limit of SRC Lactic acid of 48.6%).

Lastly, mean cookie diameters of the two Western nurseries were similar to those of the SRW nurseries (Table VIII cf. Table VII). In our experience, a mean difference on the order of 0.2 cm is generally near the threshold of a significant difference (Morris et al 2011). In this context, the 95% distribution range for the Western SWW nursery was dramatic (8.87-9.63 cm) as was the SWS (8.88-9.74). The lower limits of all four nurseries were similar (and poor) (~8.9 cm). Conversely, at the upper limit the “best” samples from the Eastern and Southern SRW nurseries (9.90 and 9.77 cm, respectively) out-performed those of the Western SWW and SWS nurseries (9.63 and 9.74 cm, respectively).

Specific Varieties Displaying Exceptional Characteristics
In the Eastern SRW wheat nursery the variety Z03-1281 had distinctly soft SKCS hardness (4.8), with relatively low wheat and flour protein (9.5% and 7.8%, respectively) (see Supplemental Data). This variety displayed high break flour yield (55.7%), flour yield (74.8%), milling score (89.5), and a large cookie diameter (9.59 cm). Similarly, the variety W06-89 was observed to have low grain and flour protein contents (9.5% and 7.6%), high break flour yield and milling score (53.5% and 88.1), and a large cookie diameter (9.60 cm).

In the Southern SRW wheat nursery the variety LA01139D-56-1 showed distinctly low SRC responses for Carbonate and Sucrose (65.3% and 89.6%), as well as a low flour-swelling volume (15.6 mL/g). These appeared to contribute to the large cookie diameter observed for this variety (9.74 cm). Similarly, the variety GA001492-7E9 also exhibited low SRC responses for Carbonate and Sucrose (66.8% and 90.1%), as well as high break flour yield (52.6%), flour yield (72.6%) and milling score (90.3). These appeared to be associated with a large cookie diameter (9.53 cm).

In the Western Regional SWW wheat nursery, the club wheat variety Chukar exhibited very low flour SDS sedimentation (6.4 mL/g) characteristic of weak club wheat gluten, and low SRC responses for all four measurements (Water, Carbonate, Sucrose and Lactic acid, 51.8%, 68.5%, 82.6% and 70.0%, respectively). Chukar exhibited very high break flour yield (52.4%), flour yield (73.6%) and milling score (91.3). In a presumptive association, these traits contributed to a large cookie diameter (9.48 cm). The variety ID98-19010A displayed some exceptional soft wheat quality attributes with high test weight (62.3 g/hL), low SKCS hardness (32.9), high break flour yield (53.5%), and low SRC Water (50.9%). The mean cookie diameter of this variety was relatively large (9.52 cm).
Two varieties stood out with exceptional cookie diameter values in the Western SWS wheat nursery: Diva and Louise. Both had high SKCS weight (42.1 and 43.1 mg) and diameters (2.98 and 3.04 mm). Diva also exhibited a low SRC Sucrose value (89.5%). The average flour SDS sedimentation value of Diva and Louise were 14.0 mL/g and 13.8 mL/g, respectively. The average SRC Lactic acid response of these two varieties was 115% and 120%, Diva and Louise, respectively. Alpowa appeared to be the strongest SWS wheat variety overall (SDS sedimentation of 16.9 mL/g and SRC Lactic acid of 146.2%), but had poor cookie diameter (9.03 cm). Previous research (Finnie et al. 2008; Kiszonas et al in press) has shown that Alpowa has among the highest total and water-soluble arabinoxylans.

**DISCUSSION**

**Grain Quality**

Location was generally more influential on grain quality characteristics than was variety across the Uniform Eastern and Southern Soft Red Winter Wheat Nurseries (Table II). This trend did not hold true for the two Western Regional Nurseries, which displayed more variability in model contribution, but overall had often stronger varietal than location influences to overall trait variability (Table IV). The influence of one model component over the other depended on the trait in question. The grain quality traits exhibited clear differences in the role of genetics and environment, with varying contributions of influence from variety and location. Guttieri et al. (2002) observed similar varying levels of environmental influence, due largely to climatic conditions when comparing only two varieties.

Whereas in the SRW nurseries, genetic contribution to SKCS kernel hardness variation was low, the Western Regional Nurseries displayed a much higher level of genotype contribution
to variation in kernel hardness (Tables III and IV). Overall, wheat grown in the Western SWW and SWS wheat nurseries tended to have higher SKCS hardness values (especially the winter germplasm). Grain protein levels were similar across all four nurseries (albeit highly variable among individual samples). The independence of kernel hardness and protein was consistent with the observations of Simmonds (1974).

**Milling Quality**

A similar relationship between variety and location contributions to variation was evident in the milling quality characteristics (Tables V and VII). In all milling traits examined, the SRW wheat nurseries showed strong location influences, whereas in the Western nurseries there was a much more variable contribution of variety and location. Despite similar break flour yields across all four nurseries, flour yields were markedly lower in the Southern SRW nursery than in the other three. Flour ash content was high in the Eastern SRW nursery; however, this did not appear to adversely impact milling scores. Milling score takes into account the trade-off between flour yield and flour ash. Differences in flour ash may be ascribed to differences in milling quality but also likely result from differences in edaphic and climatic conditions. Morris et al. (2009) found that there was very little genetic contribution to grain ash content. Both Western nurseries displayed higher milling scores, and, in the case of the Western SWS nursery, the 95% range was greater than the average milling scores for both of the Uniform SRW nurseries.

**Gluten Strength**

The four regional soft wheat nurseries were ranked for mean flour SDS sedimentation volume as follows: Eastern SRW (lowest), Western SWW, Southern SRW, Western SWS.
(Tables VII and VIII). Mean SRC Lactic acid values provided the same nursery rank order. Both of these flour/baking quality response variables which reflect gluten “strength” showed high levels of variability within each nursery. Very low G:E ratios indicated that much of this variability may be due to environmental effects (and may be reflected in protein differences). Flour SDS sedimentation values were highly variable across all four nurseries, with little apparent correlation to other traits measured except grain protein content. Notably, however, the Eastern SRW wheat nursery exhibited the lowest flour SDS sedimentation responses as well as the lowest lactic acid SRC responses. The SRC Lactic acid measurement relates to the glutenin characteristics of flour (Gaines et al 2000). The nurseries with the highest SRC Lactic acid responses (Southern SRW nursery and Western SWS nursery) also displayed the highest levels of wheat and flour protein. While protein content per se has little direct relationship to glutenin composition, protein content does influence SRC Lactic acid (Guttieri et al 2002).

The present results highlight a key feature of U.S. soft wheat quality which is best illustrated in the two Western nurseries, that is, that unlike U.S. hard wheats, there is an extremely large range in genetic gluten strength. Included in the Western SWW nursery are club wheats, a key component to the export class ‘Western White Wheat’ (USDA 2006). Club wheat varieties are expressly bred and selected for very weak gluten. The variety ARS970163-4C was similar to Chukar (see above) in having “weak” gluten, with SDS sedimentation volume of 3.8 mL/g and SRC Lactic acid of 72.1%. The variety KW990161-3049 had an exceptionally low SRC Lactic acid value of 52%. These values were the lowest encountered at any time in this study. Conversely, among the Western SWS varieties, in addition to Diva and Louise (see above), Alpowa was notable as being quite strong, with corresponding values of 16.9 mL/g SDS sedimentation volume and SRC Lactic acid of 146.2%. These and other varieties included in the
present study could provide breeders with genetic resources to modify end-use quality traits across the U.S. and elsewhere.

**Flour and Baking Quality**

Despite similar flour swelling volumes across the four nurseries, there was a wide range of responses for peak-pasting viscosity, as measured by the RVA (Tables VII and VIII). While the average peak-pasting viscosity in the Southern SRW wheat nursery was not particularly notable compared to the other three nurseries, this nursery showed a dramatic range of responses, exhibiting both the highest and lowest, by far, peak-pasting viscosities.

Water relationships are particularly important in soft wheat products. Many products are baked to very low moisture contents (e.g. crackers), whereas others are prepared from high water content batters (e.g. pancakes, Finnie et al 2006) (Morris and Rose 1996). The mean SRC Water measurement was relatively consistent among nurseries, but showed wide ranges in variability. While SRC Carbonate had one exceptionally low response (Western SWS nursery), there was no correlation with any other quality trait. SRC Sucrose, which reflects pentosan and gliadin characteristics, was observed to be more variable across the four nurseries. The Western SWW nursery had a markedly lower mean SRC Sucrose value, while the Southern SRW nursery had a notably high average response.

Despite some notable observations in the SRC measurements, they did not appear to correspond well with mean cookie diameter, which was greatest in the Eastern SRW wheat nursery, and lowest in the Western SWW nursery. In contrast to the prevailing research (Kweon et al 2011), which indicated that a low SRC Sucrose value correlates with a greater cookie diameter, the nursery with the lowest mean SRC Sucrose value also had the smallest cookie
diameter (Western SWW nursery). In this study, the SRC measurements did not appear to have any strong predictive quality for determining cookie diameter. This is not to say that they do not have value in predicting variation in other end-product or processing traits, or that they might be used in combination to build multi-variate predictive models. Cookie diameter appeared to have stronger correspondence with high break flour yields, high flour yields, and low wheat and flour protein contents, as seen for the Eastern SRW wheat nursery.

The observation in the Eastern SRW wheat nursery for the capacity of break flour yield, flour yield, and protein content to generally predict cookie diameter was strengthened by the identification of several varieties with exceptional end-use quality in this nursery. The varieties with the largest cookie diameters displayed notably low SKCS hardness values, low grain and flour protein, high break flour and flour yields, and high milling scores. Similarly, the varieties with the smallest (poorest) cookie diameters had high protein, low break flour and flour yields, low milling scores, high SKCS hardness values, and high flour ash contents. In the Uniform Southern SRW wheat nursery, it appeared that the SRC measures had more predictive power for cookie diameter. Those varieties with low SRC values tended to have larger cookie diameters than those with high SRC responses.

Chukar club wheat in the Western Regional SWW nursery exemplified a near-perfect “model” of weak-gluten soft wheat quality, with a low flour SDS sedimentation volume and low SRC responses combined with high break flour and straight-grade flour yields, a high milling score, and a large cookie diameter. This model was shown to be largely independent of starch parameters in the variety ID98-19010A, which had low SKCS hardness, protein and SRC Water, and large cookie diameter, but notably high responses for Flour Swelling Volume and RVA
peak-paste viscosity. Those varieties with very small cookie diameters were more predictable, having high SKCS hardness and flour ash, and poor milling scores.

In the Western SWW nursery the variety Diva had a low SRC Sucrose value and large cookie diameter. Smaller cookies were produced by varieties with low flour yields and milling scores, high protein, and high SRC responses.

Location Evaluations

A western U.S. location (Aberdeen, ID) was included for the Uniform Eastern and Southern SRW wheat nurseries to examine the effects of a dis-similar environment on end-use quality. For the Uniform Eastern SRW wheat nursery, kernel hardness was dramatically greater from Aberdeen (40.5) compared to Urbana (19.7) and Composite (10.7). Flour yield and break flour yield were markedly lower from Aberdeen (67.2% and 43.3%) compared to Urbana (75.8% and 54.6%) and Composite (72.2% and 51.5%). However, flour ash was considerably lower from Aberdeen (0.38%) compared to Urbana (0.51%) and Composite (0.47%). Consequently, mean Milling Scores for Wooster and Aberdeen were not statistically different. Wheat and flour protein contents were also notably higher from Aberdeen, and likely contributed to the higher flour SDS sedimentation volume (10.98 mL/g) compared to Urbana (9.36 mL/g) and Composite (7.42 mL/g). All of the SRC parameters, however, were lowest in Aberdeen. Yet, the mean cookie diameter from Aberdeen was markedly smaller than that from the Composite (9.15 vs. 9.57cm) (cookie data were not available from Urbana). Consequently, differences in cookie diameter between these nurseries may be related to differences in kernel hardness and break flour yield, as opposed to SRC parameters, which would otherwise indicate higher soft wheat quality from Aberdeen. All other quality traits were similar across all three growing locations.
Less dramatic differences in overall quality were observed in the Uniform Southern SRW wheat nursery due to location. Kernel hardness from Aberdeen was considerably higher than that from the Interior and Coastal locations (37.1 cm, 17.4 cm, and 15.4 cm, respectively). Flour yield appeared to be unaffected by the differing growing environments, but break flour yield was markedly lower from Aberdeen (42.7%) compared to the Coastal and Interior locations (50.8 and 50.8%). Despite higher wheat and flour protein levels from Aberdeen, flour SDS sedimentation was similar for all three locations. Similar to the Uniform Eastern SRW wheat nursery, the SRC responses were lowest from Aberdeen. In contrast to the Uniform Eastern SRW wheat nursery, however, mean cookie diameter for Aberdeen (9.31 cm) was quite similar to the Interior and Coastal locations (9.42 cm and 9.26 cm, respectively). Location mean cookie diameters in this SRW nursery appeared to be unrelated to the large differences in kernel hardness and break flour yield.

**Quality Predictions**

The variation attributed to variety and location was not consistent across the four nurseries surveyed, nor across the various grain, milling, flour and baking quality traits. The quality characteristics that tended to be the best indicators of large cookie diameter were high break flour yield and low wheat and flour protein levels. Despite these predictive properties of high end-use quality, as was noted by Guttieri et al. (2002), predictive models for cookie diameter were a challenge to create with consistency across multiple varieties. These predictive factors also appeared to depend on the region in which the wheat was grown, suggesting that environment also contributed to those factors which were used to predict overall wheat quality (also observed by Baenziger et al 1985). The overall conclusion of this study is that whereas on
average, soft wheat quality is similar across the U.S., within every region, there exists a large amount of variation that results from both genetic and environmental contributions. Consequently, it would seem reasonable that wheat breeders could strive to reduce this variability directly through crossing and selection, and indirectly through greater resistance to biotic and abiotic stresses. The end-user is faced with attempting to predict quality through one or more small-scale tests, “blending to the mean” (to reduce variation), or dealing with variability through process/formula adjustments.

ACKNOWLEDGMENTS

We would like to acknowledge the technical staff of the USDA-ARS Western Wheat Quality Laboratory, Doug Engle, Mary Baldridge, Bozena Paszczynska, Mishelle Lenssen, Eric Wegner, Bill Kelley, Patricia Boyer, Gail Jacobson and Anna Hansen. Stacey Sykes and Shawna Vogl assisted in the preparation of the manuscript. This project was supported by the Agriculture and Food Research Initiative Grant no. 2009-02347 from the USDA National Institute of Food and Agriculture.
LITERATURE CITED


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## TABLE I
Summary of nurseries, locations, and number of soft wheat varieties grown in each regional nursery

<table>
<thead>
<tr>
<th>Nursery</th>
<th>Contributor</th>
<th>Locations</th>
<th>No. Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniform Eastern Soft</td>
<td>H. E. Bockelman</td>
<td>Aberdeen, ID</td>
<td>42</td>
</tr>
<tr>
<td>Red Winter Wheat</td>
<td>F. L. Kolb</td>
<td>Urbana, IL&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. Sneller/E. Souza</td>
<td>Composite&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Uniform Southern Soft</td>
<td>H.E. Bockelman</td>
<td>Aberdeen, ID</td>
<td>41</td>
</tr>
<tr>
<td>Red Winter Wheat</td>
<td>S. A. Harrison</td>
<td>Interior Composite&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.A. Harrison</td>
<td>Coastal Composite&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Western Regional Soft</td>
<td>B. Brown</td>
<td>Parma, WA</td>
<td>34</td>
</tr>
<tr>
<td>White Winter Wheat</td>
<td>K. Garland-Campbell</td>
<td>Pullman, WA</td>
<td></td>
</tr>
<tr>
<td>Western Regional Soft</td>
<td>L. E. Talbert</td>
<td>Bozeman, MT</td>
<td>15</td>
</tr>
<tr>
<td>White Spring Wheat</td>
<td>G. B. Shelton</td>
<td>Pullman, WA</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Grain samples from this location were limited; no SRC Water, SRC Lactic acid or cookie baking was performed.

<sup>b</sup> Composite of Owensville, IN, Urbana, IL, Wooster, OH, in approximate proportion of 10:10:80, respectively. Grain samples were limited; no SRC Water or SRC Lactic acid was performed.

<sup>c</sup> Composite of Warsaw, VA, Battle Ground, IN, and Belle Mina, AL

<sup>d</sup> Composite of Blacksburg, VA, Plains, GA, Greenville, MS, and Winnsboro, LA
### TABLE II
Response variables and their corresponding units

<table>
<thead>
<tr>
<th>Category</th>
<th>Response Variable</th>
<th>Units</th>
<th>AACCI Approved Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain</td>
<td>Test weight</td>
<td>kg/hL</td>
<td>55-10.01</td>
</tr>
<tr>
<td></td>
<td>SKCS&lt;sup&gt;a&lt;/sup&gt; hardness</td>
<td>-</td>
<td>55-31.01</td>
</tr>
<tr>
<td></td>
<td>SKCS weight</td>
<td>mg</td>
<td>55-31.01</td>
</tr>
<tr>
<td></td>
<td>SKCS diameter</td>
<td>mm</td>
<td>55-31.01</td>
</tr>
<tr>
<td></td>
<td>Wheat protein</td>
<td>per cent&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39-25.01</td>
</tr>
<tr>
<td></td>
<td>PPO&lt;sup&gt;c&lt;/sup&gt; (L-DOPA&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>absorbance at 475nm</td>
<td>22-85.01</td>
</tr>
<tr>
<td>Milling&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Break flour yield</td>
<td>per cent&lt;sup&gt;f&lt;/sup&gt;</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Flour yield</td>
<td>per cent&lt;sup&gt;f&lt;/sup&gt;</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Flour ash</td>
<td>per cent&lt;sup&gt;f&lt;/sup&gt;</td>
<td>08-01.01</td>
</tr>
<tr>
<td></td>
<td>Milling score</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Baking</td>
<td>Flour protein</td>
<td>per cent&lt;sup&gt;f&lt;/sup&gt;</td>
<td>39-11.01</td>
</tr>
<tr>
<td></td>
<td>Flour swelling volume</td>
<td>mL/g</td>
<td>56-21.01</td>
</tr>
<tr>
<td></td>
<td>Flour SDS&lt;sup&gt;g&lt;/sup&gt;</td>
<td>mL/g</td>
<td>56-60.01</td>
</tr>
<tr>
<td></td>
<td>SRC&lt;sup&gt;h&lt;/sup&gt; Water</td>
<td>per cent</td>
<td>56-11.02</td>
</tr>
<tr>
<td></td>
<td>SRC Carbonate</td>
<td>per cent</td>
<td>56-11.02</td>
</tr>
<tr>
<td></td>
<td>SRC Sucrose</td>
<td>per cent</td>
<td>56-11.02</td>
</tr>
<tr>
<td></td>
<td>SRC Lactic acid</td>
<td>per cent</td>
<td>56-11.02</td>
</tr>
<tr>
<td></td>
<td>Peak-pasting viscosity (RVA)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>centipoise x 12</td>
<td>76-21.01</td>
</tr>
<tr>
<td></td>
<td>Cookie diameter</td>
<td>cm</td>
<td>10.52.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Single Kernel Characterization System

<sup>b</sup> Adjusted to a 12% moisture basis
Polyphenol oxidase

L-3,4-dihydroxyphenylalanine

Milling was performed following the modified Quadrumat Sr. method of Jeffers and Rubenthaler (1977)

Adjusted to a 14% moisture basis

SDS sedimentation volume

Solvent Retention Capacity

Rapid Visco-Analyzer
### TABLE III

Grain end-use quality response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Uniform Eastern and Southern Soft Red Winter (SRW) wheat nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Uniform Eastern SRW</th>
<th>Uniform Southern SRW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio $^a$</td>
</tr>
<tr>
<td>Test weight</td>
<td>0.93</td>
<td>0.016</td>
</tr>
<tr>
<td>SKCS hardness</td>
<td>0.96</td>
<td>0.015</td>
</tr>
<tr>
<td>SKCS weight</td>
<td>0.85</td>
<td>3.35</td>
</tr>
<tr>
<td>SKCS diameter</td>
<td>0.87</td>
<td>0.33</td>
</tr>
<tr>
<td>Wheat protein</td>
<td>0.90</td>
<td>0.011</td>
</tr>
<tr>
<td>PPO (L-DOPA)</td>
<td>0.86</td>
<td>0.25</td>
</tr>
</tbody>
</table>

$^a$Ratio of variety mean square:location mean square; all models, and the model components ‘variety’ and ‘location’ were significant at $P \leq 0.05$
**TABLE IV**
Grain end-use quality response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Western Regional Soft White Winter (SWW) and Soft White Spring (SWS) wheat nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Western Regional SWW</th>
<th></th>
<th>Western Regional SWS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio$^a$</td>
<td>Mean</td>
<td>95% Range</td>
</tr>
<tr>
<td>Test weight</td>
<td>0.94</td>
<td>0.079</td>
<td>77.7</td>
<td>72.6 - 82.8</td>
</tr>
<tr>
<td>SKCS hardness</td>
<td>0.93</td>
<td>15.6$^b$</td>
<td>34.8</td>
<td>16.6 - 52.9</td>
</tr>
<tr>
<td>SKCS weight</td>
<td>0.89</td>
<td>1.36</td>
<td>35.7</td>
<td>28.3 - 43.1</td>
</tr>
<tr>
<td>SKCS diameter</td>
<td>0.89</td>
<td>15.1$^b$</td>
<td>2.78</td>
<td>2.54 - 3.02</td>
</tr>
<tr>
<td>Wheat protein</td>
<td>0.52ns</td>
<td>0.89</td>
<td>10.8</td>
<td>9.7 - 11.8</td>
</tr>
<tr>
<td>PPO (L - DOPA)</td>
<td>0.91</td>
<td>0.16</td>
<td>0.72</td>
<td>0.33 - 1.12</td>
</tr>
</tbody>
</table>

$^a$ Ratio of variety mean square:location mean square; unless otherwise indicated, the overall model, and the model components ‘variety’ and ‘location’ were significant at $P \leq 0.05$

$^b$ Location not significant at $P \leq 0.05$

'ns’ indicates that the overall model was not significant with $P > 0.05$
<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Uniform Eastern SRW</th>
<th>Uniform Southern SRW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio $^a$</td>
</tr>
<tr>
<td>Break flour yield</td>
<td>0.92</td>
<td>0.015</td>
</tr>
<tr>
<td>Flour yield</td>
<td>0.93</td>
<td>0.009</td>
</tr>
<tr>
<td>Flour ash</td>
<td>0.92</td>
<td>0.014</td>
</tr>
<tr>
<td>Milling Score</td>
<td>0.70</td>
<td>0.21</td>
</tr>
</tbody>
</table>

$^a$Ratio of variety mean square:location mean square; all models, and the model components ‘variety’ and ‘location’ were significant at $P \leq 0.05$
## TABLE VI
Milling response variables, ANOVA model $R^2$, and the ratio of variety:location mean squares for the Western Regional Soft White Winter (SWW) and Soft White Spring (SWS) wheat nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Western SWW</th>
<th>Western Regional SWS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio$^a$</td>
</tr>
<tr>
<td>Break flour yield</td>
<td>0.95</td>
<td>50.3$^b$</td>
</tr>
<tr>
<td>Flour yield</td>
<td>0.91</td>
<td>0.14</td>
</tr>
<tr>
<td>Flour ash</td>
<td>0.93</td>
<td>0.0023$^c$</td>
</tr>
<tr>
<td>Milling Score</td>
<td>0.93</td>
<td>0.0057</td>
</tr>
</tbody>
</table>

$^a$Ratio of variety mean square:location mean square; unless otherwise indicated, the overall model, and the model components 'variety' and 'location' were significant at $P \leq 0.05$

$^b$Location not significant at $P \leq 0.05$

$^c$Variety not significant at $P \leq 0.05$
### TABLE VII

Baking response variables, ANOVA model $R^2$, and the ratio of variety:location mean squares for the Uniform Eastern and Southern Soft Red Winter (SRW) wheat nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Uniform Eastern SRW</th>
<th>Uniform Southern SRW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>Ratio $^a$</td>
</tr>
<tr>
<td>Flour protein</td>
<td>0.92</td>
<td>0.011</td>
</tr>
<tr>
<td>Flour Swelling Volume</td>
<td>0.84</td>
<td>0.12</td>
</tr>
<tr>
<td>Peak-pasting viscosity</td>
<td>0.90</td>
<td>0.68</td>
</tr>
<tr>
<td>Flour SDS</td>
<td>0.82</td>
<td>0.045</td>
</tr>
<tr>
<td>SRC Water</td>
<td>0.88</td>
<td>0.037</td>
</tr>
<tr>
<td>SRC Carbonate</td>
<td>0.86</td>
<td>0.045</td>
</tr>
<tr>
<td>SRC Sucrose</td>
<td>0.85</td>
<td>0.23</td>
</tr>
<tr>
<td>SRC Lactic acid</td>
<td>0.82</td>
<td>0.79</td>
</tr>
<tr>
<td>Cookie diameter</td>
<td>0.89</td>
<td>0.011</td>
</tr>
</tbody>
</table>

$^a$Ratio of variety mean square:location mean square; unless otherwise indicated, the overall model, and the model components

$^b$Location not significant at $P \leq 0.05$
TABLE VIII
Baking response variables, ANOVA model $R^2$, and the ratio of variety:location mean squares for the Western Regional Soft White Winter (SWW) and Soft White Spring (SWS) wheat nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Western SWW</th>
<th></th>
<th>Western Regional SWS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio$^a$ Mean 95% Range</td>
<td>Model $R^2$</td>
<td>Ratio$^a$ Mean 95% Range</td>
</tr>
<tr>
<td>Flour protein</td>
<td>0.59ns</td>
<td>0.64 9.1 8.1 - 10.1</td>
<td>0.94</td>
<td>0.21 9.6 7.9 - 11.2</td>
</tr>
<tr>
<td>Flour Swelling Volume</td>
<td>0.92</td>
<td>0.0098 18.6 14.2 - 23.1</td>
<td>0.90</td>
<td>44.1$^b$ 21.6 17.8 - 25.4</td>
</tr>
<tr>
<td>Peak-pasting viscosity</td>
<td>0.85</td>
<td>0.13 121 80 - 163</td>
<td>0.91</td>
<td>210$^b$ 160 84 - 236</td>
</tr>
<tr>
<td>Flour SDS</td>
<td>0.93</td>
<td>0.038 9.4 2.5 - 16.3</td>
<td>0.97</td>
<td>0.13 14.7 8.0 - 21.5</td>
</tr>
<tr>
<td>SRC Water</td>
<td>0.93</td>
<td>0.42 54.8 50.6 - 59.1</td>
<td>0.71ns</td>
<td>2.27 53.7 48.6 - 58.8</td>
</tr>
<tr>
<td>SRC Carbonate</td>
<td>0.59ns</td>
<td>14.3 72.0 64.7 - 79.2</td>
<td>0.73</td>
<td>7.85$^b$ 68.9 61.1 - 76.7</td>
</tr>
<tr>
<td>SRC Sucrose</td>
<td>0.94</td>
<td>0.023 90.7 79.5 - 102</td>
<td>0.83</td>
<td>1.03$^b$ 94.1 85.0 - 103</td>
</tr>
<tr>
<td>SRC Lactic acid</td>
<td>0.94</td>
<td>0.042 88.8 48.6 - 129</td>
<td>.</td>
<td>. 116 77.1 - 155</td>
</tr>
<tr>
<td>Cookie diameter</td>
<td>0.84</td>
<td>0.31 9.25 8.87 - 9.63</td>
<td>0.89</td>
<td>0.47 9.31 8.88 - 9.74</td>
</tr>
</tbody>
</table>

$'^ns'$ indicates that the overall model was not significant with $P > 0.05$

$'^a$Ratio of variety mean square:location mean square; unless otherwise indicated, the overall model, and the model components $'variety'$ and $'location'$ were significant at $P \leq 0.05$

$'^b$Location not significant
CHAPTER FOUR

A Comprehensive Survey of Hard Wheat Grain Quality in U. S. Germplasm

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of other that may also be suitable.

