FACTORS IN HARD WINTER WHEAT AFFECTING WATER ABSORPTION TOLERANCE


by


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B.S., Kansas State University, 2011


A THESIS


submitted in partial fulfillment of the requirements for the degree


MASTER OF SCIENCE


Department of Grain Science
College of Agriculture


KANSAS STATE UNIVERSITY
Manhattan, Kansas


2013

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# Abstract

Hard winter wheat flour is predominantly used for bread production.  Optimal dough handling properties are critical in commercial bread production.  Variation in flour water absorption tolerance dramatically affects dough handling.  Understanding the factors which affect water absorption tolerance and the influences of genotype, environment, and their interaction on those factors will improve breeding efforts and production practices to improve the quality of bread wheat. A previous study found the γ-gliadin protein fraction correlated highly with water absorption tolerance.  The objectives of this study were to confirm the effect of γ-gliadin and investigate the effects of genetics and environment on water absorption tolerance. Nineteen hard winter wheat cultivars consisting of released varieties and experimental breeding lines from the Pacific Northwest grown in 2011 in 2 locations in Oregon (Pendleton and Arlington) were obtained. Quality evaluations including flour yield, test weight, kernel weight, kernel diameter, and kernel hardness were conducted on the wheat kernels and water absorption tolerance, protein content, moisture content, ash content, Solvent Retention Capacity (SRC), starch damage, and protein composition analyses were preformed on the resulting flours.  The mixograph water absorption tolerance behavior of each sample was ranked as high, medium or low. Farnum and OR2080156H grown at Arlington had the highest water absorption tolerance range (1.6mL) while Farnum, Eddy, Paladin and OR2080227H grown at Pendleton exhibited the lowest interval (0.4mL). No wheat or flour parameters measured showed high correlation with water absorption tolerance.  Moderate correlation was observed between water absorption tolerance and kernel weight ($r = 0.39$), kernel diameter ($r = 0.37$), starch damage ($r = 0.33$), and the extractable and unextractable polymeric protein fractions ($r = 0.32$).

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# Acknowledgements

I appreciate the fully funded Grain Science Departmental offer to continue research on water absorption tolerance started by Gabriela Rattin under the direction of Dr. Miller and Dr. Faubion. I would like to recognize all the help Gabriela provided in the form of procedures, correspondence, and data throughout the research process and collaboration. I would like to thank my major professor, Dr. Rebecca Miller, for academic, professional, and experimental guidance, encouragement, and support. I appreciate the time and effort expended to help me learn and develop into a capable young professional scientist. Dr. Jon Faubion, thank you for peaking my interest in Grain Science with your intriguing lectures, encouraging me to continue my education in the field by entering graduate school, and serving on my masters committee. Thank you Dr. Michael Tilley for serving on my masters committee, giving experimental advice, and offering the opportunity to work at the USDA Center for Grain and Animal Health. I would also like to thank Sushma Prakash for patiently teaching me the protein extraction and HPLC analysis procedure. I am grateful to Zhining Ou for statistical analysis assistance and aid with understanding the statistical output. Thank you to Mary Knapp for finding reliable climate data pertinent to the growing sites. I would like to acknowledge the Wheat Quality Lab student workers for assistance with ash analysis.

Thank you Dr. Aramouni for generously providing many opportunities to learn through hands-on experience and attending industry conferences during my undergraduate degree and your friendship throughout my masters. I would like to acknowledge the friendship of fellow graduate students for which I am thankful. Finally, I would like to thank my family for their encouragement throughout my education and loving support.

# Chapter 1 - Introduction

Hard winter wheat is the predominant wheat of choice for bread production; however, not all varieties create dough with optimum handling properties (Ross and Bettge 2009). Water absorption is one property of flour that greatly affects dough machinability. Flour with a low water absorption tolerance becomes sticky, slack, and difficult to process in a commercial bakery if the water absorption is slightly off target (too high or too low) while flours possessing high water absorption tolerance are more robust, thus are capable of producing a machinable dough. Substantial changes in the mixing curves and dough handling properties occur with flours possessing a low water absorption tolerance (1 or 2%, flour basis, change in water level). Flours with high water absorption tolerance show minor changes in the mixing curve and dough handling characteristics over a wider range of water application levels. Bread produced from wet or dry dough is inferior in volume and texture to bread produced with optimum water absorption.