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ABSTRACT

Wheat (*Triticum aestivum* L.) quality may be evaluated in terms of grain, milling, and baking characteristics. Individual traits within each of these categories are variably influenced by genetic and environmental factors producing a summation of end-use quality. Breeding advances usually include increased grain yield and adaptation to biotic and abiotic stresses. Advances may also include improved end-use quality. The interaction of genetics and environment and the diversity of protocols used to measure quality necessitate that a survey of the end-use quality of wheat germplasm be preferably conducted in a single laboratory. This study surveyed 180 hard wheat varieties (cultivars and advanced breeding lines) grown in six regional cooperative nurseries from representative environments across the United States (Great Plains and Pacific Northwest). The quality parameters included kernel hardness, wheat and flour protein, flour yield, break flour yield, Mixograph parameters, and bread loaf volume. Our findings indicate that the relative importance of location and variety depended upon the particular set of varieties and the particular location being evaluated as well as on the trait being measured. A high level of variation was observed among varieties and growing regions. Differences in the extent to which variety and location influenced the overall quality also existed among the parameters studied. The contribution of variety or location to mean separation within a growing region varied among the nurseries. Relationships among quality traits were observed to be unique to the specific nursery in which they were studied—the relationships did not correlate well across nurseries. However, the factors that appeared to predict high bread loaf volume were high flour protein, high flour yield, and high Mixograph water absorption, and a trend towards longer mixing times. Low loaf volume responses were simpler to define, characterized by low wheat and flour protein, low SDS sedimentation volumes, and short mixing times. The highly variable influence of
variety and location necessitate the consideration of specific growing regions when evaluating wheat germplasm for end-use quality. Considerable genetic variation exists in the germplasm present in all U.S. regional nurseries, such that selection could provide a higher and more consistent level of end-use quality.
INTRODUCTION

Throughout history, crop improvement has been the driving force of agricultural development. The advent of cross breeding in wheat (*Triticum aestivum* L.) at the close of the 19th century accelerated this venture (Bonjean and Angus 2001). Currently, parental selection and subsequent evaluation of progeny begin this process. Intense end-use quality assessment begins with the F5 or F6 generation and continues for 4-6 years as the breeding line undergoes consideration for commercial release. Currently, regional cooperative nurseries in the U.S. include the most advanced-generation lines for assessment. These nurseries are delineated by growth habit (winter or spring) and broad geographical region. Multiple locations provide assessments of adaptation and disease resistance. End-use quality evaluations are generally of more limited scope and are performed at regional quality laboratories of the United States Department of Agriculture.

The standardization and improvement of end-use quality assessment has long been a function of AACC International. Despite the standardization of methods, milling and baking evaluations are difficult to replicate across laboratories, and thus, evaluation of grain, milling, and baking quality of wheat grown across broad geographic regions, such as this study, is best performed at a single laboratory for the most uniformly consistent and directly comparable results.

Wheat end-use quality is a product of genetics (G), environment (E), and their interaction (GxE). The relative importance of these factors in any given study is a function of (1) the genetic diversity of varieties, (2) the diversity of environments sampled, and (3) the relative extent to which any given trait is influenced by genetics and environment. G, E, and GxE are highly significant factors in variation in milling and baking quality in soft red winter wheats grown in
the southeastern U.S. (Baenziger et al 1985; Kiszonas et al in press). The high correlation observed among variety performance in any given environment suggests that rank order of performance remained consistent. The influences of G, E, and GxE similarly affect hard red winter wheat quality (Peterson et al 1992, 1998). The quality of hard red winter wheat varieties was influenced by the environment, but similar to soft red winter wheat, rank performance was highly consistent. While both environment and genetics influenced flour quality, it was observed that environment had a larger contribution to flour quality variation in a study of two soft wheat cultivars (Guttieri et al 2002). In a multi-environment study conducted in Kazakhstan and Siberia with many varieties, environmental impacts on quality traits played a variable role (Gómez-Becerra et al 2010). The GxE interaction varied in an Austrian study depending upon the quality trait studied (Grausgruber et al 2000). Mediterranean wheat exhibited a similar phenomenon, showing that genetic variation is widespread across many different growing conditions (Rharrabti et al 2003).

It is common practice to compare only varieties targeted for areas with similar climatic and edaphic conditions and abiotic/biotic stresses. Morris et al (1997) described the potential challenges when using only a small environmental area to study wheat quality and demonstrated that environmental influence depended on which specific varieties were studied. In order to obtain results with marketing applicability, environmental influence is generally evaluated within a specific area of adaptation. In a study utilizing eastern and western U.S. soft wheat cultivars at two locations in Washington State, Morris et al (2004, 2005) observed varietal differences across and within regional germplasm. The eastern varieties were not grown in their intended region of commercial production and thus, their end-use quality did not reflect the influences of their region of adaptation.
Grain, milling, and baking quality are common sub-divisions of overall wheat end-use quality (Morris and Rose 1996; Wrigley and Morris 1996). Genetic differences in individual traits comprising end-use quality are widely observed (Baenziger et al 1985; Peterson et al 1992, 1998; Ram and Singh 2004; Morris et al 2004, 2005, 2009; Finnie et al 2006; Li et al 2009; Kiszonas et al in press). Individual traits taken in combination facilitate the study of predictive relationships and correlations to further understand wheat quality variation across genetic and environmental factors.

The most widely utilized first determinant of end-use quality for wheat is kernel hardness (Morris and Rose 1996). Despite common belief, kernel texture is largely unrelated to protein content (Simmonds 1974) and is a product of genetic control (Morris 2002). Hard wheat fractures along cell walls in the milling process, whereas soft wheat fractures through cell walls (Hoseney et al 1988). Consequently, soft wheat has finer particles and less starch damage due to this unique fracture pattern (Hoseney et al 1988). Cookies, pancakes, cakes, and other similar products often utilize soft wheat, while hard wheat is better suited for bread production (Huebner et al 1999).

Water absorption is a critical component of hard wheat functionality, and is influenced by several physical-chemical factors, including damaged starch, soluble starch, pentosans, protein content and flour particle size. Dextrins are released from damaged starch, and become solubilized when flour is mixed with water. Particle size is also a critical component to water absorption relationships. Damaged starch is often highly correlated to larger flour particle size distribution (Yamamoto et al 1996). Pentosans, specifically those that are water-extractable, have been observed to increase bread dough consistency and dough stiffness (Courtin and Delcour 2002). Generally, high levels of water-extractable pentosans increase baking absorption.
Improved dough characteristics tend to be positively correlated with water-extractable pentosans, but negatively correlated with the total pentosan content (Courtin and Delcour 2002).

Wheat grain and flour protein are often highly influential determinants of end-product functionality and use. Gluten protein formation is a key element to successful bread making, and is generally associated with high protein levels in hard wheats. Soft wheat products, however, are often negatively impacted by gluten formation, necessitating lower protein levels and/or weaker gluten proteins in soft wheat products (Gaines 2004). Graybosch et al (1993) found dough strength and bread loaf quality to be primarily a function of flour protein concentration. Whereas protein content is primarily a function of environment, protein composition is genetically determined. Both the quality and content of protein must be optimized for the highest level of end-use functionality. These two work in concert to impart gluten “strength”. Gluten strength is the most critical component in optimizing bread making. The gluten network is responsible for creation of cohesive, viscoelastic dough and the ability of dough to retain gas during fermentation (Hoseney 1998). This gas retention and viscoelastic character is critical to the development of high-quality pan bread. Gluten strength is primarily responsible for variation in gas retention and ultimately loaf volume (Hoseney 1998). There are several methods commonly employed to predict or estimate the gluten strength of bread dough, two of the most prominent being flour SDS sedimentation volume and the Mixograph.

Two Mixograph parameters provide particularly useful data to predict overall bread making performance: Mixograph water absorption and mixing time. Mixograph absorption describes the optimum flour water absorption and is generally considered to be a function of protein content and the other physical-chemical factors, and encompasses contributions from varietal and environmental influence (Finney and Shogren 1972). Mixograph mixing time is the
time in minutes required to mix the flour and water other bread dough constituents to the optimum condition for bread baking to the point of minimum mobility, evidenced by the ‘peak’ of the mixing curve (Finney and Barmore 1945). Bake water absorption is an estimate of the amount of water required to make a dough of proper consistency for bread baking when mixed to optimum conditions (Finney 1945). Bake water absorption and mixing time have been shown to be influenced by flour protein content (Ohm et al 1998). These characteristics are also generally well correlated to loaf volume (Ohm et al 1998). Dowell et al (2008) also determined that the best predictive models for overall bread quality (loaf volume) incorporated grain or flour protein in addition to bake water absorption and bake mixing time.

There were three objectives for this research. The first was to survey hard wheat end-use quality across the contiguous United States and analyze the regional and varietal differences in grain, milling and baking quality. The second objective was to evaluate potential relationships among different traits across a broad range of elite varieties and environments. The third objective was to determine predictive factors for end-use quality based on observed relationships among grain, milling and baking quality traits. In the present report, we document the quality of hard spring and winter wheat germplasm from the United States drawn from regional nurseries harvested in 2009. Prior to this study, no such comprehensive survey had been undertaken.

MATERIALS AND METHODS

Grain Samples

A total of 264 hard wheat samples representing 180 different genotypes were included in this study. These wheat samples were grown at seven locations (Table I), spanning the major hard wheat producing areas of the continental United States, in the 2009 crop year (winter

Quality Measurements

The analyses performed on these grain samples can be grouped into three categories: grain, milling, and baking quality. The quality parameters were assessed using AACC International Approved Methods (2010). Table II provides a list of these parameters along with the units of measurement for each. The grain quality parameters studied were: test weight, wheat grain protein, single kernel characterization system (SKCS) parameters of kernel hardness, weight, and size, and polyphenol oxidase (PPO) activity. The milling quality characteristics surveyed were: flour yield, break flour yield, milling score, flour protein, and flour ash. The
baking quality parameters studied were: Mixograph water absorption, bake water absorption, flour SDS sedimentation volume, mixing time (the time to reach optimal dough consistency), and loaf volume (100 g ‘pup’ loaf pan bread). The parameters of mechanically determined mixing time to peak mixing strength, the height at the midline of peak mixing strength, width at the peak, and width of the lines two minutes (to determine resistance to over-mixing) following optimum mixing time from the Mixograph were determined using the Mixsmart (Mixsmart for Windows Version 1.0.404, Lincoln, NE).

### Statistical Analysis

The statistical analyses were performed using SAS v.9.2 (SAS Institute, Cary, NC). The ANOVAs were performed using PROC GLM. Each nursery was analyzed separately with varieties and locations as fixed effects. No interaction term was included in the model due to the lack of quality analyses on multiple field replicates. The absence of analyzing more than one field plot replicate is a near universal limitation in end-use quality analysis of wheat breeding programs, as a simple two- vs. one-replicate system would necessarily double the number of analyses. The model consisted of determining the variation due to location and varietal differences within nurseries having two locations. Type III mean squares were used to calculate variety:location ratios. As such, a ratio of 1 indicates equal contributions from variety and location. The 95% range was calculated by adding or subtracting two standard deviations from the mean. Nurseries were analyzed separately because in general, each nursery was a unique set of germplasm with few if any entries appearing in more than one nursery.
RESULTS

Grain Quality Characteristics – Hard Spring Wheats

Variation in grain quality characteristics of the Uniform HRS and Western Spring wheat nurseries was well modeled in the ANOVA using ‘variety’ (= G) and ‘location’ (≈ E) as model main effects with whole model $R^2$ values ranging from 0.76 to 0.97 (Table III). Both nursery whole models were significant, as was ‘variety’ for all grain quality traits. The high model $R^2$ values indicated that GxE was likely a minor source of variation, although it could not be accurately estimated due to lack of in-lab analysis of field plot replicates.

To examine the relative contribution of ‘genotype’ (variety) vs. ‘environment’ (location), ratios of the respective mean squares were calculated and are presented in Table III. Only test weight and PPO activity in the Uniform HRS nursery and SKCS hardness and PPO activity in the Western Spring nursery were more highly influenced by varietal differences; the other grain quality traits were more heavily impacted by location differences.

Test weights and their respective 95% ranges were similar between the two nurseries with an overall advantage in the Western Spring nursery (Table III). The SKCS hardness, however, was notably higher in the Uniform HRS (77.0) nursery as compared to the Western Spring nursery (67.5). This trait was highly influenced by varietal differences in the Western Spring nursery. Conversely, the SKCS weight was higher, on average, in the Western Spring nursery, although the low end of the ranges was similar. Mean SKCS diameter and PPO activity were similar for the two nurseries, although the range of PPO activity was considerably larger in the Western Spring nursery. The low PPO activity of some varieties in the Western Spring nursery was associated with the hard white varieties in this nursery. The hard red wheat varieties in the Western Spring nursery had an average PPO activity of 0.72 absorbance units (AU),
whereas the hard white varieties had an average PPO activity of 0.50 AU. This clustering of red vs. white varieties likely reflects the breeding efforts directed toward decreasing PPO activity in hard white wheat in the Pacific Northwest (Morris and Rose 1996).

The grain protein content of the Uniform HRS nursery (14.2%) was somewhat higher than in the Western Spring nursery (13.5%), although the upper ends of their 95% ranges were similar. The difference in means may be attributed to several low-protein content varieties in the Western Spring nursery. Grain protein was heavily influenced by environment in both nurseries, reflected in low genotype:environment ratios, indicating that the differences were primarily due to environmental factors rather than genetics. The two locations of the Western Spring nursery exhibited a larger difference in mean grain protein content (0.79%) than did those of the Uniform HRS nursery (0.50%) (data not shown).

**Grain Quality Characteristics – Hard Winter Wheats**

All grain quality response variable models in the Western Winter nursery (Table IV) showed high levels of significance, with \( R^2 \) values ranging from 0.86 to 0.96, indicating that varietal and location differences captured the majority of the variation, with minor variety x location interaction. In this nursery, however, SKCS hardness was also strongly impacted by differences among the varieties, with much less contribution from location. In the Western Winter nursery, the other SKCS parameters of kernel weight and diameter were also strongly influenced by variety, with much less contribution to variation due to location. Based on the G:E ratio, PPO activity was more heavily influenced by varietal differences than by location differences, in a fashion similar to that seen in the spring nurseries.
The samples available for the Kansas WWPT nursery, Northern RP nursery, and Southern RP nursery were from single locations, and thus could not be analyzed for varietal vs. location contributions in the overall statistical model. The Western Winter nursery and Kansas WWPT nursery had mean test weights notably higher than those in the Northern and Southern RP nurseries. All four nurseries exhibited similar SKCS kernel diameters and corresponding ranges. The SKCS hardness was markedly higher in the Kansas WWPT nursery with a narrower 95% range. The SKCS hardness of the Northern RP nursery was markedly lower than the other three nurseries; the Western Winter nursery and Southern RP nursery had similar values. The two waxy wheat varieties in the Northern RP nursery exhibited markedly lower SKCS hardness (31.3 and 14.9) than the nursery average (48.5).

Grain protein content was also substantially higher in the Kansas WWPT nursery, with a mean of 14.3%, as compared to the Western Winter nursery (13.2%), and the Northern (11.2%) and Southern (12.1%) RP nurseries. The corresponding 95% range in the Kansas WWPT nursery was very small, suggesting high levels of consistency among the varieties. The large differences in protein content are not surprising based on the well-established large environmental influence on wheat protein content.

The PPO activity present in the Western Winter nursery, Northern RP nursery, and Southern RP nursery germplasm revealed a varying degree of separation between hard red and hard white wheat varieties, as previously discussed for spring nurseries. In the Western Winter nursery, the hard red wheats had an average of 0.81 AU for PPO activity, whereas the hard white wheat varieties had an average of 0.57 AU. In the Northern and Southern RP nurseries, there was less separation between the two classes; the hard red wheat varieties had an average PPO activity
of 0.88 and 0.81 AU, respectively, whereas the hard white wheat varieties had a PPO activity of 0.88 and 0.68 AU, respectively.

**Milling Quality Characteristics – Hard Spring Wheats**

Of the four milling characteristics surveyed (Table V), only break flour yield and flour yield exhibited overall model significance in the Uniform HRS nursery, while all response variables showed overall model significance in the Western Spring nursery ($R^2$ values 0.76-0.93). In the Uniform HRS nursery, flour ash and milling score did not have sufficient varietal or location differences to allow for mean separation among any of the data. It is also quite possible that in those cases, there was a GxE interaction which could not be directly quantified. For both flour ash and milling score, however, the ratio indicated that there was a greater location or “environmental” contribution to overall variation than variety, but neither was sufficient to give model significance. In all milling characteristics surveyed, location effects contributed predominantly to the overall model, with very little varietal influence. In the Uniform HRS nursery, differences in break flour yield were overwhelmingly the product of location differences; the varietal contribution was not significant to the overall model. Flour yield showed a similar pattern of strong location differences, but less dramatic than in break flour yield.

Break flour yield and flour yield and their respective 95% ranges were similar for both spring wheat nurseries (Table V). The 95% distribution ranges for both break flour yield and flour yield were especially large in both nurseries, and in combination with the high location contribution to these variables, indicates a wide range of environmental influence on these varieties. Flour ash was notably higher in the Uniform HRS nursery (0.40 vs. 0.35%), which was
likely the main cause of a markedly lower milling score (79.9) as compared to the Western Spring nursery (84.2).

**Milling Quality Characteristics – Hard Winter Wheats**

All milling traits showed strong model significance in the Western Winter nursery, with $R^2$ values ranging from 0.94 to 0.97 (Table VI), indicating that a large portion of the variation in the data was captured by varietal and location differences, with little contribution from an interaction between the two factors. Break flour yield and flour yield in the Western Winter nursery was heavily influenced by varietal differences. There was no significant contribution of location to break flour yield in the Western Winter nursery, which indicated that this was in effect, essentially a varietal characteristic.

Among the four winter nurseries, (Table VI), the milling quality means and 95% ranges varied greatly. Break flour yield was similar among the Western Winter, Northern RP and Southern RP nurseries with correspondingly similar ranges, as compared to a lower break flour yield in the Kansas WWPT nursery. Flour yield was also similarly higher in the Western Winter nursery and the two RP nurseries as compared to the Kansas WWPT nursery (71.7, 67.0, and 69.0 vs. 64.3%, respectively). The two waxy wheat varieties in the Northern RP nursery had notably lower flour yields (52.9 and 59.6%) as compared to the nursery average (67.0%). Flour ash was similar across the four winter nurseries, with the Western Winter nursery and two RP nurseries exhibiting only slightly lower levels of bran contamination and/or intrinsic endosperm ash than in the Kansas WWPT nursery. The milling scores, however, reflected the higher break flour yield and flour yield of the Western Winter nursery and Southern RP nursery, with mean milling scores of 88.6 and 85.1 respectively, and the Northern RP nursery and Kansas WWPT
nursery exhibiting lower mean scores of 79.1 and 82.9, respectively. To that end, the upper limit of the 95% range for the Kansas WWPT nursery was lower than the mean milling scores observed in the Western Winter and Southern RP nurseries. The two waxy wheat varieties of the Northern RP nursery had lower milling scores, in one case dramatically lower (67.3 and 79.0) than the nursery average (82.9).

**Baking Quality – Hard Spring Wheats**

The varietal and location differences generated the majority of the variation in the baking parameters, with $R^2$ values ranging from 0.70 to 0.95 (Table VII). Based on the G:E ratios, location differences had a much greater influence on baking traits with the exception of flour SDS and loaf volume. Bake water absorption, in particular, had no significant varietal differences and was thus predominantly controlled by location differences in this model. Flour SDS was negligibly influenced by location differences. Loaf volume was more heavily controlled by varietal differences than location differences in both spring wheat nurseries, supporting the assertion that loaf volume is largely a genetically controlled characteristic, though a certain amount of environmental confounding may also play a small role in loaf volume variation. The Uniform HRS nursery exhibited a slightly higher flour protein content than the Western Spring nursery, with a narrower 95% range (Table VII). Mixograph water absorption and bake water absorption were similar, though the Uniform HRS nursery had slightly higher responses for these characteristics. The mixing time was slightly longer in the Western Spring nursery (4.92 vs. 4.25 min). There was a notably large range of mixing times observed in the Uniform HRS nursery and Western Spring nursery (2.12-6.37 min and 2.32-7.52 min). The additional Mixograph parameters of time to peak, height of the midline at peak, curve width at
the peak, and width two minutes after the peak did not exhibit any appreciable correlation to loaf volume (data not shown). The flour SDS sedimentation volume was only assessed in the Uniform HRS nursery, and was correlated to mixing time ($r = 0.57$) and width of the curve at peak ($r = 0.61$).

Mean loaf volume of the Western Spring nursery was markedly higher than in the Uniform HRS nursery. In the Western Spring nursery, there was a strong division in loaf volume between the hard red and the hard white wheat varieties. The hard red varieties had considerably larger loaf volumes on average (1040 cm$^3$) than the hard white varieties (967cm$^3$), with the notable exception of one high-performing hard white wheat variety (A01458S-D-2, 1095 cm$^3$).

**Baking Quality – Hard Winter Wheats**

All $R^2$ values were significant in the Western Winter nursery (Table VIII), ranging from 0.91 to 0.96, indicating that varietal and location differences captured the majority of the data variation, with very little interaction between the two factors. Both variety and location had significant impacts on the models in all baking traits. Based on the G:E ratios, loaf volume was the only trait influenced more by genetics than by environment.

The Northern and Southern RP nurseries exhibited the lowest flour protein (9.6 and 10.5%, respectively), the Western Winter nursery was intermediate, and the highest flour protein was present in the Kansas WWPT nursery (Table VIII). Flour SDS sedimentation volume, an indicator of gluten strength, was notably higher in the Kansas WWPT nursery (23.0 mL/g) than in the Northern and Southern RP nurseries (16.6 and 18.9 mL/g). The two waxy wheat varieties in the Northern RP nursery exhibited considerably lower flour SDS sedimentation volumes (6.4 and 8.7 mL/g) than the nursery average (16.6 mL/g).
The nursery rank order of Mixograph absorption and bake water absorption followed that of flour protein; the Northern and Southern RP nurseries had the lowest Mixograph absorption values, the Western Winter nursery was intermediate, and the Kansas WWPT nursery had the highest response. The Western Winter nursery had a very low mean mixing time of 2.83 min, whereas the Kansas WWPT nursery, Southern RP nursery, and Northern RP nursery were more similar with mixing times of 4.99, 5.08, and 5.12 min, respectively. The Southern RP nursery also exhibited the greatest 95% range of responses from 1.82 to 8.35 min, a much larger range than that observed in the other three nurseries. The additional Mixograph parameters determined by the Mixsmart Software did not exhibit any appreciable correlation to loaf volume (data not shown). Higher correlations were observed between flour SDS sedimentation volume and mixing time ($r = 0.54, 0.52, \text{ and } 0.33$) for the Kansas WWPT, Northern RP, and Southern RP nurseries, respectively, and the curve width at peak ($r = 0.59, 0.56, \text{ and } 0.37$) for the Kansas WWPT, Northern RP, and Southern RP nurseries, respectively.

The Northern RP nursery had the lowest mean loaf volume of 843 cm$^3$, the Western Winter nursery and Southern RP nursery had intermediate loaf volumes of 900 and 904 cm$^3$, and the Kansas WWPT nursery had the highest mean loaf volume of 979 cm$^3$. The largest difference between mean loaf volumes for hard red and hard white wheat varieties within a nursery was observed in the Western Winter nursery, 973 vs. 854 cm$^3$, respectively. The Southern RP nursery also exhibited a large difference between red and white wheat varieties (917 vs. 850 cm$^3$). The difference was much less dramatic between the two market classes in the Kansas WWPT nursery. Notably, the mean loaf volume of white wheat varieties in the Northern RP nursery was 20 cm$^3$ greater than the mean loaf volume of red wheat varieties. The two waxy wheat varieties in the Northern RP nursery had notably the lowest loaf volumes (585 and 660 cm$^3$), considerably
lower than the nursery average of 843 cm³, and undoubtedly adversely affected the red wheat mean.

**Gluten Strength**

Our analysis also specifically examined the utility of flour SDS to estimate or predict loaf volume. Figure 1 depicts the relationship between flour SDS sedimentation volume and loaf volume for the Uniform HRS nursery (two locations), Kansas WWPT nursery, Northern RP nursery, and Southern RP nursery. The second order polynomial curve captures the complicated nature of the relationship well, with an $R^2$ value of 0.50. When the contributions from each nursery to the relationship are examined individually, the only apparent trend was that the Kansas WWPT nursery responses were concentrated in the upper portion of the flour SDS sedimentation volume and loaf volume curve. Beyond this, the nurseries were well-distributed along the curve.

**Specific Varieties Displaying Exceptional Baking Quality – Spring Wheats**

Two Uniform HRS varieties showed exceptional loaf volume, which is the primary determinant of a high-quality hard wheat variety: SD4011 and MN06018. Neither of these exhibited many other outstanding characteristics in the other response variables measured. The protein content was notably high in SD4011, and the bake water absorption was high in MN06018.

Three Western Spring varieties at the upper end of the loaf volume spectrum were identified as having outstanding results: Kelse, Hank, and WA8027. The variety Kelse exhibited high flour protein, and high Mixograph and bake water absorption. Kelse had low PPO activity
and a short optimum mixing time. The variety Hank exhibited high responses for SKCS weight and size, and no low values for any variable. The variety WA8027 displayed high wheat protein, flour protein, and Mixograph water absorption, while it had no particularly low responses.

**Specific Varieties Displaying Exceptional Baking Quality – Winter Wheats**

Three varieties were identified as having exceptionally large loaf volumes in the Western Winter nursery: ID660, Finley, and ID826. The variety ID660 had a low SKCS kernel weight (2.77 mg). The variety Finley had high SKCS kernel size, break flour yield, and milling score, while it exhibited a low SKCS hardness score (54.5). The trend seen in this nursery of higher overall performance of hard red wheat varieties as compared to hard white wheat varieties was mirrored in the stand-out variety performance, the exception being ID660, which was a hard white variety, exceptionally large loaf volume in comparison to the nursery mean.

There were five varieties with large bread loaf volumes in the Kansas WWPT nursery: Overley, HV9W03-539R, Ike, Armour, and TAM112, which were all hard red winter varieties. Overley had low SKCS hardness, high flour protein, and a high optimum mixing time. The variety HV0W03-539R exhibited a high bake water absorption, but low test weight, break flour yield, and milling score. Ike had a high break flour yield and high flour protein. Interestingly, although hard white wheat varieties had overall lower loaf volumes than hard red varieties in this nursery, those at either end of the loaf volume distribution were primarily hard red.

Several varieties exhibited exceptionally large loaf volumes in the Northern RP nursery: SD07126, NE06436, and CA9W07-818. SD07126, NE06436, and CA9W07-818 exhibited high kernel hardness, high wheat and flour protein, and loaf volumes of 1035, 940 and 910 cm$^3$, respectively. The varieties SD07126 and CA9W07-818 also exhibited high flour SDS.
sedimentation values, although the response for NE06436 was similar to the mean flour SDS sedimentation value for the nursery. The mixing times also varied greatly; the highest loaf volume (SD07126) had a mixing time of 3.9 min, whereas the other two had considerably longer mixing times of 6.1 min.

Three varieties from the Southern RP nursery exhibited exceptional loaf volumes; all three were hard red winter. Those with large loaf volume were: AP05T2413, HV9W05-1125R, OK05212. The three varieties with exceptionally large loaf volumes exhibited only moderate kernel hardness (59.7-61.5), which was only slightly above the Southern RP nursery mean. These varieties were also characterized by high wheat and flour protein contents. Their flour SDS sedimentation volumes ranged from 19.2 to 20.9 mL/g, and exceeded the nursery mean of 18.9 mL/g. There was no discernible pattern in Mixograph absorption or mixing time among these three varieties. The loaf volumes for AP05T2413, HV9W05-1125R, and OK05212 were 1050, 1040, and 1030 cm$^3$, respectively.

**DISCUSSION**

In the Uniform HRS nursery and Western Spring nursery, location differences had an overall greater effect on quality than did varietal differences (Table III). The Western Winter nursery was more divided; roughly half of the response variables were more highly influenced by location, while the other half of the quality characteristics were dominated by varietal differences (Table IV). Most of the varietal differences were observed among the grain and milling characteristics, while baking characteristics were most heavily influenced by location differences, with the notable exception of loaf volume. These findings indicate that the relative importance of location and variety depend upon the trait being measured, as well as on the
particular set of varieties and the particular location being evaluated. Therefore no universal conclusions can be drawn about wheat grown in all hard wheat regions in the United States. The same pattern of variation was observed in a survey of U.S. soft wheat germplasm (Kiszonas et al in press). Nevertheless, robust statistical models (Tables III-VIII) indicated that in most cases there was likely little GxE interaction such that variety rank order would be consistent across locations. This point is especially relevant for breeding and germplasm selection in furtherance of cultivar development activities. Following is a discussion of results across all nurseries for the three sub-divisions of quality traits.

**Grain Quality**

Among the grain quality traits, there was little consistent influence in terms of a greater contribution to variation from location or variety across nurseries (Tables III and IV). Guttieri et al (2002) also observed varying levels of environmental influence, due largely to climatic conditions. The test weight of varieties in the Northern and Southern RP nurseries were notably lower than in the other four nurseries, with correspondingly smaller 95% ranges. The SKCS kernel hardness of the Uniform HRS nursery and Kansas WWPT nursery were substantially higher than that observed in the two Western nurseries. The Northern and Southern RP nurseries were, on average, the softest. Kernel hardness was highly associated with varietal differences in both the Western Spring and Winter nurseries, indicating that in the western growing regions, location differences played a less prominent role in determining grain hardness variation. The high level of varietal influence in these nurseries mirrors that observed in Western soft wheat nurseries (Kiszonas et al in press), suggesting that for both hard and soft wheats, there is less environmental influence over kernel hardness, and most variation can be attributed to genetics.
Some of the variation observed in kernel hardness may be the result of different puroindoline alleles (Giroux and Morris 1997, 1998; Morris et al 2001; Morris 2002; Martin et al 2006). Kernel weight varied among the six spring and winter nurseries. The Western Winter nursery had the highest kernel weight (37.5 mg) of the six nurseries. Kernel diameter, however, was similar among all six nurseries. There was also little concurrence between SKCS hardness and protein content, reflecting a similar finding by Simmonds (1974).

The one characteristic that was influenced heavily by varietal differences was PPO activity. The present results indicate that variation in PPO activity is overwhelmingly the result of genetic differences. PPO activity varied greatly among the six nurseries, however, likely in some part because each nursery contained a different make-up of red and white wheat varieties, which tended to exhibit differing levels of PPO activity (see below). This feature likely reflects a varying degree of selection pressure exerted by breeders due to end-use quality objectives.

**Milling Quality**

Among the hard spring wheat nurseries, location differences heavily influenced the statistical models (Table V), whereas in the Western Winter nursery, flour yield and break flour yield were dominated by varietal differences (Table VI). Break flour yield ranged from 36.3 to 44.7% across the six nurseries, with variable 95% ranges among the individual nurseries. There appeared to be a separation between the nurseries with the Kansas WWPT nursery, Uniform HRS nursery, and Western Spring nursery having lower values of 36.3, 37.3, and 38.1% break flour yield. The Western Winter nursery, Northern RP nursery, and Southern RP nursery exhibited markedly higher break flour yields of 42.9, 44.7, and 43.4%. Break flour yield was primarily genetically controlled in Western soft wheat nurseries (Kiszonas et al in press).
Flour yield was similarly varied among the six nurseries, although the responses across the nurseries were more evenly distributed, ranging from the Kansas WWPT nursery (64.3%) to the Western Winter nursery (71.7%). Flour yields were consistently higher in the two Western nurseries as compared to the remaining four nurseries from the U.S. Great Plains (Tables V and VI).

Although flour ash varied modestly across the six nurseries, milling score varied dramatically. This contrasting range of responses was notable because milling score is influenced by both break flour yield and flour ash, both of which were similar across all nurseries. The high level of variation in milling scores is consistent with that observed in soft wheat varieties grown across the U.S. (Kiszonas et al in press). Milling scores were lowest in the Kansas WWPT nursery, which corresponded to low break flour yield, flour yield, and relatively high flour ash content. Conversely, the Western Winter nursery had the highest milling score, along with high break flour yield, flour yield, and low flour ash content.

**Baking Quality**

Flour protein content mirrored grain protein; the rankings of nurseries and varieties within each nursery were nearly identical between the two measurements (Tables VII and VIII). However, there was a wide range of flour protein contents across the six nurseries. The Northern RP nursery had the lowest mean flour protein content of 9.6%, whereas the Uniform HRS nursery had the highest flour protein content, 12.9%. In terms of baking quality, a 3% difference is considerable. Flour SDS sedimentation volume also varied widely across the nurseries. The Kansas WWPT nursery and the Uniform HRS nursery had high mean flour SDS sedimentation
volumes, whereas lower volumes were observed in the Northern and Southern RP nurseries. These trends generally corresponded to the flour protein contents observed in these nurseries.

Mixograph water absorption followed this same trend, with those nurseries exhibiting low flour protein and low flour SDS sedimentation volume also having low Mixograph and bake water absorptions. The highest Mixograph absorption and bake water absorption values were observed in the Kansas WWPT nursery. The six nurseries had the same rank for Mixograph and bake water absorptions from lowest to highest: Northern and Southern RP nurseries, Western Winter and Spring nurseries (equivalent), Uniform HRS nursery, and Kansas WWPT nursery. The 95% range exhibited within each of the nurseries was also similar in magnitude. In all six nurseries, location differences had a much greater effect on the overall statistical model for Mixograph and bake water absorptions than did varietal differences. These similarities indicate that across environments, varietal differences play a relatively small role in Mixograph and bake water absorption variation, but that location effects have similar influences on these two traits across all nurseries.

Mixing times were similar across five of the nurseries, ranging from 4.25 to 5.12 min; the Western Winter nursery exhibited a substantially shorter mixing time of 2.83 min. This was the only notable baking characteristic among the traits surveyed for the Western Winter nursery; all other traits fell within the range of the other nurseries. Similar to the Mixograph and bake water absorptions, mixing time was largely influenced by location differences across the three nurseries for which ‘location’ model contributions were analyzed.

Loaf volume was heavily influenced by varietal differences in the three multi-location nurseries. In both the Uniform HRS nursery and the Western Spring nursery, location differences were not significant, indicating that the statistical model was influenced more heavily by varietal
differences. Because variety overwhelmingly contributed to the model, it would appear as if location had little impact on loaf volume, despite the fact that location influenced other quality traits known to affect loaf volume (i.e., protein content, Mixograph and bake water absorption, and mixing time). Mean loaf volumes ranged from 843 cm$^3$ in the Northern RP nursery to 1005 cm$^3$ in the Western Spring nursery, with the remaining four nurseries falling within this range.

**Traits Predicting Bread Quality**

When the quality traits most often associated with loaf volume were examined, there were several apparent trends. Within and across nurseries, wheat and flour protein content were the best overall predictors of loaf volume ($r = 0.31$ and 0.32). However, not surprisingly, high protein did not in-and-of-itself guarantee good bread performance. High Mixograph and bake water absorptions also had value in predicting large loaf volume. Whether this observation was directly related to dough mass or other intrinsic factors (such as protein or damaged starch) could not be ascertained in the present study. Longer mixing times tended to also be associated with better bread, but there were exceptions.

Conversely, poor bread as evidenced by small loaves was almost always associated with low protein, low water absorption, short mixing time, and/or low SDS sedimentation volume. A closer examination of the data revealed that on occasion, especially high values of one or more of the preceding traits were also associated with lower loaf volume. Two varieties in particular exhibited this phenomenon of high gluten strength decreasing overall bread volume: Hawken and Fuller in the Kansas WWPT nursery. They had moderate-to-high flour protein levels (13.0 and 12.2%), a high flour SDS sedimentation volume (both 27.3 mL/g), and relatively long mixing times (7.2 and 6.3 min, respectively), yet had the lowest and fifth lowest loaf volumes of the
nursery (745 and 715 cm³). In these varieties it appeared that the dough (≈gluten) was actually too strong; a trait described by bakers as “bucky” doughs with poor extensibility. It should be noted, however, that some availability of this type of variety is highly valued in the U.S. market to “blend up” the strength of weaker varieties.

The analysis of SDS sedimentation and loaf volume (Fig. 1) supported the view that there may be a threshold level of gluten strength necessary for optimum loaf volume. Figure 1 indicates that there is a minimum level of flour SDS sedimentation volume, or gluten strength, necessary for large loaf volume. Once this level of strength has been achieved, further increases do not markedly increase the loaf volume and may in fact decrease volume. The non-linearity of the least squares line indicates that there is a more complex relationship between SDS sedimentation volume (≈gluten strength) and loaf volume. The figure also shows a more linear relationship at the lower end of the SDS sedimentation volume and loaf volume responses, and a more highly varied relationship at the higher levels of SDS sedimentation volume and loaf volume. It should be noted that an SDS sedimentation value approaching 30 mL/g essentially indicates no settling of the sediment in the assay, i.e. the entire tube volume is occupied by swollen hydrated sample.

Overall, it appeared as if there were no defining characteristic(s) indicative of high bread-making quality as quantified by loaf volume. There were few trends even within a nursery to serve as predictive factors for high loaf volume. Characteristics such as high wheat and flour protein, and high Mixograph and bake water absorption appeared several times across the nurseries in conjunction with high loaf volume. This trend followed closely that observed by Dowell et al (2008) and Ohm et al (1998). Low loaf volume, however, was more easily correlated to other quality traits surveyed. Low loaf volume was generally related to low
responses in flour yield, SKCS hardness, flour protein, mixing time, and flour SDS sedimentation volume.

**Red and White Wheat Varieties**

The lower PPO activity among some hard white varieties reflects a specific breeding effort aimed at decreasing PPO activity in hard white wheat to appeal to consumers and food processors – specifically the Asian noodle market. The Asian noodle market prefers less discoloration as attributed to PPO activity (Fuerst et al 2010). In all nurseries, the average PPO levels were higher in hard red wheats and lower in hard white wheats, but to a varying degree.

In five of the six nurseries with both hard red and hard white varieties, loaf volume was higher, on average, for the hard red wheat varieties. The degree of separation between the two classes, however, differed among the nurseries. Notably, in the Northern RP nursery the white wheat varieties exhibited a higher mean loaf volume than did the red wheat varieties. The Western Winter and Spring nurseries exhibited strongly contrasted loaf volumes between hard red wheat and hard white wheat, the hard red varieties displaying considerably larger loaf volumes than the hard white varieties. This difference may reflect the breeding model for hard white wheat adopted in the early 1980s wherein hard white varieties were aimed at Asian noodle uses and were thought to require a lower level of gluten strength (Morris 1998). These differences were less pronounced in the Kansas WWPT nursery, exhibiting small mean differences between the color classes. In this nursery, there was a slight advantage in the hard red wheats (18 cm³).

**Conclusion**

Overall, the traits that best predicted large loaf volume were high flour protein, high flour yield, and high Mixograph water absorption, and a trend towards longer mixing times. The low
loaf volume responses were simpler to define, characterized by low wheat and flour protein, low SDS sedimentation volumes, and short mixing times. The variation in grain, milling, and baking quality appeared to be region-dependent, indicating that environmental contributions to variation influenced the overall assessment of wheat quality. This interplay between genetics and environment across regions on wheat quality was also observed by Baenziger et al (1985). While bakers would typically prefer a shorter mixing time to achieve full gluten development for cost and time savings, this study observed moderate to somewhat longer mixing times were associated with larger bread loaf volumes. This relationship, however, was dependent upon the nursery, vis-a-vis the growing region. Nevertheless, with the caveat of stronger-than-optimum varieties, the current laboratory methods provided a good assessment of end-use quality, with low error variances and robust statistical models. As such, this study provides a valuable survey that further enhances the understanding of variation in U.S. hard wheat quality as it relates to genetics, environmental factors, and the influence they have on grain, milling, and baking quality.

ACKNOWLEDGMENTS

We would like to acknowledge the technical staff of the USDA-ARS Western Wheat Quality Laboratory, Doug Engle, Mary Baldridge, Bozena Paszczynska, Mishelle Lenssen, Eric Wegner, Bill Kelley, Patricia Boyer, Gail Jacobson, and Anna Hansen. Stacey Sykes and Shawna Vogl assisted in the preparation of the manuscript. This project was supported by the Agriculture and Food Research Initiative Grant no. 2009-02347 from the USDA National Institute of Food and Agriculture.
LITERATURE CITED


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interaction. Field Crops Res. 80:141-146.


TABLE I

Summary of regional nurseries, locations, and color class of varieties used to survey hard wheat end-use quality in the U.S.

<table>
<thead>
<tr>
<th>Nursery</th>
<th>Location</th>
<th>Varieties/Location</th>
<th>Varieties/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniform Hard Red Spring Wheat Regional</td>
<td>Fargo, ND</td>
<td>37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pullman, WA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Regional Hard Spring Wheat</td>
<td>Bozeman, MT</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Pullman, WA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Regional Hard Winter Wheat</td>
<td>Aberdeen, SD</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Bozeman, MT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kansas Winter Wheat Performance Trials</td>
<td>Garden City, KS</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Northern Regional Performance (winter wheat)</td>
<td>Palmer, KS</td>
<td>22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>Southern Regional Performance (winter Wheat)</td>
<td>Salina, KS</td>
<td>37</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Three of these entries were Canadian Western Red Spring (CWRS) varieties  
<sup>b</sup>Two of these varieties were waxy
<table>
<thead>
<tr>
<th>Category</th>
<th>Response Variable</th>
<th>Units Measured</th>
<th>AACCI Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain</td>
<td>Test weight</td>
<td>kg/hL</td>
<td>55-10.01</td>
</tr>
<tr>
<td></td>
<td>Wheat protein</td>
<td>per cent\textsuperscript{e}</td>
<td>46-30.01</td>
</tr>
<tr>
<td></td>
<td>SKCS\textsuperscript{a} hardness</td>
<td>-</td>
<td>55-31.01</td>
</tr>
<tr>
<td></td>
<td>SKCS weight</td>
<td>mg</td>
<td>55-31.01</td>
</tr>
<tr>
<td></td>
<td>SKCS size</td>
<td>mm</td>
<td>55-31.01</td>
</tr>
<tr>
<td></td>
<td>LDOPA\textsuperscript{b} (PPO\textsuperscript{c})</td>
<td>abs. at 475nm (AU)</td>
<td>22-85.01</td>
</tr>
<tr>
<td>Milling</td>
<td>Flour yield</td>
<td>per cent\textsuperscript{f}</td>
<td>26-50.01</td>
</tr>
<tr>
<td></td>
<td>Break flour yield</td>
<td>per cent\textsuperscript{f}</td>
<td>26-50.01</td>
</tr>
<tr>
<td></td>
<td>Milling score</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Flour protein</td>
<td>per cent\textsuperscript{f}</td>
<td>46-30.01</td>
</tr>
<tr>
<td></td>
<td>Flour ash</td>
<td>per cent\textsuperscript{f}</td>
<td>08-01.01</td>
</tr>
<tr>
<td>Baking</td>
<td>Mixograph absorption</td>
<td>per cent\textsuperscript{f}</td>
<td>54-40.02</td>
</tr>
<tr>
<td></td>
<td>Bake water absorption</td>
<td>per cent\textsuperscript{f}</td>
<td>10-11.01</td>
</tr>
<tr>
<td></td>
<td>Flour SDS\textsuperscript{d}</td>
<td>mL/g</td>
<td>56-60.01</td>
</tr>
<tr>
<td></td>
<td>Mixing time</td>
<td>minutes</td>
<td>10-11.01</td>
</tr>
<tr>
<td></td>
<td>Loaf volume</td>
<td>cm\textsuperscript{3}</td>
<td>10-11.01</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Single Kernel Characterization System
\textsuperscript{b}L-3, 4-dihydroxyphenylalanine
\textsuperscript{c}Polyphenol oxidase
\textsuperscript{d}SDS sedimentation volume
\textsuperscript{e}Adjusted on a 12% moisture basis
\textsuperscript{f}Adjusted on a 14% moisture basis
### TABLE III

Grain end-use quality response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Uniform Hard Red Spring and Western Spring nurseries

| Response Variable | Uniform Hard Red Spring | | | | Western Spring | | | |
|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                   | $R^2$ | Ratio a | Mean | 95% Range | $R^2$ | Ratio a | Mean | 95% Range |
| Test Weight       | 0.79  | 5.98 b  | 80.10 | 77.0-83.10 | 0.92  | 0.07 | 81.60 | 78.5-84.7 |
| SKCS Hardness     | 0.86  | 0.320   | 77.00 | 66.3-87.70 | 0.91  | 5.65 b | 67.50 | 51.5-83.6 |
| SKCS Weight       | 0.87  | 0.062   | 30.40 | 24.5-36.20 | 0.94  | 0.055 | 35.80 | 26.8-44.8 |
| SKCS Diameter     | 0.90  | 0.034   | 2.76  | 2.55-3.00  | 0.93  | 0.035 | 2.90  | 2.59-3.21 |
| Wheat Protein     | 0.76  | 0.140   | 14.20 | 12.8-15.60 | 0.90  | 0.25  | 13.50 | 11.2-15.8 |
| PPO (L-DOPA)      | 0.86  | 2.77 b  | 0.75  | 0.43-1.08  | 0.97  | 12.1 b | 0.62  | 0.14-1.09 |

*aRatio of variety mean square:location mean square

bLocation not significant at $P \leq 0.05$
## TABLE IV

Grain end-use quality response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Western Regional Hard Winter Wheat Nursery, and response variables for the Kansas WWPT Nursery and Northern and Southern Regional Performance Nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Western Winter</th>
<th>Kansas WWPT</th>
<th>Northern RP</th>
<th>Southern RP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio</td>
<td>Mean</td>
<td>95% Range</td>
</tr>
<tr>
<td>Test Weight</td>
<td>0.96</td>
<td>0.06</td>
<td>79.60</td>
<td>75.4-83.80</td>
</tr>
<tr>
<td>SKCS Hardness</td>
<td>0.93</td>
<td>157.40b</td>
<td>61.70</td>
<td>51.0-72.50</td>
</tr>
<tr>
<td>SKCS Weight</td>
<td>0.94</td>
<td>2.38</td>
<td>37.50</td>
<td>31.1-43.90</td>
</tr>
<tr>
<td>SKCS Diameter</td>
<td>0.86</td>
<td>1.21</td>
<td>2.95</td>
<td>2.76-3.14</td>
</tr>
<tr>
<td>Wheat Protein</td>
<td>0.92</td>
<td>0.34</td>
<td>13.20</td>
<td>11.5-14.80</td>
</tr>
<tr>
<td>PPO (L-DOPA)</td>
<td>0.96</td>
<td>3.58</td>
<td>0.66</td>
<td>0.12-1.20</td>
</tr>
</tbody>
</table>

*aRatio of variety mean square:location mean square
bLocation not significant at $P \leq 0.05$
TABLE V
Milling response variables, ANOVA model $R^2$, and the ratio of variety:location mean squares for the Uniform Hard Red Spring Wheat Nursery and Western Regional Hard Spring wheat nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Uniform Hard Red Spring</th>
<th>Western Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio$^a$</td>
</tr>
<tr>
<td>Break flour yield</td>
<td>0.74</td>
<td>0.033$^c$</td>
</tr>
<tr>
<td>Flour yield</td>
<td>0.80</td>
<td>0.037</td>
</tr>
<tr>
<td>Flour ash</td>
<td>0.49ns</td>
<td>0.71</td>
</tr>
<tr>
<td>Milling score</td>
<td>0.60ns</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^a$Ratio of variety mean square:location mean square
$^b$Location not significant at $P \leq 0.05$
$^c$Variety not significant at $P \leq 0.05$
TABLE VI

Milling response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Western Regional
Hard Winter Wheat Nursery, and response variables for the Kansas WWPT Nursery and Regional Performance Nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Western Winter</th>
<th></th>
<th>Kansas WWPT</th>
<th></th>
<th>Northern RP</th>
<th></th>
<th>Southern RP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio$^a$</td>
<td>Mean</td>
<td>95% Range</td>
<td>Mean</td>
<td>95% Range</td>
<td>Mean</td>
<td>95% Range</td>
</tr>
<tr>
<td>Break flour yield</td>
<td>0.94</td>
<td>4.33$^b$</td>
<td>42.9</td>
<td>38.0-47.7</td>
<td>36.3</td>
<td>31.8-40.9</td>
<td>44.7</td>
<td>38.8-50.5</td>
</tr>
<tr>
<td>Flour yield</td>
<td>0.96</td>
<td>2.39</td>
<td>71.7</td>
<td>68.2-75.1</td>
<td>64.3</td>
<td>61.3-67.3</td>
<td>67.0</td>
<td>59.6-74.5</td>
</tr>
<tr>
<td>Flour ash</td>
<td>0.97</td>
<td>0.17</td>
<td>0.36</td>
<td>0.27-0.44</td>
<td>0.39</td>
<td>0.34-0.45</td>
<td>0.37</td>
<td>0.32-0.42</td>
</tr>
<tr>
<td>Milling score</td>
<td>0.96</td>
<td>0.57</td>
<td>88.6</td>
<td>83.1-94.2</td>
<td>79.1</td>
<td>74.5-83.6</td>
<td>82.9</td>
<td>75.2-90.6</td>
</tr>
</tbody>
</table>

$^a$Ratio of variety mean square:location mean square
$^b$Location not significant at $P \leq 0.05$
$^c$Variety not significant at $P \leq 0.05$

‘ns’ indicates that the overall model was not significant with $P > 0.05$
### TABLE VII

Baking response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Uniform Hard Red Spring Wheat Nursery and Western Regional Hard Spring Wheat Nursery

| Response Variable | Uniform Hard Red Spring | | | Western Spring | | | |
|-------------------|-------------------------|------------------|------------------|-------------------------|------------------|------------------|
|                   | Model $R^2$ | Ratio$^a$ | Mean | 95% Range | Model $R^2$ | Ratio$^a$ | Mean | 95% Range |
| Flour protein     | 0.80        | 0.50      | 12.9 | 11.4-14.5 | 0.95        | 0.18      | 12.2 | 9.80-14.6 |
| Flour SDS         | 0.90        | 2.39$^b$  | 20.0 | 10.9-29.2 | -            | -         | -    | -         |
| Mixograph abs     | 0.91        | 0.015     | 64.4 | 61.3-67.4 | 0.82        | 0.098     | 63.7 | 60.3-67.2 |
| Bake water abs    | 0.70        | 0.057$^c$ | 67.8 | 64.5-71.1 | 0.71        | 0.20      | 66.9 | 63.2-70.7 |
| Mixing time       | 0.86        | 0.31      | 4.25 | 2.12-6.37 | 0.82        | 0.17      | 4.92 | 2.32-7.52 |
| Loaf volume       | 0.74        | 15.3$^b$  | 862  | 751-972   | 0.78        | 1.07$^b$  | 1005 | 888-1122  |

$^a$Ratio of variety mean square:location mean square

$^b$Location not significant at $P \leq 0.05$

$^c$Variety not significant at $P \leq 0.05$
TABLE VIII

Baking response variables, ANOVA whole model $R^2$, and the ratio of variety:location mean squares for the Western Regional Hard Winter Wheat Nursery, and response variables for the Kansas WWPT Nursery and Regional Performance Nurseries

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Western Winter</th>
<th>Kansas WWPT</th>
<th>Northern RP</th>
<th>Southern RP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model $R^2$</td>
<td>Ratio$^a$</td>
<td>Mean 95% Range</td>
<td>Mean 95% Range</td>
</tr>
<tr>
<td>Flour protein</td>
<td>0.91</td>
<td>0.50</td>
<td>11.7 10.2-13.3</td>
<td>12.4 11.0-13.9</td>
</tr>
<tr>
<td>Flour SDS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23.0 17.2-28.7</td>
</tr>
<tr>
<td>Mixograph abs</td>
<td>0.94</td>
<td>0.11</td>
<td>63.8 60.2-67.4</td>
<td>65.2 62.0-68.3</td>
</tr>
<tr>
<td>Bake water abs</td>
<td>0.96</td>
<td>0.079</td>
<td>66.8 62.6-71.0</td>
<td>68.5 64.7-72.3</td>
</tr>
<tr>
<td>Mixing time</td>
<td>0.91</td>
<td>0.75</td>
<td>2.83 0.73-4.92</td>
<td>4.99 3.18-6.81</td>
</tr>
<tr>
<td>Loaf volume</td>
<td>0.94</td>
<td>2.44</td>
<td>900 736-1064</td>
<td>979 835-1123</td>
</tr>
</tbody>
</table>

‘ns’ indicates that the overall model was not significant with $P > 0.05$

$^a$Ratio of variety mean square:location mean square

$^b$Location not significant at $P \leq 0.05$

$^c$Variety not significant at $P \leq 0.05$
Fig. 1. Relationship between SDS flour sedimentation volume and bread loaf volume.
CHAPTER FIVE

Relationship of Arabinoxylan Content to Grain, Milling, and Baking Quality in United States Soft Wheat Germplasm

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of other that may also be suitable.