Typically commercial bakeries set flour specifications to regulate dough consistency instead of running mixing tests for each lot of flour; therefore, consistent flour quality between lot changeovers is important to bakers. Since commercial bakery production line equipment is set to produce large volumes of product at a high production rate, there is no time allowance for process modification and often the product simply cannot be salvaged once outside of desired parameters for the processing line (i.e. too weak or too strong). This results in large quantities of lost time and lost product. By using flours with a high water absorption tolerance, more stable dough handling properties could be introduced to today's highly automated bakeries and waste could be reduced. Such robust wheat varieties would be of great interest to wheat breeders and the commercial baking industry because final product quality would not be affected by small variations in water level addition.

Many studies have examined the effect of flour components on optimum water absorption level and mixing time but no published research has defined water absorption tolerance, determined the factors affecting the phenomenon or related the trend with bread quality in HWW varieties. Previous, thesis work conducted by Gabriela Rattin (2011) using five HRW wheat varieties grown in six Kansas locations showed the γ-gliadins, from the extractable polymeric protein fraction, to be significantly correlated with water absorption tolerance.

# Objectives

The purpose of this study was to examine the physiochemical flour constituents of 20 hard winter Pacific Northwest wheat varieties grown in 2 locations in Oregon to determine the roles that environment (growing conditions) and genetics (variety) have on water absorption tolerance.

The objectives of this study were:

1. Determine genetic vs environmental effect on water absorption tolerance.
2. Verify the correlation between extractable polymeric protein and water absorption tolerance.
3. Analyze and identify the extractable polymeric protein bands.

# Chapter 2 - Literature Review

## 2.1 – Wheat Kernel and Flour Characteristics

### 2.1.1 Wheat Kernel Composition

The wheat (*Triticum aestivum*) kernel is a single seeded caryopsis with rounded dorsal side and longitudinal ventral-side crease (Delcour and Hoseney 2010). Wheat is classified as red or white depending on the intensity of red pigmentation found in the seed coat, hard or soft, and Spring or Winter (Gooding 2009). The grain consists of 10.4-20.0% bran, 74.9-86.5% endosperm, and 0.99-3.8% embryo (germ) (MacMasters et al 1971). The outer protective pericarp layer encompasses the entire grain and is easily removed; while, the inner pericarp layer contains intermediate cells, densely fit cross-cells circling the kernel, and tube cells aligning parallel to the vertical axis (Autio and Salmenkallio-Marttila 2003). The three-layer seed coat is firmly attached to a tube cell layer followed by the nucellar epidermis layer and the single cell thick aleurone layer covering the starchy endosperm and germ (Delcour and Hoseney 2010). Pericarp, seed coat, nucellar epidermis, and aleurone layers are removed by the shearing action of milling and constitute bran, a by-product high in cellulose, hemicellulose, and ash (Eliasson and Larsson 1993). The germ is high in protein and lipid content and comprised of the embryonic axis with rudimentary root, shoot and the scutellum, a storage organ (Delcour and Hoseney 2010). With particle size reduction, the endosperm starch-protein matrix becomes flour. During synthesis, glutenin, gliadin, and globulins are deposited in protein bodies (Bechtel et al 2009). As large lenticular starch granules fill out, the protein bodies are compressed into a continuous network and small spherical starch granules form in remaining voids creating a densely packed mature endosperm. Puroindoline proteins are correlated with soft endosperms (Greenwell and Schofield 1986) and interrupt the starch-protein network interface by binding to the starch granules surface (Dubreil et al 1998).

### 2.1.2 Wheat Flour

Due to the unique viscoelastic capability of dough made from wheat flour, it is used for a wide range of products including bread, cakes, cookies, doughnuts, bagels, pizza, flat breads, pasta, noodles, and chapatti (Wrigley 2009). Kernel hardness effects milling capacity, flour yield, starch damage, particle size, and determines flour application (Eliasson and Larsson 1993).

The primary goal of milling is to produce the highest yield of flour possible from the endosperm without excessive contamination by bran or germ. A 70% flour extraction rate typically contains 0.41-0.43% ash, 1.1-1.2% lipids, 8-12% protein, 71-75% starch and trace amounts of fiber on a 14% moisture basis. As the extraction rate increases baking performance deteriorates because more lipids, ash, and fiber originating in the germ and bran are included (Morrison 1989).