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ABSTRACT

End-use quality in soft wheat (*Triticum aestivum* L.) can be assessed by a wide array of measurements, generally categorized into grain, milling, and baking characteristics. These characteristics are variably influenced by genetics and environment. The objective of this study was to assess the relationship of several select end-use quality parameters and determine the role that arabinoxylans (AX) play in end-use quality of soft wheat in four distinct growing regions as represented by four regional nurseries. The four regional nurseries were the Uniform Eastern and Southern Soft Red Winter wheat nurseries, the Western Regional Soft White Winter, and the Western Regional Soft White Spring wheat nurseries. The selected parameters were: kernel hardness, break flour yield, milling score, flour protein, flour SDS sedimentation volume, solvent retention capacity (SRC) parameters, total and water-extractable AX (TAX and WEAX), and cookie diameter. Exceptionally high and low performing varieties were selected on the basis of their responses to the aforementioned characteristics in each nursery. The relationship between high and low performing varieties varied across growing regions for the selected parameters. The Uniform Eastern and Southern SRW wheat nurseries exhibited high levels of correlation among the selected traits and a high level of variation between high and low performing varieties. Arabinoxylans detrimentally influenced cookie diameter. There were weaker relationships among the quality parameters and AX in the two Western Regional nurseries. The genetic and environmental influence on TAX and WEAX was evaluated in the Uniform Eastern and Southern SRW wheat nurseries. The TAX content was highly dependent on genetic differences, whereas the genetic and environmental influence over WEAX differed between the nurseries. This work further emphasizes the need to consider the characteristics of a specific growing region to better understand how genetics and environment influence end-use quality.
INTRODUCTION

Overall wheat quality is the product of grain, milling, and baking characteristics (Wrigley and Morris 1996). These characteristics help to define the optimum end-product usage for specific wheat varieties and growing environments. Many different parameters within these three categories can be used to evaluate end-use quality. Determining the importance of each parameter on the overall end-use quality as defined by the consumer product can prove challenging given the genetic diversity of wheat and the highly variable environments in which wheat is grown across the United States (Baenziger et al 1985). It is crucial to identify which quality assessments provide the most accurate and stable view of end-use quality in order to optimize product quality. Understanding the relationship among these qualities, genetic influence, and environmental diversity is critical in order to achieve optimum, consumer-appealing products. Along with the standard grain, milling, and baking assessments, quantification of arabinoxylans (AX) may provide additional information useful in quality assessments.

Arabinoxylans are made up of a β-1,4 linked D-xylopyranosyl backbone with substituted monomeric α-L-arabinofuranoside units at the second and/or third carbon positions of the xylose residues. Ferulic acid may be attached to the fifth carbon of the arabinose moiety (Courtin and Delcour 2002). Multiple factors determine the three-dimensional structure of AX: length of the xylan backbone, the ratio of arabinose to xylose, the substitution pattern on the backbone, and ferulic acid coupling to other AX molecules or the cell wall (Courtin and Delcour 2002). The somewhat flexible structure of AX (Dervilly et al 2000) and the large molecular weight (65,000) (Andrewartha et al 1979) contribute to empirically derived subfractions based on extractability. The two main extractable categories of total AX (TAX) are water-extractable (WEAX) and
water-unextractable (WUAX) arabinoxylans (Izydorczyk and Biliaderis 1992; Courtin and Delcour 2002).

There are differing reports on the influence of genetics and environment on TAX and WEAX levels. The overwhelming conclusion of several multi-cultivar studies is that both TAX and WEAX are heavily controlled by genetics (Li et al 2009; Finnie et al 2006; Hong et al 1989; Saulnier et al 1995; Dornez et al 2008; Lempereur et al 1997; Saulnier et al 2007). One study did contradict these findings; Andersson et al. (1993) did not observe a relationship between non-starch polysaccharide content and variety, and that the differences in non-starch polysaccharides in one variety grown at different locations over several years equaled differences observed across samples of different varieties.

Among the studies exhibiting high heritability of AX, however, there is a wide range of conclusions as to the role environmental differences play in TAX and WEAX content. Li et al. (2009) observed no significant impact of environment on TAX content in hard spring wheat, and that environmental differences varied between TAX and WEAX content. Although Finnie et al. (2006) observed great genetic control of TAX and WEAX; environment did contribute to some of the variation observed among cultivars at different growing locations. There was also a difference observed in the contribution of genetics and environment across market classes and sample type; genetics played a larger role in TAX content in soft spring wheat flour, but environment was more influential in TAX content differences in soft spring wheat meal. There was a more equivalent contribution of genetic and environmental influence on soft winter wheat (Finnie et al 2006). The content of WEAX, however, was overwhelmingly influenced by genetic differences, with very little environmental contribution (Finnie et al 2006). Differences in the genetic influence on TAX and WEAX were observed by Dornez et al. (2008), with more
variation in WEAX content, observed to be primarily genetically determined. A similar phenomenon was observed by Saulnier et al. (2007) across 90 wheat lines; higher variation in WEAX than TAX, with the high heritability of WEAX attributed to oligo- or polygenic control. In particular, Martinant et al. (1999) determined high heritability coefficients in both WEAX content and the arabinose/xylose ratio. These data suggested that genes likely controlled the relative proportion of arabinose and xylose in WEAX molecules more so than the actual content of WEAX, although the quantity of WEAX in a given variety was also apparently genetically controlled (Martinant et al 1999).

Two studies highlighted the environmental influence of AX as the result of drought conditions. Though Lempereur et al. (1997) observed a strong genetic influence on TAX and WEAX, water-stress conditions during the growing season resulted in higher levels of TAX. A positive relationship was also observed between TAX accumulation and drought conditions by Coles et al. (1997). The work of Li et al. (2009) also suggested the importance of quantifying WEAX on the same genotypes across multiple environments to better understand the varied influence of genetics and environment on different wheat market classes and preparation method (flour vs. meal).

Arabinoxylan content has been linked to other measures of wheat quality. There was an observed negative correlation between WEAX and single kernel characterization system (SKCS) hardness (Li et al 2009). A similar study found that with increasing grain hardness, the TAX, WEAX, and WUAX contents also increased across several market classes (Hong et al 1989). Within a market class, grain hardness was also observed to increase as TAX and WEAX content increased (Hong et al 1989). In hard wheat fractions, grain hardness was minimally affected by AX content, but in soft wheat samples, AX had a clear hardness-modifying effect, which also
translated into end-use quality variation (Bettge and Morris 2000). Particularly influential were membrane-associated AX in relationship to grain texture (Bettge and Morris 2000). This influence on grain texture was evident in a significant, negative correlation between grain hardness and membrane-associated AX, although these observations did not confer end-use quality differences (Bettge and Morris 2000). In a contradicting study, Li et al. (2009) did not observe any relationships between TAX and grain hardness, wheat protein, or test weight. In this study, however, WEAX was negatively correlated with grain hardness (Li et al 2009).

Arabinoxylans have long been observed to influence end-use quality (Bettge and Morris 2000; 2007, Ramseyer et al 2011a; 2011b, Courtin and Delcour 2002, Izydorczyk and Biliaderis 1995). The main influence of AX in baking is the result of their high water absorption capability (Jelaca and Hlynka 1971), which creates a strong competition with other ingredients or constituents for water (Izydorczyk and Biliaderis 1995). Arabinoxylans have the unique ability to form oxidative cross-links, producing a gel (Niño-Medina et al 2010, Izydorczyk and Biliaderis 1992), which gives AX very distinct impacts on end-use quality (Bettge and Morris 2007; Ramseyer at al 2011a, 2011b). Along with the covalent cross-links formed, non-covalent interactions between AX chains may also contribute to the gel structure formation (Niño-Medina et al 2010). The gelation properties of AX are dependent upon the structural characteristics of the AX molecules involved in the cross-links (Niño-Medina et al 2010). The density of cross-linking determines the ability of AX to bind water; increasing cross-linkages increases the water-binding capacity of the gel network up to an optimum level, beyond which the water-holding capacity decreases (Izydorczyk and Biliaderis 1995). It is this cross-linking which is most responsible for increasing batter viscosity (Bettge and Morris 2007). The increase in viscosity due to cross-linking is closely related to decreases in cookie diameter (Bettge and Morris 2007). The
increased viscosity prevents cookies from spreading adequately and helps to explain variation in cookie diameter otherwise unexplained by WEAX content and high protein levels (which are also known to decrease cookie diameter) (Bettge and Morris 2007). The factors of WEAX content and viscosity increases resulting from oxidative cross-linking are shown to influence the control of water in dough, impacting the final cookie diameter (Bettge and Morris 2000). Along with a decrease in cookie diameter, dough plasticity has been observed to decrease as a result of sugar syrup sequestration or increased bake-out time, increasing the incidence of checking, which are stress fractures in low-moisture products resulting from over-baking to optimize moisture content (Bettge and Morris 2007).

Along with cookie diameter, other end-use quality assessments can indirectly measure the impact of TAX and WEAX on the final product. The solvent retention capacity (SRC) parameters of Lactic acid (to measure viscosity) and Sucrose aid in evaluation of the gliadin content and the influence of AX on viscosity (Bettge and Morris 2007, Slade and Levine 1994). The flour SDS sedimentation volume can also provide information about viscosity and gluten strength, both of which are detrimental characteristics in soft wheat products such as cookies and Japanese sponge cakes (Bettge and Morris 2007, Ramseyer et al 2011a).

Kiszonas et al. (in press) identified several key factors in determining ideal soft wheat quality traits. High break flour yield in combination with low kernel hardness and flour protein were all observed to contribute to high cookie diameter measurements (Kiszonas et al in press). The SRC parameters studied did not conclusively show any predictive capacity for end-use quality (Kiszonas et al in press), contradictory to several other studies (Gaines et al 2000; Guttieri et al 2001; Guttieri and Souza 2003; Ram and Singh 2004; Moiraghi et al 2011; Kweon et al 2011, 2012; Souza et al 2012). This discord in the predictive capability of SRC
measurements may have been the result of multiple growing environments, and the observation that most end-use quality parameter surveyed are not able to accurately predict end-use quality across highly variable growing regions (Kiszonas et al *in press*).

The objective of this study was to evaluate TAX and WEAX differences among soft wheat grain samples displaying highly varied grain, milling, and baking characteristics. Several varieties from each of four nurseries were identified as having exceptional end-use quality characteristics and were analyzed for TAX and WEAX content to evaluate a pattern of AX content in these notable varieties.

**MATERIALS AND METHODS**

**Grain Samples**

In Kiszonas et al. (*in press*), a collection of 132 soft wheat varieties from four regional nurseries across the contiguous United States was surveyed for grain, milling, and baking quality. Of these 132 varieties, 24 varieties were identified as having exceptional baking characteristics, with either very good or very poor performance (Table I). Six varieties with exceptionally high or low kernel hardness, break flour yield, or solvent retention capacities were chosen from each nursery. The Uniform Eastern Soft Red Winter Wheat Nursery and Uniform Southern Soft Red Winter Wheat Nursery had three location replications and the Western Regional Soft White Winter and Soft White Spring Wheat Nurseries each had one location represented. The 24 varieties were surveyed at all locations with available samples.

**Quality Measurements**

The quality analyses performed on these grain samples can be classified into three categories: grain, milling, and baking quality. The quality parameters were assessed using AACC approved methods (2000). Table II contains a list of these parameters along with the units of
measurement for each. The grain quality was assessed using the single kernel characterization system (SKCS) hardness parameter. Milling quality was evaluated using break flour yield and milling score. The baking quality parameters were: flour protein, flour SDS sedimentation volume, solvent retention capacity (SRC) parameters (Water, Carbonate, Sucrose, and Lactic acid), and cookie diameter.

**Arabinoxylan Quantification**

This procedure was adapted from Englyst and Cummings (1984) and Courtin et al. (2000).

**WEAX Hydrolysis**

Water-extractable AX quantification was performed beginning with between 350 and 700 mg of the sample in a centrifuge-safe 12-mL test tube. Seven mL H₂O was added to each flour sample, and mixed with a vortex mixer for 7 sec. The tubes were continuously shaken in a 7 ºC refrigerator for one hour. The tubes were centrifuged at 1600 X g for 5 min and 0.875 mL of the supernatant was removed and placed in another centrifuge-safe 12-mL test tube. To this mixture, 0.875 mL trifluoroacetic acid (TFA; 4 N) was added and the solution was mixed for 7 sec with a vortex mixer. The samples were incubated for 60 min at 110 ºC in a Fisher Isotemp Dry Bath Model 145 heating block (Thermo Fisher Scientific, Santa Clara, CA). The block was made of anodized aluminum with 48 cylindrical wells 1.7cm in diameter and 4.4cm deep. The samples were immediately transferred into an ice bath until the test tubes were cool to the touch. The samples were then centrifuged at 1600 X g for 5 min. At this point, the WEAX and TAX samples were treated identically.

**TAX Hydrolysis**

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The TAX was quantified starting with 10.5 – 35 mg of sample weighed into centrifuge-safe 12-mL test tubes. To the samples, 1.75 mL TFA (4 N) was added and mixed for 7 sec with a vortex mixer. The samples were incubated at 110 ºC for 60 min and immediately following incubation, transferred into a nice bath until the test tubes were cool to the touch. The samples were centrifuged at 1600 X g for 5 min.

**WEAX and TAX Derivatization**

From the centrifuged product, 1 mL of the supernatant was removed and placed into a 12-mL test tube. An internal standard of allose (0.35 mL of a 1 mg/mL in H₂O solution) was added to each test tube. The test tubes were placed in an ice bath and 0.55 mL NH₃ was added to each and the test tubes were mixed with a vortex mixer for 7 sec. Each sample solution was pH tested to verify basicity. If the solution was not basic, aliquots of 100 µL NH₃ were added until a pH of over 7 was achieved. Following this verification, 7 µL of 2-octanol was added to each test tube. At this point, a suspension of NaBH₄ in 3 M NH₃ was made (200 mg NaBH₄ / mL NH₃). It is critical to wait until this time period to make the suspension so that it is fresh before adding 70 µL to each sample. Once the NaBH₄ suspension was added to each sample, the test tubes were mixed with a vortex mixer for 7 sec before being incubated in a heating block at 40 ºC for 30 min. Following incubation, the samples were placed in an ice bath briefly before the addition of 140 µL glacial acetic acid and mixed for 7 sec with a vortex mixer. One hundred, seventy-five microliters of this solution was transferred to a fresh 12-mL test tube. To this, 175 µL 1-methylimidazole and 1.75 mL acetic anhydride were added. After letting the solution set for 10 min, 1 mL ethanol was added and immediately mixed with a vortex mixer for 7 sec. The solution was allowed to set for 5 min before the addition of 3.5 mL H₂O and another 5 min waiting period. Following the addition of 175 µL bromophenol blue, the test tubes were transferred to an
ice bath. To the solutions, two aliquots of 1.75 mL concentrated KOH were added, with a brief resting period between aliquots. The solutions undergo a phase separation following the addition of KOH, the top layer is the desired layer and appears yellow-ish in color. The bottom layer appears dark blue. Letting the solutions remain in the ice bath for a few minutes allows the phases to separate more completely for an easier extraction of the top layer. The top layer was transferred to a fresh test tube and anhydrous Na₂SO₄ was added to dry the solution. Once the water was been removed from the solutions with the desiccant, the solutions were transferred to GC vials for analysis using the GC-FID.

**GC-FID Analysis**

The samples were injected into the GC into a capillary column with a split-splitless injector. The samples then entered the flame ionization detector to detect the components of the samples. The column used in the GC was a Supelco SP-2380 polar column (Sigma-Aldrich, Bellefonte, PA) in an Agilent 6890 Series Chromatograph (Agilent, Santa Clara, CA) with an autosampler and a splitter injection port (injection volume dependent upon the type of sample; split ratio 1:20). Detection with the FID was performed using a carrier gas of hydrogen. Sample injection occurred at 210 ºC, separation beginning at 237 and ending at 260 ºC, and detection at 240 ºC.

**Statistical Analysis**

The statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). The general analyses were done using PROC GLM. Each regional nursery was analyzed separately with varieties and locations as fixed effects. Due to the lack of field replication, no interaction term was included in the model. Because there were no replicated varieties across nurseries, each nursery was analyzed separately. The Uniform Eastern and Southern SRW wheat nurseries
included three growing locations in each nursery, whereas the Western Regional nurseries had one growing location. The six exceptional varieties identified in each nursery were analyzed at all available growing locations, resulting in 18 samples each for analysis in the Uniform Eastern and Southern SRW wheat nurseries, and six samples each for analysis in the Western Regional nurseries.

**RESULTS**

**Correlations**

Several characteristics of soft wheat were identified in Kiszonas et al. (*in press*) as being potentially predictive of quality as determined by cookie diameter. These characteristics were: SKCS hardness, break flour yield, milling score, flour protein, flour SDS sedimentation volume, and the four SRC parameters of Water, Sucrose, Carbonate, and Lactic acid. The responses for these traits were correlated using the varieties identified as having exceptional performance (high or low, Kiszonas et al *in press*).

The Uniform Eastern SRW wheat nursery exhibited numerous strong correlations for the selected traits (Table III). Several factors were highly correlated with cookie diameter: kernel hardness (-0.84), break flour yield (0.84), flour protein (-0.81), and flour SDS sedimentation volume (-0.69). The only notable correlations of TAX were with SRC Carbonate (0.51) and SRC Sucrose (0.65). Water-extractable AX, however, was highly correlated with break flour yield (-0.54), milling score (-0.83), SRC Water (0.53), SRC Carbonate (0.62), SRC Sucrose (0.84), and cookie diameter (-0.63). A strong relationship was evident between TAX and WEAX content (0.65). The SRC parameters were also closely related to each other. The high correlation
between flour protein and flour SDS sedimentation volume (0.69) indicates an accurate prediction of gluten strength.

The Uniform Southern SRW wheat nursery also exhibited a high level of correlation between the selected traits (Table IV), albeit slightly weaker relationships than those observed in the Uniform Eastern SRW wheat nursery. In this nursery, cookie diameter was closely related to milling score (0.46), SRC Carbonate (-0.49), and SRC Sucrose (-0.49). The TAX content exhibited high levels of correlation with kernel hardness (0.57), break flour yield (-0.62), milling score (-0.88), SRC Water and Carbonate (0.74), SRC Sucrose (0.71), and cookie diameter (-0.72). In this nursery, higher correlations were observed with TAX than with WEAX, which did exhibit close relationships to break flour yield (-0.74), milling score (-0.62), flour protein (0.72), SRC Water (0.62), cookie diameter (-0.53), and TAX (0.62). The SRC parameters exhibited similarly high correlations amongst themselves, as also seen in the Uniform Eastern SRW wheat nursery.

The Western Regional Soft White Winter wheat nursery exhibited several high correlations (Table V); however, their $P$-values were substantially lower than in the Uniform Eastern and Southern SRW wheat nurseries, likely the result of the smaller number of varieties grown in this nursery. Despite the smaller sample size, there were strong correlations observed with cookie diameter, such as: kernel hardness (-0.63), break flour yield (0.73), flour SDS sedimentation volume (-0.53), SRC Water (-0.66), and SRC Lactic acid (-0.47). SRC Sucrose was particularly highly correlated with milling score (-0.83). The TAX content exhibited only two high levels of correlation: flour protein (-0.82) and SRC Carbonate (0.68). The WEAX content, however, was more closely related to the selected traits. High levels of correlation were observed between WEAX and kernel hardness (0.91), break flour yield (-0.74), SRC Water
(0.87), and cookie diameter (-0.71). In this nursery, TAX and WEAX were not closely related, exhibiting a correlation coefficient of only 0.11. The SRC parameters did not show close relationships with each other, their correlation coefficients no higher than 0.53, which was observed between SRC Water and Sucrose.

Similar to the Western Regional Soft White Winter wheat nursery, the Western Regional Soft White Spring wheat nursery did not exhibit high levels of significance (Table VI), in part because of the small sample size. Contrary to the Western Regional Soft White Winter wheat nursery, though, there were fewer high levels of correlation observed in the Western Regional Soft White Spring wheat nursery. Kernel hardness was closely related to break flour yield with a negative correlation of 0.66. Cookie diameter was most closely related to flour protein (-0.58), flour SDS (-0.66), SRC Sucrose (-0.52), and WEAX (-0.56). Flour SDS sedimentation volume was also strongly correlated with SRC Lactic acid (0.78). The TAX content did not exhibit any correlations stronger than 0.47 (flour SDS sedimentation volume). The WEAX content did not correlate strongly with characteristics other than SRC Water (0.71) and cookie diameter (-0.56). The correlation between TAX and WEAX was not statistically significant at 0.41.

**Performance Comparison**

The selected varieties were categorized as “high” or “low” performing varieties across multiple characteristics. The varietal responses to these selected characteristics were analyzed to determine if high and low performing varieties exhibited significant differences.

The Uniform Eastern SRW wheat nursery exhibited differences in variety performance in break flour yield, milling score, SRC Water, SRC Carbonate, SRC Sucrose, cookie diameter, and both TAX and WEAX (Table VII). The high performing varieties had higher break flour yield,
milling scores, and cookie diameter responses, with lower responses for the SRC parameters, TAX, and WEAX. Despite a large difference in kernel hardness between the high and low performing varieties (14.7 vs. 28.6), the difference was not statistically significant.

The Uniform Southern SRW wheat nursery exhibited similar differences between the high and low performing varieties (Table VIII) as compared to those observed in the Uniform Eastern SRW wheat nursery. Differences between high and low performing varieties were observed in all measured parameters with the exception of flour protein, flour SDS sedimentation volume, SRC Lactic acid, and WEAX content. Particularly large differences were observed in milling score, SRC Water, and TAX content. The high performing varieties had higher responses in break flour yield, milling score, and cookie diameter, with lower responses in kernel hardness, SRC Water, Carbonate, and Sucrose, and TAX content.

Few differences were observed between the high and low performing varieties in the Western Regional Soft White Winter wheat nursery (Table IX). Kernel hardness and SRC Water were markedly lower in the high performing varieties as compared to the low performing varieties. Break flour yield was higher in the high performing varieties in relation to the low performing varieties (52.2 vs. 45.9%, respectively). Although cookie diameter was larger in the high performing varieties (9.50cm) than in the low performing varieties (9.16cm), the difference was not significant, likely due, in part, to the small number of total responses.

Similar to the Western Regional Soft White Winter wheat nursery, the Western Regional Soft White Spring wheat nursery did not exhibit many differences in quality characteristics between the high and low performing varieties (Table X). Only break flour yield and SRC Water differed between the two categories of varieties. In contrast to the other three nurseries, the high performing varieties exhibited a higher response for SRC Lactic acid as compared to the low
performing varieties (115.8 and 112.9%, respectively). The responses for TAX and WEAX were very similar, with a smaller range in WEAX responses between the varietal performance categories in comparison to the other nurseries.

In a comparison across the four nurseries (Tables VII - X), the largest appreciable differences occurred in kernel hardness, and TAX and WEAX content. Kernel hardness in the high performing varieties of the Uniform Eastern and Uniform Southern SRW wheat nurseries was 14.7 and 14.3, respectively, while the high performing varieties in the Western Regional Soft White Winter and Western Regional Soft White Spring wheat nurseries had average hardness responses of 20.4 and 17.2, respectively. The low performing varieties were similarly lower in kernel hardness in the Uniform Eastern and Uniform Southern SRW nurseries as well. The TAX content was markedly lower for all varieties in the Uniform Eastern and Uniform Southern SRW wheat nurseries compared to the Western Regional nurseries. Both the Uniform Eastern and Uniform Southern SRW wheat nurseries exhibited mean separation between the two classes of varieties based on performance, whereas no mean separation was possible in the Western Regional nurseries for TAX and WEAX content. This comparison across nurseries mirrored differences in cookie diameter between nurseries as well. Only the Uniform Eastern SRW wheat nursery showed differences between WEAX content in the high and low performing varieties.

**Arabinoxylan Variation**

The high and low performing varieties of the Uniform Eastern and Uniform Southern SRW wheat nurseries were surveyed for TAX and WEAX content across multiple locations to determine the relative influence of genetics and environment, as assessed by varietal and location
differences. In order to determine the relative contribution of variety and location in the overall statistical model, the ratio of the mean squares of variety and location was calculated. As such, a ratio of 1 would indicate equivalent contribution from variety and location to overall TAX and WEAX differences.

In the Uniform Eastern SRW wheat nursery, varietal differences were significant for both TAX and WEAX at the $P \leq 0.01$ level \((data not shown)\). The ratio of variety:location mean squares in TAX and WEAX content were 1.95 and 23.72, respectively. Locations exhibited statistically similar responses in both types of AX quantification. In the Uniform Southern SRW wheat nursery, varietal differences were significant in both TAX and WEAX measurements \((P \leq 0.01\), and location was not observed to influence TAX content differences. In contrast to the Uniform Eastern SRW wheat nursery, location had a high level of influence over WEAX content in the Uniform Southern SRW wheat nursery. Whereas the ratio of variety:location mean squares for TAX was 2.06, this same ratio was 0.52 for WEAX content. All three locations exhibited differing WEAX content responses.

**DISCUSSION**

**Correlations**

The Uniform Eastern SRW wheat nursery exhibited numerous high levels of correlation among the selected quality traits. These high correlations indicate that the selected characteristics served as accurate predictors of end-use quality across both high and low performing varieties. This particular nursery was governed largely by location, or environmental influence (Kiszonas et al \textit{in press}). Because of the overwhelming environmental rather than genetic influence, in
combination with the high levels of correlation, it would appear as if environmental factors influenced the quality traits similarly, leading to the high levels of correlation.

In this study, the final product to be evaluated was cookie diameter, as this is the product that is ultimately marketed for consumer appeal. Cookie diameter was strongly linked to the characteristics of kernel hardness, break flour yield, flour protein, flour SDS sedimentation volume, and WEAX content. These factors were consistently highly predictive of both high and low cookie performance based on their responses to all of the selected traits. In this way, cookie diameter may be consistently modeled upon these traits at both ends of the quality spectrum, showing their robust character in predicting final end-use quality. Flour SDS sedimentation volume, in particular, had a high, negative correlation with cookie diameter, indicating that this measurement accurately predicted the detrimental effects of gluten strength on cookie diameter. The observation of this close relationship concurs with the findings of Bettge and Morris (2007) and Ramseyer et al. (2011a).

In the evaluation of whether or not AX quantification can enhance the understanding of overall wheat quality, the results from this nursery indicate that AX can be a useful tool in modeling end-use quality. Both TAX and WEAX content were highly correlated with SRC Sucrose (0.65 and 0.84, respectively), which is consistent with prior reports of a close relationship between these parameters (Bettge and Morris 2007, Slade and Levine 1994). The close, negative relationship of WEAX and milling score indicates that the level of bran contamination and milling quality influences the level of apparent WEAX, perhaps due to the AX heterogeneity throughout the wheat kernel (Philippe et al 2006). Higher levels of bran contamination may contribute to both a lower milling score and higher levels of WEAX, depending on the individual make-up of the AX in a particular wheat variety.
The Uniform Southern SRW wheat nursery exhibited similar correlations to the Uniform Eastern SRW wheat nursery, as well as a high level of location or environmental influence, as determined by Kiszonas et al (in press). Cookie diameter was highly correlated with a number of traits, including milling score, SRC Water, Carbonate, and Sucrose, as well as TAX and WEAX content. There were significant, but lower levels of correlations between cookie diameter and break flour yield, flour protein, and flour SDS sedimentation volume. In a similar manner to the Uniform Eastern SRW wheat nursery, in this nursery, TAX and WEAX were highly correlated with SRC Sucrose, and also notably with SRC Water and Carbonate. The close relationship with SRCs that has been previously described (Bettge and Morris 2007, Slade and Levine 1994) was consistent with the observations in this nursery.

Both TAX and WEAX exhibited close relationships with all measured characteristics, with the exception of flour SDS sedimentation volume and SRC Lactic acid. This observation indicates that in these growing environments, TAX and WEAX are useful indicators of performance in a number of quality traits, most notably cookie diameter. The negative relationship between both TAX and WEAX with milling score strengthens the proposal that bran contamination may lead to higher AX content in flour as well as a decrease in milling score.

The Western Regional Soft White Winter wheat nursery exhibited fewer significant correlations in comparison to the Uniform Eastern and Southern SRW wheat nurseries. The variation of responses in this nursery was variably influenced by varietal (genetic) and location (environmental) differences (Kiszonas et al in press). Despite an overall decrease in correlations observed in this nursery as compared to the others surveyed, cookie diameter did exhibit close relationships to several other characteristics. Those parameters which were most closely linked to cookie diameter were: kernel hardness, break flour yield, flour SDS sedimentation volume,
SRC Water, and SRC Lactic acid. Cookie diameter also exhibited a correlation of -0.71 with WEAX, but was not statistically significant, likely due to the small sample size. The notable characteristics correlating with cookie diameter are primarily consistent with the other nurseries surveyed.

In contrast to the Uniform Eastern and Southern SRW wheat nurseries, the Western Regional Soft White Winter wheat nursery did not exhibit a high correlation between AX content (of either form) with SRC Sucrose, but TAX was highly correlated with SRC Carbonate (0.68), as was also observed in the Uniform Southern SRW wheat nursery. The TAX and WEAX content were not similarly correlated with any trait, but each did display high levels of correlation with particular end-use quality traits. The TAX content was highly, negatively correlated with flour protein, and highly, positively correlated with SRC Carbonate. The WEAX content was highly, positively correlated with kernel hardness, SRC water, and to a lesser extent SRC Lactic acid. Those traits with a high, negative correlation to WEAX were break flour yield and cookie diameter. Notably, the correlation between TAX and WEAX was very low (0.11). This discord in relationships to other traits is unique to this particular nursery; the other three nurseries exhibited more similar relationships between TAX, WEAX, and the other surveyed characteristics. The differing relationship between TAX and WEAX across multiple growing regions warrants further exploration to better understand genetic and environmental influences on TAX and WEAX.

Similar to the Western Regional Soft White Winter wheat nursery, the Western Regional Soft White Spring wheat nursery did not exhibit as many high levels of correlation as the Uniform Eastern and Southern SRW wheat nurseries. Another notable difference between this nursery and the other three is that the majority of the quality traits were observed to be
genetically influenced more so than environmentally influenced (Kiszonas et al in press). Cookie
diameter exhibited four notable correlations: flour protein (-0.58), flour SDS sedimentation
volume (-0.66), SRC Sucrose (-0.52), and WEAX content (-0.56). The relationship of cookie
diameter to flour SDS sedimentation volume and WEAX is consistent with those relationships
observed in the other three nurseries. The TAX and WEAX content did not exhibit many notable
correlations. Neither TAX nor WEAX was closely related to the SRC parameters of Carbonate,
Sucrose, or Lactic acid, although WEAX was positively correlated with SRC Water (0.71),
similar to the Uniform Southern SRW wheat nursery.

Across all four nurseries, there was a clear distinction in the levels of correlation between
the Uniform Eastern and Southern SRW wheat nurseries, and the Western Regional nurseries.
Despite the high level of environmental influence over the Uniform Eastern and Southern SRW
wheat nurseries, they exhibited high levels of correlation between the selected traits. This may
suggest that the growing environments played a large role in synergistically influencing end-use
quality. The Western Regional nurseries, however, were more variably influenced by genetics
and environment, but exhibited distinctly lower levels of correlation between the selected traits.
This further emphasizes the large role that environment, in combination with genetics, can play
in impacting end-use quality.

Several characteristics consistently exhibited high levels of correlation across the four
nurseries. Break flour yield tended to have a high, positive correlation with cookie diameter,
while flour protein and flour SDS sedimentation volume had high, negative correlations with
cookie diameter. The levels of TAX and WEAX were variably related to cookie diameter across
the four nurseries; however, it did appear that lower WEAX levels consistently related to larger
cookie diameters, if to a varying extent. The role of TAX in influencing cookie diameter was less
clear across the four nurseries. In all but the Western Regional Soft White Winter wheat nursery, the relationship between TAX and cookie diameter was negative, though the level of correlation differed. In the Western Regional Soft White Winter wheat nursery, TAX and cookie diameter were positively correlated. Related to this, the Western Regional Soft White Winter wheat nursery exhibited a very low correlation between TAX and WEAX content (0.11), whereas the other three nurseries exhibited a stronger relationship (0.41-0.65). This may suggest that the discord between TAX and WEAX in the Western Regional Soft White Winter wheat nursery stems from more structural heterogeneity, or more varied levels of water-unextractable AX, thereby changing the water relationships and causing other AX factors to influence cookie diameter more strongly.

As a result of these varied relationships across growing regions, it is imperative to understand the nature of quality relationships in the desired growing region to be evaluated. Characteristics such as break flour yield, flour protein, and flour SDS sedimentation volume can provide robust and specific information about the overall end-use quality of particular varieties, and must be considered in the context of the other quality assessments. To that end, AX quantification can be used to enhance the understanding of overall wheat quality, but the particular influence of AX on wheat quality in a particular growing region must be taken into consideration.

**Performance Comparison**

The results of the performance comparison portion of the study mirror those of the correlations: the Uniform Eastern and Southern SRW wheat nurseries exhibited a high level of difference across nearly every selected trait according to variety performance, whereas the
Western Regional nurseries exhibited few discernible differences between high and low performing varieties across the selected traits. All four nurseries, however, were observed to exhibit a high level of difference between high and low performing varieties for break flour yield and SRC Water. In this context, these two traits could be considered the two most influential parameters in predicting end-use quality across many varieties and growing environments. In this way, these two appear to be stable measured traits.

In the Uniform Eastern and Southern SRW wheat nurseries, the quality characteristics that appeared to be predictive of end-use quality were break flour yield, milling score, TAX, and the SRC parameters of Water, Carbonate, and Sucrose. The WEAX content in the Uniform Eastern SRW wheat nursery also exhibited a high level of difference between the two categories of varieties. In the Uniform Southern SRW wheat nursery, kernel hardness also appeared to be highly varied between high and low performing varieties.

Overall, the TAX content was considerably lower in the Uniform Eastern and Southern SRW wheat nurseries in all varieties (2.76-3.72) as compared to the Western Regional nurseries (4.15-4.89). The WEAX content was similar across all four nurseries and both categories of varieties. Cookie diameter was also larger in the high performing varieties of the Uniform Eastern and Southern SRW wheat nurseries (9.56-9.59cm) as compared to the high performing varieties in the Western Regional nurseries (9.45-9.50). This observation may be related to the lower levels of TAX in the Uniform Eastern and Southern SRW wheat nurseries and the higher correlations in AX content to cookie diameter also observed in these nurseries. The WEAX content did not appear to have an influence on cookie diameter differences across the four nurseries, indicating that WEAX had a negligible effect on cookie baking performance across multiple growing environments. In each nursery, however, WEAX content did appear to
negatively influence cookie diameter, albeit to a varying extent. These observations are consistent with the detrimental impact of WEAX on soft wheat products as reported by Bettge and Morris (2000, 2007) and Ramseyer et al. (2011a).

**Arabinoxylan Variation**

The high level of genetic influence over TAX content was evident in the high ratios of variety:location mean squares in both the Uniform Eastern and Southern SRW wheat nurseries. The varietal contribution to variation was roughly twice the variation attributed to environmental differences. To that end, location did not play a significant role in variation between the high and low performing varieties in either nursery. These observations are in agreement with numerous previous studies which emphasized the high level of genetic influence on TAX (Li et al 2009; Finnie et al 2006; Hong et al 1989; Saulnier et al 1995; Dornez et al 2008; Lempereur et al 1997; Saulnier et al 2007).

The WEAX content, conversely, was variably influenced by variety and location in the two nurseries surveyed. In the Uniform Eastern SRW wheat nursery, the varietal influence was over twenty-three times that of the environmental influence on WEAX content. Strongly contrasting this, location differences in WEAX content were twice that of the varietal influence in the Uniform Southern SRW wheat nursery. Both varietal and location factors, however, made significant contributions to WEAX variation. While Finnie et al. (2006) did observe a higher level of genetic influence over WEAX content; this work did show some environmental influence over WEAX. In this way, these observations are in partial agreement with those of Finnie et al. (2006). The high level of environmental influence over WEAX content can perhaps explain the lack of separation in WEAX content between the high and low performing varieties.
observed in the Uniform Southern SRW wheat nursery. These results suggest that TAX is heavily influenced by genetics, but WEAX content can be variably influenced by genetics and environment, depending on the growing environment. Therefore, it is critical to understand the nature of the growing environment when attempting to examine the influence of WEAX on end-use quality.

CONCLUSION

The relationships among selected traits were highly polarized between the Uniform Eastern and Southern SRW wheat nurseries and the Western Regional nurseries. While the selected end-use quality parameters exhibited close relationships among themselves in the Uniform Eastern and Southern SRW wheat nurseries, such strong relationships were not observed in the Western Regional nurseries. The two characteristics which exhibited high, stable correlations with cookie diameter across all four nurseries were break flour yield and flour SDS sedimentation volume, with a fairly stable, negative relationship to cookie diameter also observed in flour protein. These observations suggest that these three parameters offer the highest level of prediction power across multiple varieties and growing environments. These results also emphasize the variable nature of predictive capacity to end-use quality across growing environments. Particularly highlighting this variable nature was the role of AX in end-use quality. In some environments, AX content can be another useful tool in modeling end-use quality as evaluated by cookie diameter, while in others; it does not add any appreciable value to the end-use quality assessments. The influence of genetics and environment is similarly variable on WEAX content across multiple growing environments. As a result, it is critical to understand the nature of the growing environment when assessing quality traits.
ACKNOWLEDGMENTS

We would like to acknowledge the technical staff of the USDA-ARS Western Wheat Quality Laboratory, Doug Engle, Mary Baldridge, Bozena Paszczynska, Mishelle Lenssen, Eric Wegner, Bill Kelley, Patricia Boyer, Gail Jacobson and Anna Hansen. Stacey Sykes and Shawna Vogl assisted in the preparation of the manuscript. This project was supported by the Agriculture and Food Research Initiative Grant no. 2009-02347 from the USDA National Institute of Food and Agriculture.
LITERATURE CITED


Izydorczyk, M.S., and Biliaderis, C.G. 1992. Effect of molecular size on physical properties of


Philippe, S., Barron, C., Robert, P., Devaux, M-F., Saulnier, L., and Guillon, F. 2006. Characterization using Raman microspectroscopy of arabinoxylans in the walls of
different cell types during the development of wheat endosperm. J. Agric. Food Chem. 54:5113-5119.


### TABLE I
Summary of nurseries, locations, and number of soft wheat varieties grown in each regional nursery

<table>
<thead>
<tr>
<th>Nursery</th>
<th>Contributor</th>
<th>Locations</th>
<th>No. Varieties</th>
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<td>H. E. Bockelman</td>
<td>Aberdeen, ID</td>
<td>42</td>
</tr>
<tr>
<td>Red Winter Wheat</td>
<td>F. L. Kolb</td>
<td>Urbana, IL&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>C. Sneller/E. Souza</td>
<td>Composite&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Uniform Southern Soft</td>
<td>H. E. Bockelman</td>
<td>Aberdeen, ID</td>
<td>41</td>
</tr>
<tr>
<td>Red Winter Wheat</td>
<td>S. A. Harrison</td>
<td>Interior Composite&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. A. Harrison</td>
<td>Coastal Composite&lt;sup&gt;d&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Western Regional Soft</td>
<td>B. Brown</td>
<td>Parma, WA</td>
<td>34</td>
</tr>
<tr>
<td>White Winter Wheat</td>
<td>K. Garland-Campbell</td>
<td>Pullman, WA</td>
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<tr>
<td>Western Regional Soft</td>
<td>L. E. Talbert</td>
<td>Bozeman, MT</td>
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<tr>
<td>White Spring Wheat</td>
<td>G. B. Shelton</td>
<td>Pullman, WA</td>
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<sup>a</sup> Grain samples from this location were limited; no SRC Water, SRC Lactic acid or cookie baking was performed.

<sup>b</sup> Composite of Owensville, IN, Urbana, IL, Wooster, OH, in approximate proportion of 10:10:80, respectively. Grain samples were limited; no SRC Water or SRC Lactic acid was performed.

<sup>c</sup> Composite of Warsaw, VA, Battle Ground, IN, and Belle Mina, AL

<sup>d</sup> Composite of Blacksburg, VA, Plains, GA, Greenville, MS, and Winnsboro, LA
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<th>AACCNI Approved Method</th>
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<td>per cent\textsuperscript{c}</td>
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<td>SRC Lactic acid</td>
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<td>Water-extractable arabinoxylans</td>
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\textsuperscript{a}Single Kernel Characterization System
\textsuperscript{b}Milling was performed following the modified Quadruplet Sr. method of Jeffers and Rubenthaler (1977)
\textsuperscript{c}Adjusted to a 14% moisture basis
\textsuperscript{d}SDS sedimentation volume
\textsuperscript{e}Solvent Retention Capacity
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<th>WEAX</th>
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* *P ≤ 0.05, **P ≤ 0.01, ***P < 0.0001
Table IV
Uniform Southern Soft Red Winter Wheat Nursery

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<th>WEAX</th>
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* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
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<th>Milling Score</th>
<th>Flour Protein</th>
<th>Flour SDS</th>
<th>SRC Water</th>
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* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
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<tr>
<th></th>
<th>Break Flour Yield</th>
<th>Milling Score</th>
<th>Flour Protein</th>
<th>Flour SDS</th>
<th>SRC Water</th>
<th>SRC Carbonate</th>
<th>SRC Sucrose</th>
<th>SRC Lactic Acid</th>
<th>Cookie Diameter</th>
<th>TAX</th>
<th>WEAX</th>
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<td>Lactic acid</td>
<td>Cookie</td>
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* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
## Table VII
Uniform Eastern Soft Red Winter Wheat Nursery

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<th>Single Kernel Hardness</th>
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<th>Milling Flour</th>
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<th>SRC Sucrose</th>
<th>SRC Lactic Acid</th>
<th>Cookie Diameter</th>
<th>TAX</th>
<th>WEAX</th>
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<tr>
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<td>12.24**</td>
<td>20.31**</td>
<td>71.77***</td>
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* *P ≤ 0.05, **P ≤ 0.01, ***P < 0.0001*
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<th>SRC Sucrose</th>
<th>Lactic Acid</th>
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<th>WEAX</th>
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<td>11.5</td>
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<td>9.56</td>
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* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
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<th>Single Kernel Hardness</th>
<th>Break Flour Yield</th>
<th>Milling Score</th>
<th>Flour Protein</th>
<th>Flour SDS</th>
<th>SRC Water</th>
<th>SRC Carbonate</th>
<th>SRC Sucrose</th>
<th>SRC Lactic Acid</th>
<th>Cookie Diameter</th>
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<td>79.9</td>
<td>9.16</td>
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* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
Table X
Western Regional Soft White Spring Wheat Nursery

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<th>Single Break</th>
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<th>Flour Milling</th>
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<th>Flour Protein</th>
<th>Flour SDS</th>
<th>SRC Lactic</th>
<th>SRC Water</th>
<th>SRC Carbonate</th>
<th>SRC Sucrose</th>
<th>SRC Lactic Acid</th>
<th>Cookie Diameter</th>
<th>TAX</th>
<th>WEAX</th>
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<tr>
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<td>9.21</td>
<td>4.66</td>
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</table>

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
CHAPTER SIX

Arabinoxylan Content and Relationships throughout the Baking Process of Pancakes

Alecia M. Kiszonas¹, E. Patrick Fuerst¹, Craig F. Morris²

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of other that may also be suitable.

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ABSTRACT
Non-starch polysaccharides, specifically, arabinoxylans (AX) are well known to have a wide-ranging influence on wheat (*Triticum aestivum* L.) end-use quality. Arabinoxylan content and structure is most often assessed in raw flour and slurries. The role AX plays in baking is then extrapolated based on molecular interactions that occur in water-based solutions. Thus far, however, no study has examined the role AX plays throughout the baking process and in the food product itself in a soft wheat product. The objective of this study was to track total and water-extractable (TAX and WEAX) content and structural relationships throughout the baking process of pancakes, traditionally a soft wheat product. The TAX and WEAX content along with the arabinose:xylose (A/X) ratio was quantified for raw flour in wholemeal and refined flour of five varieties, including both hard and soft wheats. Pancake batter and subsequent pancakes were made using the wholemeal and refined flour of the five selected varieties. The TAX, WEAX, and A/X ratio was evaluated in batter and cooked pancakes. The availability of AX for quantification varied throughout the baking process. Wholemeal samples exhibited overall decreases in AX availability throughout the baking process, while refined flour samples exhibited varied results between raw flour, batter, and pancakes in AX availability. In wholemeal pancakes, the TAX content of raw flour exhibited the closest relationship to pancake diameter, whereas in refined flour pancakes, the WEAX content of raw flour appeared to be more influential on pancake quality. The differences in availability of AX molecules and between wholemeal and refined flour is attributed to the complex molecular interactions of AX with other ingredients in pancakes, such as water and protein molecules. This work emphasizes the need for more intensive investigations of the molecular interactions occurring throughout the baking process, not solely focused on raw flour samples.
Little research has been done involving North American pancake quality. Traditionally a soft wheat, batter-based product, many quality attributes studied relate closely to other batter-based products such as cakes (Finnie et al 2006). However, cakes are often assessed based on cake volume because the lateral flow of the batter is restricted by a pan. Pancake batter flow, conversely, is not restricted by a pan, and thus the pancake diameter provides an assessment of quality, much like cookie diameter. Pancake height can also be assessed, which is inversely related to pancake diameter. Pancakes that are deemed too thick may take longer to cook through, creating over-browning at the pancake surface, which is an undesirable trait. The same is true for pancake weight, which can indirectly account for pancake density. Very dense pancakes may require longer cooking times and result in an undesirable level of surface darkening (Finnie et al 2006).

Arabinoxylans (AX) make up 85% of all non-starch polysaccharides in wheat. They are comprised of a $\beta$-1,4 linked D-xylopyranosyl backbone with substituted monomeric $\alpha$-L-arabinofuranoside units at the second and/or third carbon positions of the xylose residues. The fifth carbon on the arabinose may contain a ferulic acid constituent (Courtin and Delcour 2002). The three-dimensional structure of AX is determined by: the length of the xylan backbone, the arabinose:xylose (A/X) ratio, the arabinose substitution pattern, and the ferulic acid coupling to other AX molecules or the cell wall (Courtin and Delcour 2002). Two empirically derived subfractions, based on extractability, exist in large part because of the somewhat flexible nature of AX (Dervilly et al 2000) and the large molecular weight (65,000) (Andrewartha et al 1979). Total arabinoxylan (TAX) content can be subdivided based on extraction properties: water-
extractable (WEAX) and water-unextractable (WUAX) AX (Izydorczyk and Biliaderis 1992; Courtin and Delcour 2002).

Arabinoxylans have a wide range of influence on end-use quality (Bettge and Morris 2000, 2007; Courtin and Delcour 2002; Jelaca and Hlynka 1971; Izydorczyk and Biliaderis 1995; Delcour et al 1991). The heavy influence of AX in baked products is the result of their high water absorption (Jelaca and Hlynka 1971), which creates a strong competition with other ingredients or constituents for water (Izydorczyk and Biliaderis 1995). The unique ability of AX to form oxidative cross-links and subsequent gel (Niño-Medina et al 2010) give AX very distinct impacts on end-use quality (Bettge and Morris 2007; Ramseyer et al 2011a, 2011b). Non-covalent interactions between AX molecules may also play a small role in the formation of the gel network (Niño-Medina et al 2010). The properties of the gel network depend on the structural characteristics of the cross-linking in AX molecules (Niño-Medina et al 2010). Cross-linked AX molecules are capable of binding water; the capacity to do so is dependent upon the extent of cross-linking. The water-binding capacity increases with increasing cross-linkages; however, there is an optimum level of cross-linkages for highest water-binding capacity, beyond which the capacity to retain water decreases (Izydorczyk and Biliaderis 1995). This oxidative cross-linking is the factor mostly responsible for increasing batter viscosity in soft wheat products (Bettge and Morris 2007). The increase in viscosity due to cross-linking is closely related to decreases in cookie diameter and sponge cake volume (Bettge and Morris 2007, Ramseyer et al 2011a, b). Adequate cookie spread is likely impeded by this gelation, which explains in part the variation in cookie diameter otherwise unexplained by WEAX and high protein levels, two factors also known to decrease cookie diameter (Bettge and Morris 2007). Because water relationships in cookies are influenced by viscosity increases, AX plays an influential role in the final cookie
diameter (Bettge and Morris 2000). A decrease in dough plasticity has also been observed, attributed to sugar syrup sequestration by WEAX, and in increased bake-out time. This higher bake-out time can increase the incidence of checking, or stress fractures occurring in low-moisture products as the result of over-baking to decrease the moisture content to an optimum level (Bettge and Morris 2007). Thus far, no studies have undertaken the specific impacts of TAX and WEAX in the soft wheat product of pancakes.

The overall objective of this study was to track TAX and WEAX content through the baking process for both pancakes and bread. Secondary objectives were to determine differences between AX content for wholemeal and refined flour, as well as differentiating between patterns of AX content in different varieties from different market classes.

MATERIALS AND METHODS

Grain Samples

Five varieties grown in 2009 were identified to undergo extensive studies on their arabinoxylan (AX) content throughout the baking process. The varieties were: Alpowa and Louise (soft white spring wheat), Blanca Grande and Macon (hard white spring wheat), and Westbred 926 (hard red spring wheat) (Table I). These varieties were isolated in an effort to represent several different market classes of wheat. Both wholemeal and refined flour pancakes were made and sampled for each variety, resulting in 10 total samples of starting material.

Pancake Sample Preparation Method

The pancakes were prepared according to the method developed by the AACC Soft Wheat and Flour Technical Committee with participation by Zory Quinde-Axtell, Meera Kweon, Barbara Heidolph, Sean Finnie, Diane Gannon and Art Bettge. The dry pancake mix consisted of
175g flour, 17g granulated sucrose, 5.5g dextrose, 3.16g sodium bicarbonate, 0.79g monocalcium phosphate, 3.51g sodium acid pyrophosphate, and 2.6g sodium chloride. The leavening acids and sodium bicarbonate were kept separate from the other ingredients as well as from each other until combined with other dry ingredients until immediately prior to use in order to prevent pre-reaction. The dry ingredients were mixed together at speed 1 (low) with a Hobart N5 mixer (Hobart Corporation, Troy, OH), or speed 2 on a KitchenAid mixer (KitchenAid Professional 600 Series, St. Joseph, MI) with a whisk attachment for 1min. The sides of the mixing bowl were scraped down and the ingredients were mixed for an additional 1min. In a non-running mixer, 11g canola oil was added to the dry ingredients. The mixture was mixed for 1min with a whisk attachment at the same speed as the first mixing. The sides of the bowl were scraped down and the mixture was blended for an additional minute.

Following complete mixture of the dry ingredients, 242mL water (for refined flour samples) or 266mL water (for wholemeal samples) was added to the mixing bowl and was mixed for 10sec. The sides of the mixing bowl were scraped down and the batter was mixed for an additional 10sec. The batter was allowed to rest for 3min, and a full #20 food scoop was used to pour approximately 41g batter onto the griddle (with a surface temperature of 190 °C). Four pancakes were dispensed from approximately 8cm above the griddle surface. The pancakes were then cooked for 90sec on each side. Once removed, the pancakes were allowed to cool for 10min, flipped, and cooled for an additional 10min. Three diameters of each pancake were recorded along with the height and weight of the four pancakes together.

Batter sampling was done immediately prior to placement on the griddle. The cooked pancakes then were divided into fourths and one-fourth of each pancake was subsampled for the “cake” sample. The batter and pancake samples were lyophilized using a Virtis SQ Super XL-70
freeze-dryer with a 25L condenser (SP Industries, Gardiner, NY) and prepared for GC-FID analysis according to Englyst and Cummings (1984) and Courtin et al (2000). Each flour sample (refined or wholemeal) was replicated four times.