Starch is the main constituent of flour (74-90%, dry basis) and has many roles in dough development and baking (Eliasson and Larsson 1993). Starch provides fermentable carbohydrate for yeast fermentation and fills interstitial space between the gluten gel and gaseous cells in dough. During gelatinization in baking; the starch structure swells with water absorption and leaches amylose to form an amorphous gel. During retrogradation, starch recrystallizes and the gel network rigidifies with time leading to bread staling (Wong 1982; Atwell et al 1988). Starch damage occurs during milling and is an important factor to regulate. Excessive starch damage levels increase water absorption four fold higher than the normal level (Stauffer 2009), which results in sticky dough and causes a poor bread loaf volume (Eliasson and Larsson 1993). Damaged starch is also susceptible to cleaving by alpha amylase during proofing and fermentation, which causes significant rheological changes (Stauffer 2009).

Wheat storage proteins found in the endosperm, and subsequently flour, include: albumins, globulins, gliadins, and glutenins (Eliasson and Larsson 1993). The large polymer viscoelastic gluten network that forms during mixing is composed of gliadins and glutenins.

Lipids constitute approximately 2.5% (w/w) of flour with 1% being non-polar lipids (monoglycerides, diglycerides, free fatty acids and sterol esters) and 1.5% polar lipids (galactosyl glycerides 0.6% and phospholipids 0.9%) constituting the remaining portion (Stauffer 2009). McCormack et al (1991) revealed lipids do not affect dough rheology or mixing behavior as shown by identical mixographs of defatted and original flours; however, lipids do complex with gluten proteins during mixing (Eliasson and Larsson 1993, Stauffer 2009). Through acetic acid extraction, McCann et al (2009) found glycolipids interacted with glutenins by hydrophobic forces and hydrogen bonding; whereas, phospholipids interacted with gliadins and lipid binding proteins. Thus, Pareyt et al (2011) theorized the polar lipids bound with gluten serve as a stabilizer at the gas cell interface to aid gas retention throughout dough processing.

Non-starch polysaccharides (cellulose, β-glucans, and pentosans) make up about 2-2.5% of flour. Pentosans are the main component present (1.27-2.33%) (Michniewicz et al 1990).

Pentosans are water-soluble (65%) or water-insoluble (35%). Pentosans increase viscosity by absorbing several times their weight in water and forming bonds with gluten proteins (Stauffer 2009).

Ash is the mineral content left behind after flour incineration (AACC Method 8-02) and can be used as a measurement of flour refinement (Carson and Edwards 2009).

## 2.2 Wheat Protein Characterization

According to a method developed by Osborne (1907) wheat proteins are classified into four groups based on solubility in different solutions: albumins in distilled water, globulins in dilute salt solutions, prolamins in 70% aqueous ethanol, and glutelins in dilute acid. However, due to the nature of solubility-based separation being non-discrete, fractions with components from more than one protein class result and the class system based on molecular weight was developed (Singh *et al* 1990). Proteins greater than 100 kDa are classified as high molecular weight glutenin (HMW-glu), those between 25-100 kDa are gliadin or low molecular weight glutenin (LMW-glu), and those with molecular weights less than 25 kDa are albumins and globulins (Bushuk and Wrigley 1971). Currently, both gliadins and glutenins are considered to be within the prolamin group due to a high concentration of proline and glutamine and being at least partially soluble in alcohol-water mixtures. The gluten protein components are classified as high or low molecular weight subunits of glutenin, sulfur poor (ω- gliadins), or sulfur rich (α-, β-, γ-gliadins, and LMW-glu) based on aggregative nature and sulfur content (Shewry et al 1986). Hydrated gliadins and glutenins impart plasticity and elasticity, respectively, to wheat flour dough through the continuous gluten network, which forms during mixing (Southan and MacRitchie 1999, Cornell and Hoveling 1998).

Gliadins are monomeric prolamin proteins characterized by high concentrations of glutamine, proline, and hydrophobic amino acids and constitute about 30-35% of total protein (Eliasson and Larsson 1993). In a study of 70 wheat varieties, the average amount of gliadin found was 4.6g/100g flour (Branlard and Dardevet 1985). Over 70 different gliadin fractions have been identified and classified as α-, β-, γ-, and ω- gliadins based on mobility at low pH in starch gel electrophoresis (listed highest to lowest) (Woychick et al 1961).  The molecular weight of ω-gliadins is larger (60,000-80,000 Da) than that of α-, β-, γ-gliadins (30,000-40,000 Da) (Bietz 1979, Wieser et al 1987). Gliadins vary in degree of hydrophobicity (Stauffer 1999)