**Arabinoxylan Quantification**

This procedure was adapted from Englyst and Cummings (1984) and Courtin et al. (2000).

**WEAX Hydrolysis**

For WEAX quantification, between 350 and 700 mg of the sample were weighed into a centrifuge-safe 12-mL test tube, 7 mL H₂O was added, and mixed with a vortex mixer for 7 sec. The tubes were continuously shaken in a 7 ºC refrigerator for one hour. The tubes were centrifuged at 1600 X g for 5 min and 0.875 mL of the supernatant was removed and placed in another centrifuge-safe 12-mL test tube. To this mixture, 0.875 mL trifluoroacetic acid (TFA; 4 N) was added and the solution was mixed for 7 sec with a vortex mixer. The samples were incubated for 60 min at 110 ºC in a Fisher Isotemp Dry Bath Model 145 heating block (Thermo Fisher Scientific, Santa Clara, CA). The block was made of anodized aluminum with 48 cylindrical wells 1.7cm in diameter and 4.4cm deep. The samples were immediately transferred into an ice bath until the test tubes were cool to the touch. The samples were then centrifuged at 1600 X g for 5 min. At this point, the WEAX and TAX samples were treated identically.

**TAX Hydrolysis**

The TAX was quantified starting with 10.5 – 35 mg of sample weighed into centrifuge-safe 12-mL test tubes. To the samples, 1.75 mL TFA (4 N) was added and mixed for 7 sec with a vortex mixer. The samples were incubated at 110 ºC for 60 min and immediately following
incubation, transferred into a nice bath until the test tubes were cool to the touch. The samples were centrifuged at 1600 X g for 5 min.

**WEAX and TAX Derivatization**

From the centrifuged product, 1 mL of the supernatant was removed and placed into a 12-mL test tube. An internal standard of allose (0.35 mL of a 1 mg/mL in H₂O solution) was added to each test tube. The test tubes were placed in an ice bath and 0.55 mL NH₃ was added to each and the test tubes were mixed with a vortex mixer for 7 sec. Each sample solution was pH tested to verify basicity. If the solution was not basic, aliquots of 100 µL NH₃ were added until a pH of over 7 was achieved. Following this verification, 7 µL of 2-octanol was added to each test tube. At this point, a suspension of NaBH₄ in 3 M NH₃ was made (200 mg NaBH₄ / mL NH₃). It is critical to wait until this time period to make the suspension so that it is fresh before adding 70 µL to each sample. Once the NaBH₄ suspension was added to each sample, the test tubes were mixed with a vortex mixer for 7 sec before being incubated in a heating block at 40 ºC for 30 min. Following incubation, the samples were placed in an ice bath briefly before the addition of 140 µL glacial acetic acid and mixed for 7 sec with a vortex mixer. One hundred, seventy-five microliters of this solution was transferred to a fresh 12-mL test tube. To this, 175 µL 1-methylimidazole and 1.75 mL acetic anhydride were added. After letting the solution set for 10 min, 1 mL ethanol was added and immediately mixed with a vortex mixer for 7 sec. The solution was allowed to set for 5 min before the addition of 3.5 mL H₂O and another 5 min waiting period. Following the addition of 175 µL bromophenol blue, the test tubes were transferred to an ice bath. To the solutions, two aliquots of 1.75 mL concentrated KOH were added, with a brief resting period between aliquots. The solutions undergo a phase separation following the addition of KOH; the top layer is the desired layer and appears yellow-ish in color. The bottom layer
appears dark blue. Letting the solutions remain in the ice bath for a few minutes allows the phases to separate more completely for an easier extraction of the top layer. The top layer was transferred to a fresh test tube and anhydrous Na₂SO₄ was added to dry the solution. Once the water was removed from the solutions with the desiccant, the solutions were transferred to GC vials for analysis using the GC-FID.

**GC-FID Analysis**

The samples were injected into the GC into a capillary column with a split-splitless injector. The samples then entered the flame ionization detector to detect the components of the samples. The column used in the GC was a Supelco SP-2380 polar column (Sigma-Aldrich, Bellefonte, PA) in an Agilent 6890 Series Chromatograph (Agilent, Santa Clara, CA) with an autosampler and a splitter injection port (injection volume dependent upon the type of sample; split ratio 1:20). Detection with the FID was performed using a carrier gas of hydrogen. Sample injection occurred at 210 ºC, separation beginning at 237 and ending at 260 ºC, and detection at 240 ºC.

**Statistical Analysis**

The statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). The general analyses were done using PROC GLM. Type III mean squares were used.

**RESULTS**

**Raw Flour**

The TAX and WEAX contents in wholemeal (Table II) ranged from 4.66 to 6.42% and 0.31 to 0.58%, respectively in wholemeal flour. In refined flour the ranges were 0.73-1.38% and 0.34-0.66% for TAX and WEAX. Across both flour types, the TAX contents and A/X ratio in
TAX did not statistically differ among varieties (data not shown). The WEAX contents and water-extractable A/X ratio did exhibit differences among varieties. The ranking of TAX content between wholemeal and refined flour was nearly identical, with Louise exhibiting the lowest TAX content and Blanca Grande exhibiting the highest TAX content in each flour type. In a comparison of WEAX content in wholemeal and refined flour, the variety rankings were identical, with Louise exhibiting the lowest WEAX content, and Westbred exhibiting the highest WEAX content in both flour types. Whereas the rank-order of varieties differed between TAX and WEAX content in wholemeal flour, the rank-order of varieties in refined flour was nearly identical for TAX and WEAX. Only the two highest in TAX and WEAX content (Westbred and Blanca Grande) exchanged rankings between the two types of analyses.

Arabinoxylans

The TAX and WEAX content as well as the A/X ratio in both fractions was analyzed for both wholemeal and refined flour (flour type), variety, and the product categories of raw flour, batter, and pancakes (Table III). The models of TAX, WEAX, and the A/X ratio in TAX were significant and the variation was well-modeled by the ANOVA with $R^2$ values ranging from 0.84 to 0.99. The A/X ratio in WEAX was not observed to be significant, only capturing the variation in the data at an $R^2$ level of 0.66. The TAX and WEAX content differed between wholemeal and refined flour, wholemeal flour exhibiting a higher TAX level than refined flour (4.15 vs. 1.12%, respectively). Conversely, refined flour had a higher WEAX level than did wholemeal flour (0.23 vs. 0.20%, respectively). Flour type did not have an appreciable impact on the A/X ratio in neither TAX nor WEAX.
Differences existed among varieties in TAX content, WEAX content, and the A/X ratio of WEAX (Table III). The TAX contents of Blanca Grande, Westbred, Macon, and Alpowa did not differ from one another. Louise had a lower TAX content as compared to Blanca Grande, Westbred, and Macon, but was not different from Alpowa. The WEAX content differed for each variety, with a ranking of Westbred, Blanca Grande, Macon, Alpowa, and Louise, in decreasing order. The A/X ratio in TAX was the highest in Louise and the lowest in Blanca Grande. An interaction between flour type and variety was observed in TAX and WEAX content. This interaction was the result of a change in rank order of varieties between the two flour types; however, Louise consistently exhibited the lowest TAX and WEAX content across the two flour types.

The TAX and WEAX content differed across product types (Table III). In both TAX and WEAX content, raw flour exhibited the highest responses and pancakes had the lowest AX content for both fractions. In both cases of TAX and WEAX content, raw flour had notably higher responses, but batter and pancakes did not differ. An interaction occurred between flour type and product type for TAX and WEAX. This interaction was the result of an increase in TAX and WEAX content evaluated during the baking process for several varieties, differing between wholemeal and refined flour. These differences in response of varieties throughout the baking process contributed to the interaction of variety and product type for the A/X ratio in TAX and the WEAX content.

The mean responses of wholemeal and refined flour in all five varieties and the baking process across four replications were surveyed in Table IV. In wholemeal, the TAX content decreased from raw flour to batter and finally to pancakes in the four varieties of Alpowa, Blanca Grande, Louise, and Macon. In Westbred, however, the TAX content decreased from raw flour
to batter, but increased slightly in cooked pancakes. The WEAX content decreased two- to three-fold between raw and batter, and decreased slightly between batter and pancakes in all varieties except Macon. The A/X ratio in TAX and WEAX did not exhibit any clear patterns across the five varieties.

In refined flour, the AX content was less consistent throughout the baking process (Table IV). In Alpowa, TAX did not differ between raw flour and batter, but increased in pancakes. In Blanca Grande and Westbred, the TAX content decreased between raw flour and batter, but increased in pancakes. In Louise and Macon, the TAX content increased throughout the baking process from raw flour, to batter, to pancakes, which exhibited the highest TAX content. In Alpowa and Louise, the WEAX content decreased three-fold between raw flour and batter, and continued to decrease slightly in pancakes. The variety Blanca Grande exhibited a decrease in WEAX between raw flour and batter, but did not decrease further in pancakes. In Macon and Westbred, the WEAX content decreased from raw flour to batter, but increased slightly in pancakes. Similarly to wholemeal flour, the A/X ratio in TAX and WEAX of refined flour did not exhibit any consistent or notable patterns.

**Baking Quality**

The pancake diameter, weight, and height were analyzed for varietal and flour type differences in Table V. All three ANOVA models were significant and captured the variation (as assessed by $R^2$) between 0.64 and 0.98. Flour type influenced pancake diameter and height, with refined flour exhibiting a larger average diameter, but a shorter height for the stack of four pancakes. Varietal differences existed in all three quality parameters surveyed. The average pancake diameter was largest in Louise and Alpowa (soft wheats) and smaller in the three hard
wheats (Macon, Westbred, and Blanca Grande). The rank of varieties for pancake weight was identically inverse of the rank order of pancake diameter. The rank for pancake weight was, in decreasing order, Blanca Grande, Westbred, Macon, Alpowa, and Louise. No difference in pancake weight was observed among the three hard wheats, nor was a difference evident between the two soft wheats. Pancake height differed among the varieties, with Westbred exhibiting the tallest pancake stack (61.6mm) and Louise the shortest (47.9mm).

Interactions between flour type and variety existed in the measurements of pancake diameter and height (Table V). These interactions were attributed to differences in rank order among the varieties across the two flour types. Macon and Westbred exhibited a higher relative pancake diameter in wholemeal pancakes as compared to refined flour pancakes. Conversely, both Alpowa and Blanca Grande exhibited higher relative pancake diameters in refined flour in comparison to wholemeal pancakes. In pancake height, Louise, Alpowa, and Macon retained their ranking positions as the three varieties with the shortest heights across wholemeal and refined flour pancakes. Whereas Westbred exhibited the tallest pancake stack in wholemeal and Blanca Grande the next tallest pancake stack, they exchanged ranking positions in refined flour. No interaction was evident between variety and flour type in the determination of pancake weight.

Because of the differences between flour type in TAX and WEAX content throughout the baking process, the flour types were separately correlated with raw flour, batter, and pancake TAX and WEAX along with their corresponding A/X ratios (Tables VI and VII). In the correlation analysis of wholemeal flour baking parameters and AX parameters (Table VI), average pancake diameter was most strongly correlated with raw flour TAX (-0.89). The A/X ratio in raw flour WEAX also exhibited a high, positive relationship with pancake diameter.
Other notable correlations with pancake diameter were raw flour WEAX (-0.77), the TAX A/X ratio in raw flour (0.55) and batter (0.74), batter and pancake WEAX (-0.58 and -0.54), pancake TAX (-0.59), and the A/X ratio in batter WEAX (0.45). Both raw flour and pancake WEAX exhibited a high correlation with pancake weight (0.85 and 0.80, respectively). Although there was a close relationship between TAX content and the TAX A/X ratio in raw flour (-0.61), there was a closer relationship between TAX and the A/X ratio in WEAX of raw flour (-0.88). Notably, this relationship between TAX and the WEAX A/X ratio was stronger than that of WEAX content and WEAX A/X ratio (-0.73). While the relationship between raw flour WEAX and batter WEAX was close (0.82), there was a remarkably strong relationship of raw flour WEAX to pancake WEAX (0.90). Despite high levels of correlation between TAX and WEAX content in raw flour and pancakes (0.71 and 0.63, respectively), batter TAX and WEAX exhibited a correlation of only 0.31.

In contrast to the wholemeal analysis, pancake diameter was most closely related to raw flour WEAX content (-0.88), followed closely by pancake WEAX content (-0.86), and batter WEAX content (-0.84). A strong, negative correlation did exist between pancake diameter and raw flour TAX content (-0.76). Notably, the relationship was positive between pancake diameter and pancake TAX content (0.57), and weakly positive to batter TAX content (0.30). Pancake weight was positively correlated with raw flour TAX content (0.52) and the WEAX content of raw flour, batter, and pancakes (0.57, 0.67, 0.66), but negatively correlated to pancake TAX content (-0.56) and the A/X ratio of pancake WEAX content (-0.55). Notably, the raw flour TAX content was negatively correlated with batter TAX and pancake TAX (-0.57 and -0.86). Despite these negative relationships, raw flour TAX content was highly, positively correlated to batter and pancake WEAX (0.90 and 0.89, respectively). The raw flour WEAX content was similarly
related to batter and pancake WEAX content (0.91 and 0.94). Whereas the raw flour TAX content was positively correlated to the raw flour A/X ratio in TAX (0.47), it was negatively correlated with the A/X ratio in all other products and types of AX.

**DISCUSSION**

**Raw Flour**

The soft wheat varieties (Louise and Alpowa) tended to exhibit slightly lower levels of TAX, on average, than did the hard wheat varieties (Macon, Westbred, and Blanca Grande). Macon, however, did exhibit a wholemeal TAX level negligibly lower than Alpowa, and only a slightly higher TAX level in refined flour than did Alpowa. As a result of these similar rankings and TAX in wholemeal and refined flour, there is no clear separation between hard and soft wheat varieties solely on the basis of AX content. As such, their corresponding results in the baking portion of the study appear to be a fair examination and comparison of performance.

The WEAX content did offer a clearer separation between hard and soft wheats. The soft wheat varieties, Alpowa and Louise, exhibited lower WEAX levels in both wholemeal and refined flour. In the hard wheat varieties, Macon, Blanca Grande, and Westbred, the WEAX levels were notably higher than in soft wheats in both wholemeal and refined flour. The A/X ratios observed in TAX and WEAX of both flour types did not exhibit any notable patterns, suggesting that these ratios are relatively unrelated to hardness classes.

**Arabinoxylans**

Because the bran fraction of a wheat kernel contributes considerable levels of TAX, the higher level of TAX exhibited in wholemeal flour as compared to refined flour is consistent with
the known understanding of AX heterogeneity throughout the wheat kernel (Philippe et al 2006a). The higher levels of WEAX in refined flour, however, suggest that the water-holding capacity of WEAX through oxidative cross-linking may have a stronger influence on soft wheat products made from refined flour, as opposed to wholemeal flour. The similarity of A/X ratios between wholemeal and refined flour suggests similar structural properties of the majority of AX in both types of flour.

In wholemeal flour, the TAX and WEAX content decreased from raw flour through the baking process. The magnitude of the decrease in AX content differed among the varieties, as did the pattern of AX content available for extraction from batter to pancakes. Because there is no biochemical way for AX to be destroyed during the baking process in the absence of added enzymes, the decreases observed in TAX and WEAX from raw flour to batter are the result of the formulation of the pancakes and the mixing action. The AX may be involved in oxidative cross-linking at this stage, and unavailable for extraction, and thus, quantification. The increase in TAX from batter to pancakes observed in Westbred, and the increase in WEAX from batter to pancakes in Macon appears to be the result of complex molecular interactions occurring, which make the TAX and WEAX molecules more or less available for extraction at different points throughout the baking process. The heat treatment during cooking may also contribute to differences in AX availability for extraction and quantification. These specific molecular interactions are as yet unknown, but may play a critical role in overall product functionality. In wholemeal there was no appreciable pattern throughout the baking process in the A/X ratio for TAX or WEAX.

The TAX and WEAX content throughout the baking process varied greatly in refined flour as compared to wholemeal flour. Several varieties exhibited an increase in TAX content
from raw flour to the batter and pancakes, while others had a decreasing level of TAX content throughout the baking process. This suggests that the AX molecules are behaving inherently differently throughout the baking process based on varietal and AX structural differences which are as yet unclear. The way that the AX molecules interact with the ingredients appears to differ such that their ability to be extracted varies across varieties. All varieties exhibited a decrease in WEAX content from raw flour to batter, but some showed a further decrease in WEAX, while others remained the same, or increased slightly in WEAX content. As previously stated, this does not indicate a physical increase in AX molecules, but rather an increase in their availability to be quantified. Oxidative cross-linking may decrease the availability of the AX molecules to be extracted, and therefore, are unaccounted for in the analysis. There were no patterns evident in the A/X ratio throughout the baking process for TAX and WEAX content.

**Baking Quality**

The pancake quality parameters exhibited differences between flour type and variety, along with interactions between these categories in pancake diameter and height. Wholemeal flours produced smaller pancake diameters, but higher stack heights than did refined flours. Despite additional water in the formulation of the wholemeal pancakes, they did not spread to the extent that the refined flour pancakes did, indicating that additional formulation modifications may need to be made in wholemeal pancakes to adequately account for differences in functionality, and achieve optimum quality. Soft wheat varieties had larger diameters than hard wheat varieties, which is consistent with the preference of soft wheat for pancake baking. Additional water may need to be included in formulations using hard wheat varieties to account
for additional water-holding capacities of hard wheat varieties, as evidenced by a decrease in batter spread.

Pancake diameter was inversely related to pancake diameter in both wholemeal and refined flour. This reflects the degree to which batters were able to spread; less pancake spread resulted in smaller diameters but thicker pancakes, and those batters that spread easily created thinner pancakes. Although higher pancake diameters may be desirable for many consumers, the thickness may also play a role in consumer appeal. In this way, the desired end-product may be achieved using wholemeal or refined flour, or hard or soft wheat, dependent upon the consumer’s interest. Pancake weight also exhibited an inverse relationship to pancake diameter; the pancakes with the largest diameter had the smallest weight, and conversely, the pancakes with the least amount of spread created the heaviest pancakes. The interaction between variety and flour type was reflected in a rank-order change of varieties between wholemeal and refined flour for both pancake diameter and height. These interactions emphasized the varied performance of varieties across different flour types. This interaction may be, in part, a result of AX structural differences, or a combination of several factors during baking. The water-holding capacity of the varieties may differ across flour types based on bran characteristics and purity of the refined flour.

Based on the correlations of wholemeal measured parameters, it appeared as if TAX levels were most influential in determining pancake diameter. The inverse relationship showed the detrimental effects of TAX content on pancake diameter. The strength of this correlation also serves to indicate that the AX in raw flour that is able to be extracted and quantified plays a larger role in determining pancake quality than does the AX content throughout the baking process that is available for extraction. With a high, negative correlation, the A/X ratio in WEAX also appeared to play a substantial role in pancake diameter determination. The higher level of
pancake diameter correlation with the A/X ratio, as opposed to WEAX content suggests that the role WEAX plays in wholemeal pancake quality is based on WEAX structure, rather than WEAX content. A higher level of arabinose as compared to xylose led to a larger pancake diameter; more highly substituted WEAX positively influenced pancake quality. The more highly substituted WEAX molecules may not be able to fully participate in oxidative cross-linking because of stearic hindrance. The WEAX molecules are typically smaller than WUAX (Philippe et al 2006b), thus, higher levels of substitution may prevent oxidative cross-linking from occurring, rendering them more available for extraction and quantification.

The close, negative relationship between TAX and the A/X ratio of WEAX in raw flour may indicate that increasing levels of TAX result from higher levels of more highly unsubstituted WEAX molecules. These data emphasize the high level of structural heterogeneity that exists in AX molecules across varieties. The higher level of correlation between raw flour WEAX and pancake WEAX in comparison to the raw flour WEAX correlation to batter WEAX suggests that during batter mixing, some WEAX molecules become tied up in oxidative cross-links with other AX molecules or proteins, rendering them unavailable for quantification, but become liberated through high levels of heat treatment occurring on the pancake griddle. These complex relationships among AX molecules in a batter system and the impact of heat are not well understood at this time, warranting further exploration. These complexities are further reflected in the low correlation between TAX and WEAX in batter (0.31), despite high levels of correlation between TAX and WEAX in raw flour and pancakes (0.71 and 0.63, respectively).

The difference in correlation responses between wholemeal and refined flour is likely attributed to the role that bran AX play in functional relationships. In contrast to the importance of TAX content in the determination of pancake diameter in wholemeal flour, in refined flour the
WEAX content in raw flour, batter, and pancakes exhibited the strongest correlations with pancake diameter. These correlations clearly indicate that in refined flour, WEAX plays a much more influential role than in wholemeal flour. Because there was no observable difference in the A/X ratio for TAX and WEAX between flour types, it does not appear as if the AX structure is responsible for the differing relationships in TAX and WEAX between flour types. The positive relationship among pancake diameter and pancake and batter TAX further emphasizes the differing AX relationships between wholemeal and refined flour.

The AX relationships in refined flour were substantially different than those observed in wholemeal flour. The negative relationship between raw flour TAX and the TAX level in batter and pancakes suggests that higher levels of TAX are associated with higher levels of intermolecular relationships which render TAX unavailable for extraction throughout the baking process. Because these same relationships were positive in wholemeal flour, as opposed to negative as observed in refined flour, the assertion of structural differences in AX between bran and endosperm is strengthened (Philippe et al 2006a). The high, positive correlation of TAX and WEAX in raw flour with the WEAX contents in batter and pancakes suggests that in refined flour, the water-unextractable AX may be most responsible for oxidative cross-linking, allowing the WEAX molecules to remain as available for extraction throughout the baking process as they were in raw flour. The negative relationship of raw flour TAX content to the A/X ratio observed in raw flour WEAX and all other products and types of AX confirm that the high-mass, more-highly substituted water-unextractable AX may be the molecules primarily involved in oxidative cross-linking, leaving less highly substituted AX molecules available for extraction and subsequent quantification.
CONCLUSION

Soft wheat and refined flour provided pancakes with the ideal quality, as defined by pancake diameter and height. Arabinoxylans exhibited vastly different properties and relationships between wholemeal and refined flour. Whereas raw flour TAX content was most detrimental to wholemeal pancake quality, WEAX content in the raw, refined flour exerted the strongest negative influence on pancake quality. The complex relationships of AX with other ingredients in the pancake formulation also differed greatly between wholemeal and refined flour. These complexities necessitate further exploration in order to better understand the nature of the molecular interactions occurring in batter systems, which in turn, heavily influence quality.

ACKNOWLEDGMENTS

We would like to acknowledge the technical staff of the USDA-ARS Western Wheat Quality Laboratory, Doug Engle, Mary Baldridge, Bozena Paszczynska, Mishelle Lenssen, Eric Wegner, Bill Kelley, Patricia Boyer, Gail Jacobson and Anna Hansen. Stacey Sykes and Shawna Vogl assisted in the preparation of the manuscript. This project was supported by the Agriculture and Food Research Initiative Grant no. 2009-02347 from the USDA National Institute of Food and Agriculture.
LITERATURE CITED


TABLE I.
Variety and market class of the five wheat samples used for baking and analysis

<table>
<thead>
<tr>
<th>Variety</th>
<th>Market Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpowa</td>
<td>Soft white spring</td>
</tr>
<tr>
<td>Blanca Grande</td>
<td>Hard white spring</td>
</tr>
<tr>
<td>Louise</td>
<td>Soft white spring</td>
</tr>
<tr>
<td>Macon</td>
<td>Hard white spring</td>
</tr>
<tr>
<td>Westbred</td>
<td>Hard red spring</td>
</tr>
</tbody>
</table>
**TABLE II.**

Arabinoxylan content of the five varieties surveyed in wholemeal and refined flour forms

<table>
<thead>
<tr>
<th>Variety</th>
<th>Wholemeal</th>
<th></th>
<th></th>
<th></th>
<th>Refined Flour</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAX(^a)</td>
<td>A/X(^b)</td>
<td>WE(^c)</td>
<td>A/X(^b)</td>
<td>TAX(^a)</td>
<td>A/X(^b)</td>
<td>WE(^c)</td>
<td>A/X(^b)</td>
</tr>
<tr>
<td>Alpowa</td>
<td>4.99</td>
<td>0.64</td>
<td>0.39</td>
<td>0.66</td>
<td>1.03</td>
<td>0.58</td>
<td>0.40</td>
<td>0.66</td>
</tr>
<tr>
<td>Blanca Grande</td>
<td>6.42</td>
<td>0.63</td>
<td>0.53</td>
<td>0.52</td>
<td>1.38</td>
<td>0.64</td>
<td>0.58</td>
<td>0.62</td>
</tr>
<tr>
<td>Louise</td>
<td>4.66</td>
<td>0.70</td>
<td>0.31</td>
<td>0.75</td>
<td>0.73</td>
<td>0.60</td>
<td>0.34</td>
<td>0.77</td>
</tr>
<tr>
<td>Macon</td>
<td>4.96</td>
<td>0.71</td>
<td>0.44</td>
<td>0.78</td>
<td>1.05</td>
<td>0.55</td>
<td>0.55</td>
<td>0.75</td>
</tr>
<tr>
<td>Westbred</td>
<td>5.39</td>
<td>0.70</td>
<td>0.58</td>
<td>0.58</td>
<td>1.27</td>
<td>0.62</td>
<td>0.66</td>
<td>0.66</td>
</tr>
</tbody>
</table>

\(^a\)Percent total arabinoxylan content  
\(^b\)Ratio of arabinose:xylose  
\(^c\)Percent water-extractable arabinoxylan content
### TABLE III.

Arabinoxylan content ANOVA, whole model $R^2$, including flour type, variety, and product (raw flour, batter, and pancake)

<table>
<thead>
<tr>
<th>Source</th>
<th>TAX a</th>
<th>A/X b</th>
<th>WE c</th>
<th>A/X b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Model $R^2$</strong></td>
<td>0.98</td>
<td>0.84</td>
<td>0.99</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Whole Model F value</strong></td>
<td>28.02***</td>
<td>2.65**</td>
<td>42.04***</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Flour Type F value</strong></td>
<td>479.5**</td>
<td>2.46</td>
<td>75.95**</td>
<td>8.68</td>
</tr>
<tr>
<td><strong>Variety F value</strong></td>
<td>3.22*</td>
<td>2.03</td>
<td>142.0***</td>
<td>5.12**</td>
</tr>
<tr>
<td><strong>Flour Type*Variety F value</strong></td>
<td>3.64*</td>
<td>0.76</td>
<td>8.57***</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Product F value</strong></td>
<td>13.52***</td>
<td>1.67</td>
<td>556.33***</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Flour Type*Product F value</strong></td>
<td>14.75***</td>
<td>3.01</td>
<td>4.25*</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Variety*Product F value</strong></td>
<td>0.73</td>
<td>2.36*</td>
<td>4.81**</td>
<td>0.26</td>
</tr>
<tr>
<td>**Flour Type<em>Variety</em>Product F value</td>
<td>0.42</td>
<td>1.56</td>
<td>1.21</td>
<td>0.69</td>
</tr>
</tbody>
</table>

* Percent total arabinoxylan content  
* Ratio of arabinose:xylose  
* Percent water-extractable arabinoxylan content  
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
TABLE IV.

Arabinoxylan percent of raw flour, batter, and pancakes in wholemeal and refined flour

<table>
<thead>
<tr>
<th>Variety</th>
<th>Wholemeal</th>
<th>Refined Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAX(^a) A/X(^b) WE(^c)  A/X(^b)</td>
<td>TAX(^a) A/X(^b) WE(^c) A/X(^b)</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>---------------</td>
</tr>
<tr>
<td>Alpowa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>4.99 0.64 0.39 0.66</td>
<td>1.03 0.58 0.40 0.66</td>
</tr>
<tr>
<td>Batter</td>
<td>3.93 0.67 0.15 0.76</td>
<td>1.03 0.78 0.13 0.63</td>
</tr>
<tr>
<td>Pancake</td>
<td>3.73 0.70 0.13 0.65</td>
<td>1.21 0.80 0.12 0.74</td>
</tr>
<tr>
<td>Blanca Grande</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>6.42 0.63 0.53 0.52</td>
<td>1.38 0.64 0.58 0.62</td>
</tr>
<tr>
<td>Batter</td>
<td>4.50 0.65 0.19 0.52</td>
<td>0.95 0.56 0.27 0.62</td>
</tr>
<tr>
<td>Pancake</td>
<td>4.34 0.65 0.18 0.57</td>
<td>0.97 0.69 0.27 0.64</td>
</tr>
<tr>
<td>Louise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>4.66 0.70 0.31 0.75</td>
<td>0.73 0.60 0.34 0.77</td>
</tr>
<tr>
<td>Batter</td>
<td>3.43 0.73 0.11 0.69</td>
<td>1.20 0.88 0.11 0.75</td>
</tr>
<tr>
<td>Pancake</td>
<td>3.17 0.64 0.09 0.73</td>
<td>1.34 0.88 0.10 0.76</td>
</tr>
<tr>
<td>Macon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>4.96 0.71 0.44 0.78</td>
<td>1.05 0.55 0.55 0.75</td>
</tr>
<tr>
<td>Batter</td>
<td>4.28 0.71 0.15 0.64</td>
<td>1.17 0.79 0.22 0.78</td>
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<tr>
<td>Pancake</td>
<td>3.89 0.67 0.17 0.68</td>
<td>1.20 0.76 0.23 0.75</td>
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<tr>
<td>Westbred</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>5.39 0.70 0.58 0.58</td>
<td>1.27 0.62 0.66 0.66</td>
</tr>
<tr>
<td>Batter</td>
<td>4.40 0.67 0.28 0.69</td>
<td>1.02 0.66 0.23 0.69</td>
</tr>
<tr>
<td>Pancake</td>
<td>4.42 0.68 0.27 0.69</td>
<td>1.15 0.72 0.25 0.70</td>
</tr>
</tbody>
</table>

\(^a\)Percent total arabinoxylan content
\(^b\)Ratio of arabinose:xylose
\(^c\)Percent water-extractable arabinoxylan content
TABLE V.

Pancake quality parameter ANOVA, model $R^2$, including average pancake diameter, and pancake weight and height (for four pancakes)

<table>
<thead>
<tr>
<th>Source</th>
<th>Diameter</th>
<th>Weight</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Model $R^2$</td>
<td>0.93</td>
<td>0.64</td>
<td>0.98</td>
</tr>
<tr>
<td>Whole Model F value</td>
<td>44.5***</td>
<td>6.03***</td>
<td>155.4***</td>
</tr>
<tr>
<td>Flour Type F value</td>
<td>250.5***</td>
<td>0.44</td>
<td>401.8***</td>
</tr>
<tr>
<td>Variety F value</td>
<td>31.5***</td>
<td>12.2***</td>
<td>233.3***</td>
</tr>
<tr>
<td>Flour Type*Variety F value</td>
<td>5.94**</td>
<td>1.25</td>
<td>15.8***</td>
</tr>
</tbody>
</table>

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
# TABLE VI.

Correlations between pancake quality and arabinoxylan parameters in wholemeal flour

<table>
<thead>
<tr>
<th></th>
<th>Raw</th>
<th>Batter (BTR)</th>
<th>Pancake (PC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Diameter</td>
<td>Weight</td>
<td></td>
</tr>
<tr>
<td>TAX(^a)</td>
<td>-0.47(^*)</td>
<td>0.60(^**)</td>
<td>0.06</td>
</tr>
<tr>
<td>TA/X(^b)</td>
<td>-0.89(^***)</td>
<td>0.04</td>
<td>0.63(^*)</td>
</tr>
<tr>
<td>WEAX(^c)</td>
<td>0.55(^*)</td>
<td>0.85(^***)</td>
<td>0.53(^*)</td>
</tr>
<tr>
<td>WA/X(^d)</td>
<td>-0.77(^***)</td>
<td>-0.51(^*)</td>
<td>-0.64(^**)</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td>-0.73(^**)</td>
</tr>
<tr>
<td>Raw TAX(^a)</td>
<td>-0.61(^**)</td>
<td>0.71(^**)</td>
<td>0.53(^*)</td>
</tr>
<tr>
<td>Raw TA/X(^b)</td>
<td>-0.09</td>
<td>0.64(^**)</td>
<td>-0.60(^**)</td>
</tr>
<tr>
<td>Raw WEAX(^c)</td>
<td>-0.73(^**)</td>
<td>0.53(^*)</td>
<td>-0.64(^**)</td>
</tr>
<tr>
<td>Raw WA/X(^d)</td>
<td>-0.35</td>
<td>0.81(^***)</td>
<td>-0.60(^**)</td>
</tr>
<tr>
<td>BTR TAX</td>
<td>0.35</td>
<td>0.31</td>
<td>0.51(^*)</td>
</tr>
<tr>
<td>BTR TA/X</td>
<td>-0.35</td>
<td>0.19</td>
<td>-0.62(^**)</td>
</tr>
<tr>
<td>BTR WEAX</td>
<td>-0.06</td>
<td>0.58(^**)</td>
<td>0.58(^**)</td>
</tr>
<tr>
<td>BTR WA/X</td>
<td>-0.03</td>
<td>0.15</td>
<td>-0.03</td>
</tr>
<tr>
<td>PC TAX</td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>PC TA/X</td>
<td>0.06</td>
<td>-0.16</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>PC WEAX</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Total arabinoxylan content
\(^b\)Arabinose:xylose ratio in total arabinoxylan content
\(^c\)Water-extractable arabinoxylan content
\(^d\)Arabinose:xylose ratio in water-extractable content

\( \ast P \leq 0.05, \ast\ast P \leq 0.01, \ast\ast\ast P < 0.0001 \)
### TABLE VII.

**Correlations between pancake quality and arabinoxylan parameters in refined flour**

<table>
<thead>
<tr>
<th>Weight TAX^a</th>
<th>TA/X^b</th>
<th>WEAX^c</th>
<th>WA/X^d</th>
<th>TAX</th>
<th>TA/X</th>
<th>WEAX</th>
<th>WA/X</th>
<th>TAX</th>
<th>TA/X</th>
<th>WEAX</th>
<th>WA/X</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diameter</strong></td>
<td>-0.30</td>
<td>-0.76***</td>
<td>-0.08</td>
<td>0.30</td>
<td>0.57**</td>
<td>-0.84***</td>
<td>0.13</td>
<td>0.57**</td>
<td>0.80***</td>
<td>-0.86***</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>0.52*</td>
<td>0.11</td>
<td>0.57**</td>
<td>-0.20</td>
<td>-0.05</td>
<td>-0.23</td>
<td>0.67**</td>
<td>0.04</td>
<td>-0.56*</td>
<td>-0.36</td>
<td>0.66**</td>
</tr>
<tr>
<td><strong>Raw TAX^a</strong></td>
<td>0.47*</td>
<td>0.85***</td>
<td>-0.83***</td>
<td>-0.57**</td>
<td>-0.71**</td>
<td>0.90***</td>
<td>-0.50*</td>
<td>-0.86***</td>
<td>-0.87***</td>
<td>0.89***</td>
<td>-0.56***</td>
</tr>
<tr>
<td><strong>Raw TA/X^b</strong></td>
<td>0.25</td>
<td>-0.66**</td>
<td>-0.42</td>
<td>-0.47*</td>
<td>0.30</td>
<td>-0.55*</td>
<td>-0.48*</td>
<td>-0.30</td>
<td>0.29</td>
<td>-0.46*</td>
<td></td>
</tr>
<tr>
<td><strong>Raw WEAX^c</strong></td>
<td>-0.47*</td>
<td>-0.35</td>
<td>-0.57**</td>
<td>0.91***</td>
<td>-0.10</td>
<td>-0.64**</td>
<td>-0.79***</td>
<td>0.94***</td>
<td>-0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Raw WA/X^d</strong></td>
<td>0.65**</td>
<td>0.62**</td>
<td>-0.53*</td>
<td>0.79***</td>
<td>0.74**</td>
<td>0.66**</td>
<td>-0.50*</td>
<td>0.53*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BTR TAX</strong></td>
<td>0.70**</td>
<td>-0.38</td>
<td>0.47*</td>
<td>0.52*</td>
<td>0.46*</td>
<td>-0.33</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BTR TA/X</strong></td>
<td>-0.63**</td>
<td>0.34</td>
<td>0.70**</td>
<td>0.60**</td>
<td>-0.61**</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>BTR WEAX</strong></td>
<td>-0.18</td>
<td>-0.77***</td>
<td>-0.85***</td>
<td>0.99***</td>
<td>-0.50*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BTR WA/X</strong></td>
<td>0.52*</td>
<td>0.29</td>
<td>-0.13</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PC TAX</strong></td>
<td>0.75**</td>
<td>-0.75**</td>
<td>0.60**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC TA/X</td>
<td>PC WEAX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>-0.84*** 0.42</td>
<td>-0.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ a^{\text{Total arabinoxylan content}} \]
\[ b^{\text{Arabinose:xylose ratio in total arabinoxylan content}} \]
\[ c^{\text{Water-extractable arabinoxylan content}} \]
\[ d^{\text{Arabinose:xylose ratio in water-extractable content}} \]
\[ * P \leq 0.05, ** P \leq 0.01, *** P < 0.0001 \]
CHAPTER SEVEN

Arabinoxylan Content and Availability throughout the Baking Process of Bread

Alecia M. Kiszonas¹, E. Patrick Fuerst¹, Craig F. Morris²

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of other that may also be suitable.

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**ABSTRACT**

End-use quality of wheat (*Triticum aestivum* L.) is heavily influenced in a variety of ways by non-starch polysaccharides, specifically, arabinoxylans (AX). The assessment of AX content and structure is often performed using raw flour and slurries, and extrapolated to determine the role they may play in baked products. Few studies have focused on the molecular interactions that actually occur throughout the baking process. The objective of this study was to track total and water-extractable AX (TAX and WEAX) throughout the baking process of five varieties, using both wholemeal flour and refined flour. The five varieties represent several different market classes, in order to understand AX variability in content and behavior across several market classes. The TAX and WEAX content was assessed in raw flour, mixed dough, proofed dough, and the bread loaf, separated into crumb, upper crust, and bottom crust. The ratio of arabinose:xylose (A/X) was also determined in order to understand the role of AX substitution in baking quality. The refined flour of hard wheat varieties exhibited the highest bread quality, as assessed by loaf volume. The content of TAX throughout the baking process differed between the wholemeal and refined flour samples, indicating a change in the TAX availability due to intermolecular interactions and heat treatment. Most critical to loaf volume was the WEAX content and the substitution level of these WEAX molecules. High levels of WEAX with low levels of arabinose substitution were observed to be most beneficial to achieve high loaf volumes. The upper crust of bread loaves also exhibited high levels of WEAX, suggesting that exposure to high levels of heat enhances the availability of AX molecules for extraction and quantification. The substitution level of WEAX molecules in particular was observed to be most critical in determining the level of influence WEAX content had in enhancing loaf volume.
INTRODUCTION

Bread consists of the basic ingredients of flour, water, yeast, and salt. Other ingredients may be added to increase shelf-life or to increase processability (Bloksma and Bushuk 1988). The basic steps of bread-making include mixing, dough formation, fermentation, and baking. Mixing the ingredients begins the process of dough development and creation of air cells. A combination of glutenins, gliadins, and residue proteins form an elastic and extensible matrix known as the gluten network. Fermentation serves to encourage carbon dioxide and ethanol production. It is during the fermentation phase that gas retention and the growth of gas cells is critical. Punching and molding serve to divide large gas cells into smaller ones, thereby increasing the total number of gas cells (Kulp 1988). During baking the gases expand and the volume increases. The solubility of carbon dioxide and other gases decrease as the temperature inside the developing bread loaf increase. Gelatinization of starch occurs and proteins denature, which give the crumb structure (Kulp 1988). Once the gas bubbles rupture, the spongy crumb texture is produced. Once the bread is removed from the oven, the amylose molecules retrograde and form a firm, sliceable crumb structure. Over several days, amylopectin molecules will also retrograde, causing the bread to stale (Kulp 1988). Many different intermolecular interactions influence bread quality, including arabinoxylans (Courtin and Delcour 2002).

Non-starch polysaccharides in wheat are mostly comprised of arabinoxylans (AX). Arabinoxylans are made up of a β-1,4 linked D-xylopyranosyl backbone with substituted monomeric α-L-arabinofuranoside units at the second and/or third carbon positions of the xylose residues. The fifth carbon on the arabinose may contain a ferulic acid constituent (Courtin and Delcour 2002). Several factors determine the three-dimensional structure of AX: the length of the xylan backbone, the arabinose:xylose (A/X) ratio, the arabinose substitution pattern, and
ferulic acid coupling to other AX molecules or the cell wall (Courtin and Delcour 2002). The three-dimensional structure is also influenced by the somewhat flexible nature of AX (Dervilly et al 2000) and the large molecular weight (65,000) (Andrewartha et al 1979). Total arabinoxylan (TAX) content can be subdivided based on extraction properties: water-extractable (WEAX) and water-unextractable (WUAX) AX (Izydorczyk and Biliaderis 1992; Courtin and Delcour 2002).

End-use quality is highly and variably influenced by AX (Bettge and Morris 2000, 2007; Courtin and Delcour 2002; Jelaca and Hlynka 1971; Izydorczyk and Biliaderis 1995; Delcour et al 1991). The high water absorption capability of AX is the main contributing factor in their influence on baked products (Jelaca and Hlynka 1971). This high water absorption creates a strong competition with other ingredients or constituents for water (Izydorczyk and Biliaderis 1995). The unique ability of AX to form oxidative cross-links and subsequent gel (Niño-Medina et al 2010) give AX distinct impacts on end-use quality (Bettge and Morris 2007; Ramseyer at al 2011a, 2011b). Substantial, if lesser influence on baking quality may result from non-covalent interactions between AX molecules in the formation of a gel network (Niño-Medina et al 2010). The properties of the gel network depend on the structural characteristics of the cross-linking in AX molecules (Niño-Medina et al 2010). Cross-linked AX molecules are capable of binding water, which is dependent upon the extent of cross-linking. The water-binding capacity increases with increasing cross-linkages; however, there is an optimum level of cross-linkages for highest water-binding capacity, beyond which the capacity to retain water decreases (Izydorczyk and Biliaderis 1995).

Despite a negative influence of AX on soft wheat products (Bettge and Morris 2007, Ramseyer et al 2011a, b), WEAX in particular has more beneficial influences on bread quality. While TAX has been observed to adversely affect overall dough characteristics, a higher
proportion of WEAX in TAX positively influences dough characteristics (Courtin and Delcour 2002). It has been surmised that the negative influence of TAX in bread can be attributed to the WUAX fraction, which tends to decrease bread quality (Courtin and Delcour 2002). Water-extractable AX exhibit several mechanisms of improvement in dough- and bread-properties. One critical function of WEAX is their protection of protein foams against thermal disruption (Izydorczyk et al 1992). The addition of WEAX to dough has been shown to increase the dough consistency and stiffen the dough (Jelaca and Hlynka 1972). An increased baking absorption is seen as a result of the stiffer dough, but mixing times are generally maintained or only slightly increased (Jelaca and Hlynka 1972). During baking, WEAX tends to slow the diffusion of carbon dioxide, which increases gas retention (Hoseney 1984). The dough foam is stabilized by the viscous nature of WEAX and subsequently stabilizes the liquid films around the gas cells (Gan et al 1995). It is through the stabilization of gas cells that the end of oven rise is delayed, which improves crumb homogeneity (Courtin and Delcour 2002). The high molecular weight of WEAX may lead to a secondary, weak gel network which can reinforce the gluten network. Diferulic acid bridges between WEAX molecules or with gluten proteins may increase the stabilization of the gluten network as well. The result of these complex interactions is an increased loaf volume (Courtin and Delcour 2002, Delcour et al 1991). There is an optimum level of WEAX, however, to optimize loaf volume, beyond which the dough can become too viscous and consequently decrease loaf volume (Delcour et al 1991). Also of critical importance is the character of the base flour used in the bread-making process and the molecular weight of the AX molecules (Courtin and Delcour 2002). Once bread is baked, AX has been shown to interfere with the intermolecular re-association of amylose and amylopectin, decreasing retrogradation and increasing the shelf-life of bread (Kim and D’Appolonia 1977).
In contrast to the benefits of WEAX in bread-making, WUAX molecules exhibit detrimental effects on overall bread quality. The WUAX molecules are capable of destabilizing the dough structure by forming physical barriers to the gluten network during dough development. In the fermentation stage they may form intrusions in the gas cells (Courtin and Delcour 2002). Water-unexactractable AX can perforate gas cells, increasing coalescence, and decreasing the overall gas retention (Courtin and Delcour 2002). The very high water absorption of WUAX may also outcompete the gluten network for water, further hindering gluten development and film formation. As a result of this high water absorption, WUAX molecules can lower the stability of the dough foam and eventually cause a decrease in the loaf volume (Courtin and Delcour 2002).

Despite the extensive research focused on the impacts of AX on the final bread product, much less is understood about the characteristics of AX throughout the baking process. Higher levels of WEAX have been observed in dough than in flour obtained from the same sample (Westerlund et al 1990). There was also a higher level of arabinose substitution in bread constituents as compared to flour and dough, likely the result of increased solubilization of highly substituted AX in the baking process (Westerlund et al 1990).

Throughout the bread loaf, WEAX levels were lower in the outer crust than the inner crust and crumb. This was attributed to a high level of denaturation of water-soluble proteins, decreasing the protein-extractability, and making WEAX less available for quantification (Westerlund et al 1990). Different bread fractions also exhibited differing structural characteristics. The crumb and inner crust contained higher levels of di-substituted AX as compared to flour and dough. This high level of di-substitution was attributed to increased solubilization of AX by the heating occurring during baking. This conclusion hinged on the
assumption that AX molecules which were originally insoluble had a more highly substituted structure than their counterparts which were soluble, and became more soluble or available during baking. A high level of structural variation was observed among white flour, dough, and the bread fractions, indicating a high level of structural heterogeneity throughout the baking process and ultimately the bread loaf (Westerlund et al. 1990). In contrast, D’Appolonia (1973) observed similar A/X ratios of TAX in the crumb and crust of both conventional and continuous bread. The overall conclusion from this study was that AX remained constant throughout the baking process in content, structure, and availability for extraction (D’Appolonia 1973).

The overall objective of this study was to track TAX and WEAX content through the baking process of bread. Secondary objectives were to determine differences between AX content for wholemeal and refined flour, as well as differentiating between patterns of AX content in different varieties from different market classes. In dividing bread loaves into sections and analyzing all sections, not only was the total AX content of the bread loaf quantified, but also potential migration patterns of AX could be studied.

**MATERIALS AND METHODS**

**Grain Samples**

Five varieties grown in 2009 were identified to undergo extensive studies on their arabinoxylan (AX) content throughout the baking process. The varieties were: Alpowa and Louise (soft white spring wheat), Blanca Grande and Macon (hard white spring wheat), and Westbred 926 (hard red spring wheat) (Table I). These varieties were isolated in an effort to represent several different market classes of wheat. Both wholemeal and refined flour bread loaves were made and sampled for each variety, resulting in 10 total samples of starting material.
**Bread Sample Preparation Method**

The bread dough was prepared according to the AACC-approved bread-bake testing method (AACC Method 10-10B). One subsample was taken immediately following mixing of the dough. After punching and proofing, another subsample was removed. A whole loaf was baked with no removal of dough and separated into crumb, upper crust (that section which exhibited oven spring out of the bread loaf pan), and bottom crust (that which made direct contact with the bread loaf pan). The mixed and proofed dough samples, along with the three bread loaf samples were lyophilized using a Virtis SQ Super XL-70 freeze-dryer with a 25L condenser (SP Industries, Gardiner, NY) and prepared for GC-FID analysis according to Englyst and Cummings (1984) and Courtin et al (2000). Henceforth the bread samples will be referred to as raw flour, mixed dough, proofed dough, crumb, upper crust, and bottom crust. The bread bakes occurred over the course of two days, one mixed dough and one proofed dough subsample was taken for each flour sample on four days. Four bread subsamples were obtained for each flour sample resulting in a total of twelve total bread subsamples for each flour sample (crumb, upper crust, and bottom crust).

**Arabinoyxlan Quantification**

This procedure was adapted from Englyst and Cummings (1984) and Courtin et al. (2000).

**WEAX Hydrolysis**

For WEAX quantification, between 350 and 700 mg of the sample was weighed into a centrifuge-safe 12-mL test tube, 7 mL H₂O was added, and mixed with a vortex mixer for 7 sec. The tubes were continuously shaken in a 7 °C refrigerator for one hour. The tubes were centrifuged at 1600 X g for 5 min and 0.875 mL of the supernatant was removed and placed in
another centrifuge-safe 12-mL test tube. To this mixture, 0.875 mL trifluoroacetic acid (TFA; 4 N) was added and the solution was mixed for 7 sec with a vortex mixer. The samples were incubated for 60 min at 110 °C in a Fisher Isotemp Dry Bath Model 145 heating block (Thermo Fisher Scientific, Santa Clara, CA). The block was made of anodized aluminum with 48 cylindrical wells 1.7cm in diameter and 4.4cm deep. The samples were immediately transferred into an ice bath until the test tubes were cool to the touch. The samples were then centrifuged at 1600 X g for 5 min. At this point, the WEAX and TAX samples were treated identically.

**TAX Hydrolysis**

The TAX was quantified starting with 10.5 – 35 mg of sample weighed into centrifuge-safe 12-mL test tubes. To the samples, 1.75 mL TFA (4 N) was added and mixed for 7 sec with a vortex mixer. The samples were incubated at 110 °C for 60 min and immediately following incubation, transferred into a nice bath until the test tubes were cool to the touch. The samples were centrifuged at 1600 X g for 5 min.

**WEAX and TAX Derivatization**

From the centrifuged product, 1 mL of the supernatant was removed and placed into a 12-mL test tube. An internal standard of allose (0.35 mL of a 1 mg/mL in H2O solution) was added to each test tube. The test tubes were placed in an ice bath and 0.55 mL NH3 was added to each and the test tubes were mixed with a vortex mixer for 7 sec. Each sample solution was pH tested to verify basicity. If the solution was not basic, aliquots of 100 µL NH3 were added until a pH of over 7 was achieved. Following this verification, 7 µL of 2-octanol was added to each test tube. At this point, a suspension of NaBH4 in 3 M NH3 was made (200 mg NaBH4 / mL NH3). It is critical to wait until this time period to make the suspension so that it is fresh before adding 70 µL to each sample. Once the NaBH4 suspension was added to each sample, the test tubes were
mixed with a vortex mixer for 7 sec before being incubated in a heating block at 40 °C for 30 min. Following incubation, the samples were placed in an ice bath briefly before the addition of 140 µL glacial acetic acid and mixed for 7 sec with a vortex mixer. One hundred, seventy-five microliters of this solution was transferred to a fresh 12-mL test tube. To this, 175 µL 1-methylimidazole and 1.75 mL acetic anhydride were added. After letting the solution set for 10 min, 1 mL ethanol was added and immediately mixed with a vortex mixer for 7 sec. The solution was allowed to set for 5 min before the addition of 3.5 mL H₂O and another 5 min waiting period. Following the addition of 175 µL bromophenol blue, the test tubes were transferred to an ice bath. To the solutions, two aliquots of 1.75 mL concentrated KOH were added, with a brief resting period between aliquots. The solutions undergo a phase separation following the addition of KOH, the top layer is the desired layer and appears yellow-ish in color. The bottom layer appears dark blue. Letting the solutions remain in the ice bath for a few minutes allows the phases to separate more completely for an easier extraction of the top layer. The top layer was transferred to a fresh test tube and anhydrous Na₂SO₄ was added to dry the solution. Once the water was removed from the solutions with the desiccant, the solutions were transferred to GC vials for analysis using the GC-FID.

**GC-FID Analysis**

The samples were injected into the GC into a capillary column with a split-splitless injector. The samples then entered the flame ionization detector to detect the components of the samples. The column used in the GC was a Supelco SP-2380 polar column (Sigma-Aldrich, Bellefonte, PA) in an Agilent 6890 Series Chromatograph (Agilent, Santa Clara, CA) with an autosampler and a splitter injection port (injection volume dependent upon the type of sample; split ratio 1:20). Detection with the FID was performed using a carrier gas of hydrogen. Sample
injection occurred at 210 °C, separation beginning at 237 and ending at 260 °C, and detection at 240 °C.

**Statistical Analysis**

The statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). The general analyses were done using PROC GLM. Type III mean squares were used.

**RESULTS**

**Raw Flour**

In wholemeal, the TAX and WEAX contents ranged from 4.66 to 6.42% and 0.31 to 0.58%, respectively (Table II). The refined flour TAX and WEAX ranges were 0.73-1.38% and 0.34-0.66%, respectively. In both flour types, the TAX contents and ratio of A/X in TAX did not statistically differ among varieties (data not shown). Differences did exist in the WEAX contents and A/X ratio of WEAX molecules among the five varieties. The ranking of TAX content between wholemeal and refined flour was very similar; Louise exhibited the lowest TAX content and Blanca Grande exhibited the highest TAX content in each flour type. The variety rankings of WEAX content were identical in wholemeal and refined flour, with Louise exhibiting the lowest WEAX content, and Westbred exhibiting the highest WEAX content in both flour types. Whereas the rank-order of varieties differed between TAX and WEAX content in wholemeal flour, the rank-order of varieties in refined flour was nearly identical for TAX and WEAX. Only the two highest in TAX and WEAX content (Westbred and Blanca Grande) exchanged rankings between the two types of analyses.