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with α-gliadins being the most hydrophilic and γ-gliadins the most hydrophobic (Wieser et al 1987). The ω-gliadins are considered sulfur poor (0-8 cysteine residues/100,000g of protein) while α-, β-, γ-gliadins are sulfur rich (17-22 residues/100,000g of protein) (Tatham and Shewry 1985, Charbonnier et al 1980). The ω-gliadins contain β-turns while the other gliadins exhibit α-helixes and β-sheets. Due to the low level of cysteine residues found in ω-gliadins, hydrophobic interactions and hydrogen bonding act to stabilize the molecular conformation instead of disulfide bonds as in the case of α-, β-, and γ-gliadins (Eliasson and Larsson 1993).

Glutenin makes up 40-50% of total wheat flour protein, is polymeric, and has low solubility due to a high molecular weight (Eliasson and Larsson 1993). In a study by Branlard and Dardevet (1985) 70 wheat varieties were found to have an average of 5.9g glutenin / 100g flour. The aggregative nature of glutenin causes the exact molecular weight to be evasive but sizes ranging from $3 \times 10^6$ Da to $20 \times 10^6$ Da have been reported (Kasarda 1989, Eliasson and Larsson 1993). High molecular weight glutenin subunits (HMW-GS) have molecular weights averaging 80,000-120,000 Da while the molecular weight average of low molecular weight glutenin subunits (LMW-GS) range from 40,000 to 55,000 Da (Stauffer 1999). The fractions are roughly equal on a weight basis but the molar ratio of LMW-GS to HMW-GS is 2:1(Stauffer 1999). HMW-GS are characterized by a high concentration of glycine and low proline content, relative to gliadins, while LMW-GS exhibit an amino acid composition similar to α-, β-, and γ-gliadins (Shewry et al 1986).

The helical sheet comprised of β-turns in the middle portion of the HMW-GS subunit is formed by a rather high level of proline (~14% of gluten protein residues) and is thought to be the cause of glutenin's elastic nature (Tatham et al 1985, Wieser et al 2006). Gliadins possess an even greater proline content yet do not present the same strong viscoelastic behavior as glutenin; thus, indicating disulfide bonds contribute more to dough strength (Cornell and Hoveling 1998). Cysteine residues located at both ends of HMW-GS subunits permit intra-molecular bonding and a possible manner in which to restrain the spring-like β-turn center portion. During mixing, the restraining bond breaks under extensional shear to permit elongation, aligning of subunits, and gluten network development (Ewart 1968, Cornell and Hoveling 1998). The resulting rope-like formation of associated glutenin fibrils imparts elastic strength through the non-covalent bonds between the subunits. LMW-GS subunits have cysteine residues at only one end permitting polymerization with inter-molecular di-sulfide bond formation and are referred to as chain

terminators. Intramolecular disulfide bonds stabilize the large polymer structure. Since the surface charge is relatively low in gluten proteins, direct interaction and hydrogen bond formation between amide side chains of glutamine residues is permitted in the aqueous dough (Stauffer 1999, Cornell and Hoveling 1998, Gras et al 2001).
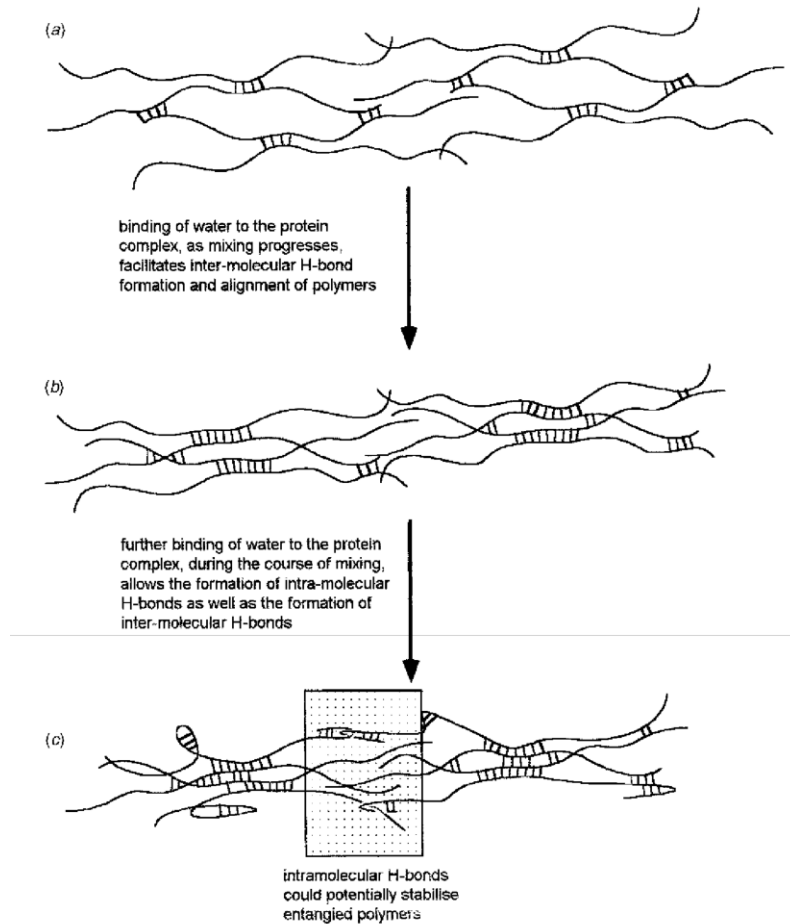