**Arabinoxylans**
The TAX and WEAX content as well as the A/X ratio in both fractions were analyzed for both wholemeal and refined flour (flour type), variety, and the product categories of raw flour, mixed dough, proofed dough, crumb, upper crust, and bottom crust (Table III). The models of TAX, WEAX, and the A/X ratio in both TAX and WEAX were significant and the variation was well-modeled by the ANOVA with $R^2$ values ranging from 0.79 to 0.98. Differences existed between wholemeal and refined flour in the TAX content as well as the A/X ratio calculated in TAX and WEAX. The mean TAX content for wholemeal flour was 4.88%, as compared to 1.28% in refined flour samples. Conversely, the A/X ratio in TAX was 0.74 in refined flour, and 0.65 in wholemeal flour. Refined flour also exhibited a higher A/X ratio in WEAX as compared to wholemeal flour (0.67 vs. 0.63, respectively). There was no observed difference in WEAX content between the two flour types. Varieties exhibited varying levels of TAX, WEAX, and the A/X ratio in both TAX and WEAX fractions.

An interaction occurred between variety and flour type for all measured parameters. The TAX rankings of refined flour products and wholemeal products differed greatly, with the only consistency being Louise, exhibiting the lowest TAX level in both refined flour and wholemeal. There were no similarities in variety rankings across wholemeal and refined flour for the A/X ratio of TAX. In a similar manner to TAX, the only similarity in varietal rankings between wholemeal and refined flour WEAX measurements was the consistently low response of Louise. The A/X ratio of WEAX exhibited slightly more consistent results, with Macon and Blanca Grande exhibiting the highest and lowest ratios, respectively, in both wholemeal and refined flour samples.

Product types differed in the four measured responses of TAX, WEAX, and the corresponding A/X ratios. The upper crust samples exhibited higher levels of TAX than did raw
flour, with crumb, bottom crust, proofed dough, and mixed dough in rank order. Mixed dough exhibited substantially lower TAX content than the other product types; the remaining fractions could not be statistically separated. The raw flour exhibited the lowest A/X ratio in TAX (0.63), but the rest of the products could not be separated by means, which ranged from 0.69 to 0.71. The WEAX content varied greatly across varieties, with raw flour having the highest content (0.48%) and the upper crust with the lowest content (0.12%). The baked products themselves exhibited nearly a two-fold range in content (0.12-0.22%, upper crust and proofed dough, respectively). The raw flour exhibited the highest A/X ratio in WEAX (0.67) and proofed dough the lowest (0.63). Despite the small range of responses, mean separation was possible between raw flour and crumb, vs. upper crust and proofed dough. The bottom crust and mixed dough fell in the middle of the range, and could not be statistically distinguished from the remaining product types.

Interactions occurred between variety and product, and among the three categories of flour type, variety, and product. These interactions can best be addressed by examination of the relationships of AX as presented in Table IV. It must be noted that changes in AX content reflect changes in AX molecule availability; AX molecules can neither be created nor destroyed without the addition of enzymes. In wholemeal flour, there was a decrease in TAX content from raw flour to mixed dough in all varieties except Westbred, which exhibited a slight increase in TAX levels. All varieties exhibited an increase in TAX content from mixed dough to proofed dough with the exception of Blanca Grande, in which the levels of TAX decreased slightly. In Alpowa and Blanca Grande, TAX levels increased again between proofed dough all of the baked bread products. Macon and Westbred had lower levels of TAX in bread products as compared to proofed dough. Louise did not exhibit a clear pattern in TAX availability between proofed dough
and baked bread products. No singular baked bread product consistently exhibited outstanding TAX levels (high or low). No patterns could be discerned in the A/X ratio in TAX and WEAX throughout the baking process. While the WEAX content decreased upon beginning the baking process starting with mixed dough, few clear patterns emerged across the varieties. Upper crust, however, did tend to exhibit the lowest levels of WEAX, and crumb had notably higher levels of WEAX content.

The TAX content exhibited a very different pattern throughout baking in refined flour as compared to wholemeal flour. In all varieties except Blanca Grande, the TAX content increased between raw flour and mixed dough. Both Alpowa and Blanca Grande experienced a decrease in TAX content between mixed dough and proofed dough, whereas the three remaining varieties exhibited an increase in TAX content from mixed dough to proofed dough. With the exception of Louise, there was an observed increase in TAX content from proofed dough to the baked bread products, although Macon exhibited a notably low TAX level in the bottom crust sample. Similar to wholemeal flour, there was no consistency across varieties for the baked bread fraction with a very high or low TAX level. The A/X ratio in TAX exhibited no clear patterns, but tended to be higher than the same ratio as observed in wholemeal flour. As with the A/X ratio in wholemeal WEAX, there were no consistent patterns of behavior across products. The WEAX levels in refined flour were similar to wholemeal flour in that they decreased from raw flour to mixed dough, but other patterns were not discernible. Upper crust, however, tended to have low WEAX levels in comparison to the other products, as was observed in wholemeal flour.

**Bread Quality**
Wholemeal flour had a smaller loaf volume for each variety in comparison to those loaf volumes of refined flour samples (Table V). In wholemeal flour, Macon had the greatest loaf volume (760cm³) and Louise the lowest (620cm³). The three hard wheat varieties had higher loaf volumes than the soft wheat varieties (716 vs. 630cm³). In refined flour, Blanca Grande had the highest loaf volume (1159cm³) and Louise had the lowest (790cm³). It is notable that the lowest loaf volume observed in refined flour was 30cm³ larger than the greatest loaf volume in wholemeal flour. The refined flour soft wheat varieties had an average loaf volume of 830cm³ as compared to the mean loaf volume in hard wheat, 1109cm³.

Because of the differences between flour types in the measurements of loaf volume and AX content, the flour types were separated for correlation analysis. Upon evaluation of the correlation analyses, the three baked bread fractions exhibited highly redundant correlation values to the mixed dough and proofed dough, thus; only the raw flour, mixed dough, and proofed dough are examined in Table VI. Loaf volume only exhibited two notable correlations with those parameters presented: raw flour WEAX (0.48) and mixed dough TAX (0.50). There was also a high correlation between loaf volume and upper crust A/X ratio in WEAX (0.74, data not shown); however, this appeared to be an anomaly. The TAX content in raw flour exhibited positive relationships with raw flour WEAX (0.71) and mixed dough TAX (0.55), but strong, negative correlations with the A/X ratio of raw flour TAX (-0.61) and WEAX (-0.88), and the A/X ratio in the WEAX of mixed dough (-0.82) and proofed dough (-0.62). The A/X ratio in raw flour TAX was positively correlated with other A/X ratios in both TAX and WEAX content.

Notably, the raw flour WEAX exhibited a number of very high correlations. This WEAX content was most closely related to mixed dough TAX (0.91), proofed dough TAX (0.78), and proofed dough WEAX (0.92). Mixed dough TAX exhibited strong, positive correlations with mixed
dough WEAX (0.77), proofed dough TAX (0.77) and proofed dough WEAX (0.86), although a negative relationship to the A/X ratio in proofed dough TAX (-0.57). Mixed dough WEAX similarly had notable correlations with proofed dough TAX (0.84), proofed dough WEAX (0.97), and the A/X ratio of proofed dough TAX (-0.77). The A/X ratio in the WEAX content of mixed dough and proofed dough were highly correlated (0.73). Both raw flour and proofed dough TAX exhibited negative relationships with their corresponding A/X ratios (-0.61 and -0.65, respectively), while mixed dough exhibited no such relationship. Notably, the A/X ratio in proofed dough TAX was highly, negatively correlated with proofed dough WEAX content (-0.84), which was not similarly observed in raw flour or mixed dough.

Refined flour exhibited many more high correlations across the variables surveyed (Table VII). Loaf volume, in particular, was highly correlated with raw flour TAX (0.84), and the WEAX content in raw flour (0.87), mixed dough (0.91), and proofed dough (0.90). Negative correlations existed between loaf volume and the A/X ratio of: raw flour WEAX (-0.47), mixed dough TAX (-0.76), and proofed dough TAX (-0.70). Raw flour TAX content exhibited high levels of correlation with all measured parameters, in particular, strong negative correlations with the A/X ratios in both TAX and WEAX of raw flour, mixed dough, and proofed dough. Raw flour WEAX was negatively correlated with mixed dough TAX (-0.66) and the A/X ratio of TAX in mixed dough and proofed dough (-0.84 and -0.70), but positively related to mixed dough and proofed dough WEAX (0.82 and 0.83). The A/X ratio of WEAX in raw flour was highly correlated with all other measured parameters, most notably to the TAX content in proofed dough (0.95) and the A/X ratio of TAX and WEAX in proofed dough (0.95 and 0.86). The A/X ratio observed in TAX and WEAX of mixed dough exhibited a number of high correlations,
particularly between the A/X ratio of TAX in mixed and proofed dough (0.94). Mixed dough WEAX and proofed dough WEAX had the highest correlation of all measured parameters (0.98).

DISCUSSION

Raw Flour

The hard wheat varieties (Macon, Westbred, and Blanca Grande) exhibited slightly higher TAX levels, on average, than the soft wheat varieties (Louise and Alpowa). Macon, however, did exhibit a wholemeal TAX level negligibly lower than Alpowa, and only a slightly higher refined flour TAX level than did Alpowa. Based on the similar rankings and TAX in wholemeal and refined flour, no clear separation between hard and soft wheat was possible on the basis of TAX content. As such, the corresponding results in the baking portion of the study appear to be a fair examination and comparison of performance.

There was a clearer separation between hard and soft wheat varieties on the basis of WEAX content. The hard wheat varieties, Macon, Blanca Grande, and Westbred, the WEAX levels were notably higher than in soft wheats (Alpowa and Louise) in both wholemeal and refined flour. No notable patterns emerged in the A/X ratios of TAX and WEAX in either flour type, suggesting that these ratios are unrelated to market class.

Arabinoxylans

The inclusion of the bran fraction in wholemeal flour potentially contributed to the higher TAX levels in wholemeal flour as compared to refined flour, consistent with the known understanding of AX heterogeneity throughout the wheat kernel (Philippe et al 2006). The higher levels of WEAX in refined flour, however, suggest that the water-holding capacity of WEAX
through oxidative cross-linking may have a stronger influence on soft wheat products made from refined flour, as opposed to wholemeal flour. The similarity of A/X ratios between wholemeal and refined flour indicates similar structural properties AX of both types of flour.

The upper crust bread fraction contained the highest levels of TAX as compared to raw flour, mixed and proofed dough, and the crumb and bottom crust of bread. Because AX can neither be created nor hydrolyzed during the mixing and baking process, it is likely that the AX molecules became more available for extraction and quantification in the upper crust. The upper crust of bread is exposed to the highest levels of heat during the baking process, which likely allows more AX molecules to become available for quantification than in raw flour and other products. The low levels of WEAX observed in the upper crust suggest that the WEAX molecules are involved in oxidative cross-linking, and the high levels of TAX more accurately reflect high levels of WUAX in the upper crust. The mixed dough exhibited markedly lower TAX levels, which could be the result of formation of oxidative cross-linkages, rendering AX molecules unavailable for extraction.

The decrease in TAX content from raw flour to mixed dough in wholemeal flour strengthens the suggestion of formation of oxidative cross-linkages once raw flour is combined with water. Because oxidative cross-linkages can occur between two AX molecules, or between AX and the tyrosine residues of proteins, there is likely an interaction of AX and gluten proteins occurring. This interaction appears to vary among varieties, as they tended to exhibit differing patterns of TAX levels throughout the baking process. These differences are likely due to structural differences among AX molecules in different varieties. The behavior of AX molecules is based on a number of factors, including the A/X ratio, length of the xylan backbone, pattern of arabinose substitution, and ferulic acid content (Courtin and Delcour 2002). It is the combination
of these factors which determines the molecular interactions occurring among AX molecules and
the gluten proteins. Because varieties are known to have heterogeneous AX characteristics, the
subsequent molecular interactions would also logically differ. Wholemeal flour includes the bran
as well, which has a very different AX profile than what is observed in the endosperm only
(refined flour).

The TAX content of refined flour was observed to increase between raw flour and mixed
dough, indicating that the addition of water in some way allowed the AX molecules to become
more available for extraction and quantification, consistent with the observations of Westerlund
et al. (1990). Two varieties exhibited a decreased in TAX between mixed dough and proofed
dough, while three had an increased TAX level in proofed dough as compared to mixed dough.
These differences are likely the result of AX heterogeneity among varieties, consequently
influencing the way the AX molecules interact with water, protein, and other AX molecules. The
increase in TAX content from proofed dough to the baked bread products was likely the result of
TAX molecules becoming more available for extraction through high levels of heat treatment.
Because of the varying nature of the AX molecules among varieties, there was no consistency in
TAX levels throughout the baked products across varieties. The A/X ratios of TAX and WEAX
did not provide additional information about the molecular interactions occurring throughout the
baking process in refined flour. Similar to wholemeal flour, the upper crust of refined flour
exhibited the lowest levels of WEAX of any bread product. This observation strengthens the
suggestion that the WEAX molecules are likely tied up in oxidative cross-linkages, and therefore
unavailable for extraction, and that the high levels of TAX in the upper crust are the result of
WUAX availability due to excessive heat.
Bread Quality

The hard wheat varieties had larger loaf volumes across both flour types than did soft wheat varieties. Hard wheats typically contain higher levels of protein and possess greater gluten strength, which are necessary for optimum loaf volume. Soft wheats are generally used for cookies, cakes, pancakes, pastries, crackers, and other products that do not necessitate such high levels of gluten development. As such, the five varieties performed as expected in the assessment of loaf volume based on their market class. The wholemeal flour samples also exhibited smaller loaf volumes than did refined flour samples.

In wholemeal flour, there were few notable correlations to loaf volume. The raw flour WEAX content and mixed dough TAX both exhibited moderate levels of correlation with loaf volume. This relationship, coupled with the high level of correlations among raw flour WEAX, mixed dough TAX, proofed dough TAX, and proofed dough WEAX indicates that immediately upon the addition of water, the WUAX molecules become tied up in oxidative cross-linkages and leave only WEAX molecules available for extraction. The mixed dough TAX was also highly correlated to mixed dough WEAX, proofed dough TAX, and proofed dough WEAX, suggesting that the majority of what was quantified as TAX in mixed dough was WEAX, as opposed to a more even split between WEAX and WUAX. It is these WEAX molecules which may, in fact stabilize protein films and enhance loaf volume, as suggested by Gan et al. (1995). Because the WEAX level decreased throughout the baking process, it can be surmised that while they do not form oxidative cross-linkages immediately upon mixing, they do interact with other AX molecules and proteins with the addition of heat. This proposal is strengthened by the particularly low WEAX levels in the upper crust, which is exposed to the highest levels of heat, and therefore may encourage more WEAX inter-molecular interactions.
The high correlation between the A/X ratio in upper crust WEAX and loaf volume, coupled with the low WEAX content of the upper crust suggests that the majority of those WEAX molecules involved in oxidative cross-linking are less highly substituted, leaving only those WEAX molecules that are very highly substituted (0.63 A/X) available for extraction. This observation suggests some structural-functional relationships of the WEAX molecules, in that the less highly substituted WEAX molecules are more likely to participate readily in oxidative cross-linking, whereas the most highly substituted WEAX molecules may have too much stearic hindrance to fully participate in the oxidative cross-linking process, either with other AX molecules, or the tyrosine residues of proteins. Therefore, the less highly substituted WEAX molecules may offer the greatest benefit to loaf volume development by interacting with gluten proteins and helping to enhance the protein foam stability, whereas the highly substituted WEAX molecules may not play a substantial role in loaf volume development. This relationship between upper crust A/X ratio of WEAX and loaf volume was only observed in wholemeal flour, suggesting that the bran layer is mostly responsible for this wide range of WEAX substitution level. The endosperm likely has a more consistent, less varied A/X substitution level in WEAX molecules.

The highly negative correlation between the A/X ratio of TAX in proofed dough and the A/X ratio of WEAX in proofed dough suggests that those WEAX molecules included in the TAX content quantification of proofed dough are considerably less substituted than the WUAX molecules also being quantified as part of the TAX evaluation. The WUAX molecules being evaluated would necessarily have high levels of substitution if the WEAX being quantified had very low levels of substitution at this phase of dough development. This observation also suggests that if the WUAX molecules are the first to react and form cross-linkages, it may be the
less-highly substituted WUAX molecules doing so, leaving the very highly substituted molecules available for extraction as part of the TAX quantification. This is consistent with the pattern observed in the upper crust WEAX molecules; those most highly substituted molecules exhibit too much stearic hindrance to effectively form cross-linkages, and therefore remain available for quantification.

Refined flour exhibited many more correlations with loaf volume than did wholemeal flour. In particular, the TAX content of raw flour and WEAX content in raw flour, mixed dough, and proofed dough exhibited high correlations with loaf volume. The negative relationship between loaf volume and the A/X ratio of raw flour WEAX, mixed dough TAX, and proofed dough TAX suggests that in a similar manner as wholemeal flour, the most highly substituted AX molecules are unable to form oxidative cross-linkages and therefore do not contribute to gluten development and improve the loaf volume of bread. The increase in A/X ratio of TAX between raw flour and mixed dough strengthens this suggestion, indicating that the less-substituted molecules are reacting first, and therefore become unavailable for reaction, causing the average A/X ratio of the mixed dough TAX to be higher. The high correlation of the A/X ratio of raw flour WEAX and the TAX content in proofed dough suggests that the less-substituted molecules are involved in oxidative cross-linkages and are unavailable for extraction, leaving the most highly substituted WEAX molecules to be quantified following extraction. A similar phenomenon was observed in the A/X ratio of TAX in mixed and proofed dough. There was no observed relationship between the upper crust WEAX content or A/X ratio in refined flour. Because the endosperm has less heterogeneous AX structures than what are observed throughout the whole wheat kernel, it is likely that the difference in substitution levels are not substantial enough to create as clear of separations in cross-linking behavior of high- and low-
substituted AX molecules. With a more consistent population of AX molecules, they would likely react more similarly and not exhibit such contrasting behavior as what was observed in wholemeal flour.

The high correlation between mixed dough WEAX and proofed dough WEAX suggests that few changes are occurring during this stage of dough development on a molecular AX level. It appears as if the majority of the oxidative cross-linking occurs during the initial mixing stage of baking, but during proofing, no further reactions take place. The changes in WEAX content observed between proofed dough and the baked bread products suggest that it is only through the addition of heat that further molecular interactions can take place. Similar structural heterogeneity throughout the baking process was observed by Westerlund et al. (1990). Across the five varieties, the trend was toward decreasing levels of WEAX as the baking process continued from proofed dough through the baked bread product. This suggests that the heat treatment encouraged more WEAX molecules to participate in oxidative cross-linking, possibly working to enhance gluten development. Blanca Grande, which had the highest loaf volume, experienced the most dramatic decrease in WEAX content from proofed dough to the baked bread products, suggesting that more WEAX molecules were able to interact with each other and proteins to enhance gluten development and increase loaf volume than in any other variety. A similar phenomenon occurred in Macon and Westbred, which had the next highest loaf volumes of the five varieties. The WEAX levels dropped dramatically in these varieties, though to a lesser extent than what was observed in Blanca Grande. It is likely that these WEAX molecules that became unavailable for extraction were in fact involved in oxidative cross-linking, potentially stabilizing the protein foams of the gluten proteins, increasing the loaf volume.
CONCLUSION

Hard wheat varieties were best-suited to bread-making as compared to soft wheat varieties. In both refined flour and wholemeal flour, the level of WEAX appeared to be crucial in determining eventual loaf volume. Along with WEAX content, the level of substitution in WEAX also heavily influenced the loaf volume. Lower levels of arabinose substitution appeared to be preferable for their lack of steric hindrance, and consequently, better availability to form oxidative cross-linkages and enhance loaf volume. The same relationship was evident in the A/X ratio of WUAX molecules, which appeared to be the first molecules to form oxidative cross-linkages in the mixed and proofed dough stages. Refined flour and wholemeal flour AX molecules exhibited vastly different relationships throughout the baking process, likely the result of increased structural heterogeneity contributed by the bran in wholemeal flour. This high level of structural heterogeneity further emphasized the beneficial influence of low substitution levels in WEAX molecules in particular, in aiding loaf volume. The addition of heat to a dough also played a large role in allowing WEAX molecules to become more reactive in oxidative cross-linkages, but also making TAX molecules potentially more available for extraction and quantification, particularly in refined flour. Overall, it is critical to understand the relationships between TAX, WEAX, and WUAX, as well as the corresponding levels of arabinose substitution and structural heterogeneity in all three classifications of AX molecules when assessing their influence on bread quality. More work is necessary to further understand the complexities of the role that oxidative cross-linking plays throughout the baking process and the influence of heat on these intermolecular interactions.
ACKNOWLEDGMENTS

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LITERATURE CITED


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Characterization using Raman microspectroscopy of arabinoxylans in the walls of different cell types during the development of wheat endosperm. J. Agric. Food Chem. 54:5113-5119.


### TABLE I.

*Variety and market class of the five wheat samples used for baking and analysis*

<table>
<thead>
<tr>
<th>Variety</th>
<th>Market Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpowa</td>
<td>Soft white spring</td>
</tr>
<tr>
<td>Blanca Grande</td>
<td>Hard white spring</td>
</tr>
<tr>
<td>Louise</td>
<td>Soft white spring</td>
</tr>
<tr>
<td>Macon</td>
<td>Hard white spring</td>
</tr>
<tr>
<td>Westbred</td>
<td>Hard red spring</td>
</tr>
</tbody>
</table>
TABLE II.

Arabinoxylan content of the five varieties surveyed in wholemeal and refined flour forms

<table>
<thead>
<tr>
<th>Variety</th>
<th>Wholemeal</th>
<th>Refined Flour</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAX&lt;sup&gt;a&lt;/sup&gt; A/X&lt;sup&gt;b&lt;/sup&gt; WE&lt;sup&gt;c&lt;/sup&gt; A/X&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>TAX&lt;sup&gt;a&lt;/sup&gt; A/X&lt;sup&gt;b&lt;/sup&gt; WE&lt;sup&gt;c&lt;/sup&gt; A/X&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alpowa</td>
<td>4.99 0.64 0.39 0.66</td>
<td></td>
<td>1.03 0.58 0.40 0.66</td>
</tr>
<tr>
<td>Blanca Grande</td>
<td>6.42 0.63 0.53 0.52</td>
<td></td>
<td>1.38 0.64 0.58 0.62</td>
</tr>
<tr>
<td>Louise</td>
<td>4.66 0.70 0.31 0.75</td>
<td></td>
<td>0.73 0.60 0.34 0.77</td>
</tr>
<tr>
<td>Macon</td>
<td>4.96 0.71 0.44 0.78</td>
<td></td>
<td>1.05 0.55 0.55 0.75</td>
</tr>
<tr>
<td>Westbred</td>
<td>5.39 0.70 0.58 0.58</td>
<td></td>
<td>1.27 0.62 0.66 0.66</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent total arabinoxylan content  
<sup>b</sup>Ratio of arabinose:xylose  
<sup>c</sup>Percent water-extractable arabinoxylan content
TABLE III.

Arabinoxylan content ANOVA, whole model $R^2$, including flour type, variety, and product (raw flour, mixed dough, proofed dough, bread crumb, bread upper crust, and bread bottom crust)

<table>
<thead>
<tr>
<th>Source</th>
<th>TAX$^a$</th>
<th>A/X$^b$</th>
<th>WE$^c$</th>
<th>A/X$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Model $R^2$</td>
<td>0.97</td>
<td>0.85</td>
<td>0.98</td>
<td>0.79</td>
</tr>
<tr>
<td>Whole Model F value</td>
<td>40.67***</td>
<td>7.29***</td>
<td>65.97***</td>
<td>5.17***</td>
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<tr>
<td>Flour Type F value</td>
<td>1002.7***</td>
<td>23.19*</td>
<td>6.54</td>
<td>36.04**</td>
</tr>
<tr>
<td>Variety F value</td>
<td>35.85***</td>
<td>23.50***</td>
<td>79.72***</td>
<td>34.83***</td>
</tr>
<tr>
<td>Flour Type*Variety F value</td>
<td>21.82***</td>
<td>7.42***</td>
<td>20.79***</td>
<td>8.32***</td>
</tr>
<tr>
<td>Product F value</td>
<td>4.38**</td>
<td>3.86**</td>
<td>680.71***</td>
<td>3.00*</td>
</tr>
<tr>
<td>Flour Type*Product F value</td>
<td>3.72**</td>
<td>12.06***</td>
<td>20.37***</td>
<td>0.66</td>
</tr>
<tr>
<td>Variety*Product F value</td>
<td>1.85*</td>
<td>3.82***</td>
<td>26.19***</td>
<td>1.79*</td>
</tr>
<tr>
<td>Flour Type<em>Variety</em>Product F value</td>
<td>1.69*</td>
<td>4.06***</td>
<td>8.65***</td>
<td>3.99***</td>
</tr>
</tbody>
</table>

$^a$Percent total arabinoxylan content  
$^b$Ratio of arabinose:xylose  
$^c$Percent water-extractable arabinoxylan content  
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
<table>
<thead>
<tr>
<th>Variety</th>
<th>Wholemeal</th>
<th></th>
<th></th>
<th>Refined Flour</th>
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<th></th>
</tr>
</thead>
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Different letters denote statistical differences at $P \leq 0.05$
TABLE VI.
Correlations Between Selected Parameters in Wholemeal Flour

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<th>Raw TA/X&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Raw WEAX&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Raw WA/X&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Mixed Dough (MD) TAX</th>
<th>Mixed Dough (MD) TA/X</th>
<th>Mixed Dough (MD) WEAX</th>
<th>Mixed Dough (MD) WA/X</th>
<th>Proofed Dough (PD) TAX</th>
<th>Proofed Dough (PD) TA/X</th>
<th>Proofed Dough (PD) WEAX</th>
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"Total arabinoxylan content
Arabinose:xylose ratio of total arabinoxylan content
Water-extractable arabinoxylan content
Arabinose:xylose ratio of water-extractable arabinoxylan content
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$
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<th>Proo</th>
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* * * denotes significance levels.
\(^a\)Total arabinoxylan content
\(^b\)Arabinose:xylose ratio of total arabinoxylan content
\(^c\)Water-extractable arabinoxylan content
\(^d\)Arabinose:xylose ratio of water-extractable arabinoxylan content

\* \( P \leq 0.05 \), \** \( P \leq 0.01 \), \*** \( P < 0.0001 \)