**Figure 2.1 The role of H-bonding in the alignment of glutenin proteins (Belton 1999).**

Gluten proteins are genetically coded by 9 major chromosomal loci: 3 loci (*Glu-A1, Glu-B1, and Glu-D1*) on the long chromosome arm of homoeologous group 1 code for HMW glutenin subunits, 3 complex loci (*Gli-A1/Glu-A3, Gli-B1/Gli-B3, Gli-D1/Glu-D3*) on the short chromosome arms of homoeologous group 1 code for ω- and γ- gliadins and LMW glutenin subunits, and 3 loci (*Gli-A2, Gli-B2,* and *Gli-D2*) on the short chromosome arms of homoeologous group 6 code for α- and β-gliadins (Law and Krattiger 1986, MacRitchie and Lafiandra 2001).

Enzymes, enzyme inhibitors, storage proteins (albumins and globulins), lipoproteins and lectins comprise the heterogeneous group of non-gluten proteins (Eliasson and Larsson 1993). These water-soluble proteins exhibit a greater mobility than gliadins (Wrigley and Bietz 1988) and have molecular weights lower than 40,000 Da (Lasztity 1984). The albumin and globulin levels present vary little between cultivars. If more nitrogen is available, as a result of fertilization for example, the portion of gluten proteins increases while the amount of metabolic (non-gluten) proteins remains constant (Eliasson and Larsson 1993, Doekes and Wennekes 1982). Their biological functions have been studied but much remains to be learned about their roles in baking.

## 2.3 Influence of Gluten Proteins on Dough and Bread Quality

Breadmaking quality of wheat flour is predominately determined by its proteins (MacRitchie 1978). Within a wheat cultivar, the protein content of hard wheat flour (9-18%) has been positively and linearly related to loaf volume (Finney and Barmore 1948). Protein quality, a trait much more difficult to quantify, must also be considered because the correlation is not seen in all wheat varieties.

Causal links between protein class and baking quality have been observed through fractionation and reconstitution experiments in which all flour components are separated and reincorporated with either varying levels of protein, protein fractions, or exchanging protein fractions of flours differing in baking quality (Eliasson and Larsson 1993). If the flour blends are reconstituted in the correct proportions, the resulting change in dough characteristics and bread quality are a result of the alteration. In a study using poor and good baking quality flour, the interchange of gliadin exhibited a minor influence as compared to interchange of the glutenin fraction (MacRitchie 1978, 1980). Altering gliadin and glutenin ratio while maintaining total protein content revealed relative glutenin quantity to be directly related to dough or gluten strength (MacRitchie 1980, 1987). MacRitchie (1987) fractionated gluten proteins by successive extraction with increasing HCl molarity and found the gliadin fraction as a whole weakened dough (as measured by peak mixograph development time) and slightly decreased loaf volume in some cases while the glutenin fraction increased dough strength and loaf volume (Singh et al 1990), and the remaining residue proteins depressed loaf strength and volume. Hydrophobic

gliadins are linked with an increase in bread loaf volume while hydrophilic gliadins are associated with a loaf volume reduction (Weegels et al 1990, Van Lonkhuijsen et al 1992).

The glutenin fraction influences baking performance by three main avenues: the gliadin/glutenin ratio, glutenin molecular weight distribution, and the presence of certain HMW glutenin subunits (Eliasson and Larsson 1993). A high proportion of HMW subunits in strong wheat varieties is causally linked to medium-long mixing time (Huebner and Wall 1976, Gupta et al 1993) and dough stickiness (Gupta et al 1990).

The nomenclature for HMW glutenin subunit was developed based on a ranking of electrophoretic mobility. As alleles were correlated with dough properties, quality scores were associated with each HMW subunit id and the Glu-1 score (summation of individual HMW subunit scores for a given variety) was developed to offer a measure of bread flour quality (Payne et al 1987). At the *Glu-1D* locus in good baking quality wheat varieties, the HMW subunits 5+10 combination have been identified while 2+12 are found in poorly performing varieties (Payne et al 1981, Wrigley and Bietz 1988). The genetic superiority is purposed to be based on the presence of an extra cysteine residue in subunit 5 (Shewry et al 1992), an additional β-turn in the central portion of subunit 10, or coding for a larger quantity of HMW subunits which shifts the molecular weight distribution to the higher range (Singh et al 1990, Southan and MacRitchie 1999). However, Singh et al (1990) found HMW subunits 5+10 in the varieties Egret and Halberd and observed weak doughs thus indicating a purely qualitative approach may be an incomplete analysis.

## 2.4 Genetic and Environmental Influences on Wheat and Flour Quality

Genetic, environmental, and agronomic practices affect the biochemistry of a developing wheat kernel and as a result milling and baking quality (Cornell and Hoveling 1998). Some grain quality characteristics are attributed to growing and harvest conditions (grain moisture, damaged grain, pesticide content, presence of weed seeds) while other attributes are a result of both genetics and environmental influences (test weight, protein content, level of sprouting, flour ash content) (Bequette 1989, Blumenthal et al 1993). Differences in wheat and flour quality due to genetic expression, environmental growing conditions, and protein content influence flour's optimum water absorption (Shogren 1997). Finlay et al (2007) found the relative environmental influence, with optimum soil fertility, to have the largest contribution to wheat quality variation

followed by genotype and lastly the genetic x environment interaction with minimal but significant importance. Many other studies (Lukow and McVetty 1991, Panozzo and Eagles 2000, Peterson et al 1992) determined the genetic by environment interaction to exhibit a smaller influence than genetics or environment on quality parameters as well. Properties such as hardness, dough strength, dough extensibility, dough mixing time, bake absorption which make a cultivar ideal for a particular product and are a result of genetics more than environmental influences (Bergman et al 1998, Blumenthal et al 1993, Bequette 1989).

Variety and protein content have been used as a long-standing classification system for flour quality. However, unexpected poor dough quality characteristics have been known to result from a cultivar with predicted desirable quality. Environmental influences are thought to be the cause of such fluctuation in flour performance. Elucidating the independent influences of genotype and environment conditions is important for wheat breeders in order to decipher the robust breeds possessing consistent dough quality properties regardless of climatic fluctuations (Peterson et al 1992, Ciaffi et al 1996). A better understanding of growing conditions and climatic effects for specific cultivars could introduce more stability in product quality, increase wheat variety marketability (Finlay et al 2007), and help meet commercial processor's tight flour quality specifications for today's highly automated bakeries (Farindi and Finley 1989).

### 2.4.1 Environment Effects

Wheat milling and flour quality variability, for a given cultivar, between locations and crop years can largely be attributed to growing conditions (Finlay et al 2007, Faridi and Finley 1989, Peterson et al 1992). Faridi and Finley (1989) conducted milling and baking evaluations of two HRW varieties (Eagle and Newton) grown in 12 Kansas locations over 10 years. They found protein content and loaf volume fluctuations in addition to the test weight, absorption, and optimum mix time decline observed was attributable to environment since the varieties were held constant.

Precipitation, late-season frosts, $CO_2$ level, soil type, and temperature are influences beyond the farmer's control but affect plant growth and consequently grain and flour quality (Randall and Wrigley 1986, Southan and MacRitchie 1999). Low rainfall and elevated $CO_2$ levels shorten the grain filling period and result in earlier maturation than in water and $CO_2$

sufficient conditions (McMaster 2009). Late-season frosts delay grain maturation and result in low test weight and poor flour functionality (Lookhart and Finney 1984).

Certain agronomic practices offer preventative measures for protecting grain quality against environmental strains. Planting time can account for periods of disease susceptibility (Faridi and Finley 1989). Grain should not be harvested with elevated moisture content or stored at a high moisture level so as to initiate germination or microbial growth (Faridi and Finley 1989). Wet grain exhibited a shorter mixing time with lower tolerance to overmixing (Lookhart and Finney 1984).

Fertilizer application allows the grower to replenish depleted organic soil matter resulting from years of cultivation (Faridi and Finley 1989). Sulfur deficiency causes changes in the grain protein composition resulting in reduced nutritional value and functional properties (Randall and Wrigley 1986) and results in greater production of sulfur poor proteins (ω-gliadins, HMW-GS) to compensate for lower sulfur rich protein production (albumins, α-, β-, γ-gliadins, LMW-GS) (Moss et al 1981, Southan and MacRitchie 1999). Grain containing less than 0.12% sulfur content or a nitrogen/sulfur ratio greater than 17 indicates sulfur deficiency (Moss et al 1981). Test baking showed sulfur-deficient grain produced less extensible doughs, smaller loaf volume, and poorer texture as compared to grain with adequate sulfur content (Randall and Wrigley 1986). The lack of viscoelastic dough properties is proposed to result from the absence of SH- and SS-bearing proteins to participate in disulfide bond formation within gluten proteins needed for gluten network formation.

For proper sulfur utilization in protein synthesis (Randall and Wrigley 1986) and a normal grain filling period (McMaster 2009), a minimum nitrogen content of 1 g nitrogen per 80-100 mg sulfur must be present (Randall and Wrigley 1986). Since the duration of grain filling is more important than the rate of growth for determining final kernel size (McMaster 1997, Johnson and Mattern 1987), a limitation on nitrogen lowers flour yield. Additional nitrogen availability with fertilization increases field yield and with heavy application a direct correlation between protein content and fertilizer level occurs (Faridi and Finley 1989), which generally is associated with higher extensibility and greater bread making potential (Southan and MacRitchie 1999). However, Gupta et al (1992) found the rate of gliadin formation increased with excessive nitrogen bioavailability causing problematic low dough strength due to the molecular weight distribution change (decrease in polymeric/monomeric protein ratio).

Since wheat is a temperate annual, periods of heat stress during grain filling are detrimental to grain yield and quality (Blumenthal et al 1993). As temperature increases, protein content increases (Kolderup 1975). The increase in protein cannot be explained by greater nitrogen bioavailability but by simply an increase in the protein/starch ratio due to suppression of starch synthesis (Blumenthal et al 1993, Ciaffi et al 1996). At a sufficiently high temperature (depending on the region and variety grown) low grain test weight results from the low level of starch synthesis and a shortened grain filling period (Hunt et al 1991). In France, the temperature threshold is 25°C (Brisson et al 2010).

Heat stress also affects protein quality as seen by an increase in dough strength (as measured by Extensigraph resistance) with mean daily temperatures up to 30°C while higher temperatures produced weaker doughs Randall and Moss (1990). Also the timing of the high temperatures proved relevant. Heat stress 20 days post-anthesis was more harmful than 36 or 50 days post-anthesis. In examination of gluten composition of heat-stressed wheat grown at temperatures >35°C in Italy and Australia, the dough weakening effect was associated with an increase in gliadin/glutenin ratio (Ciaffi et al 1996) and lower than average loaf volumes (Finney and Fryer 1958). Blumenthal et al (1990) found genes that protect against heat-shock are linked with the coding locus for gliadins thus permitting gliadin synthesis to proceed unaffected during heat stress periods. Interestingly, a gradual daily increase in temperature up to 40°C during the grain filling period resulted in a constant protein synthesis rate for each class and no change in gliadin/glutenin ratio (Bernardin et al 1995).

### *2.4.2 Genetic Effects*

Bread wheat is a hexaploid species with three genomes (A, B, D) each contributing seven chromosomal pairs to make seven homoeologous groups of chromosomes with similar genetic function (Law and Krattiger 1986). Near-isogenic wheat lines are varieties in which genes of interest at certain loci are absent, silent, or substituted with genes from another cultivar and the remaining genetic code is unchanged. Such lines occur by natural mutation or chromosomal engineering and are useful for determining the functional properties of specific proteins or subunits (MacRitchie and Lafiandra 2001). Law et al (1978) showed the *Hardness* (*Ha*) locus on the short arm of chromosome 5D is the major factor that controls grain hardness in hexaploid wheat (*Triticum aestivum*). Durum wheat lacks the *Ha* gene due to the absent short arm of 5D; as

a result its endosperm is much harder than *Triticum aestivum* wheat. The *Protein* (*Pro1* and *Pro 2)* loci on the long and short 5D chromosome arms, respectively, control the amount of protein in grain independently of yield (Law et al 1978). Southan and MacRitchie (1999) found polymeric protein formation during grain filling to be controlled by genetics; however, the exact mechanism remains to be confirmed.

The implementation of semidwarf cultivars doubled field yield due to less energy expenditure on straw production (Faridi and Finley 1989). However, from a milling and baking perspective caution must be exercised when further agronomic practices are implemented for augmenting yield because of the inverse relationship between protein level and field yield. In general, flour yield increases with test weight but higher extraction rates due to varietal differences in bran cleanup, kernel hardness, reduction, and sifting properties were also reported (Bequette 1989).

## 2.5 Methods for Flour Protein Separation and Identification

### 2.5.1 Sonication and Size Exclusion High Performance Liquid Chromatography

Sonication of wheat proteins has proven to be a simple, effective way to break down the largest glutenin fraction (Singh and MacRitchie 1989) due to sound waves splitting the polymer with mechanical shear (Bueche 1960). Subsequent size-based separation of protein species (polymeric glutenins, monomeric gliadins, and albumins/globulins) is still possible since the HWM glutenin fragments remain within the characteristic size range of LMW glutenin (Singh et al 1990). Quantification of the protein fractions using size exclusion high performance liquid chromatography (SE-HPLC) has been a successful technique due to speed, automation, and a small sample size requirement (Bietz 1986, Gupta et al 1993).  The proteins in a solvent pass through a column of chromatographic medium with the molecules larger than the pores passing through in the void area to elute first (Bietz 1986). The smaller the protein molecular weight, the more permeation into the pores occurs, resulting in longer elution time.

### 2.5.2 Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis  (SDS-PAGE)

SDS-PAGE offers a way to observe the protein distribution of unreduced and reduced protein (Singh et al 1990). With the reduction of glutenin into HMW and LMW subunits, the fractions can be studied further for polypeptide identification based on size (Eliasson and

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Larsson 1993, Cornell and Hoveling 1998). Sodium dodecyl sulfate and beta-mercaptoethanol denatures the proteins. Sodium dodecyl sulfate adds a uniform negative charge mass ratio, which acts to pull the polypeptides through an electrified gradient gel resulting in separate pure bands of settled protein (Cornell and Hoveling 1998). Comparison of migration distance with protein standards run parallel allows for molecular weight estimation.

## 2.6 Flour Absorption

Second to flour, water is a major component of bread and acts as a plasticizer and solvent during dough formation (Delcour and Hoseney 2010). Together flour and water create the structure of bread and a visco-elastic dough mass necessary for gas retention (Faubion et al 1985, Stauffer 1999). Depending on the level applied (expressed as a percentage of flour weight), a wide range of dough consistencies can be achieved for forming specific structure and texture characteristics of the final desired product (Delcour and Hoseney 2010). For example, optimum water absorption for ciabatta bread is 70-80% while that of Arabic breads is as low as 55% (Qarooni 1996). A high absorption level is valued for augmenting final pan bread yield and extending shelf life (Pyler and Gorton 2008) but the baking absorption level should also create dough with adequate handling properties during processing (mixing, fermentation, dividing, rounding) (Stauffer 1999, Ross and Bettge 2009). Thus, the proportion that balances the elastic and viscous rheological characteristics with commercial processing needs is termed the optimum or baking water absorption level (Ross and Bettge 2009).

The water absorption can be measured with the use of empirical recording dough mixers such as the Farinograph® and Mixograph®. The Farinograph® determines the amount of water required to achieve an established level of resistance (500 BU) to mixing a flour-water dough based on intrinsic flour components and gluten quality but the arbitrary consistency is not correlated with the optimum water absorption (Stauffer 1999, Ross and Bettge 2009).  Water level found offers simply a comparative measure of absorbance at a standardized consistency (Delcour and Hoseney 2010). To establish the optimum water absorption using the Mixograph®, water level is estimated based on flour protein content and altered by trial and error until a mixogram with a proper development profile is observed with operator experience as the primary determinant (Ross and Bettge 2009). Neither instrument is a replacement for test baking (Pratt 1978) but an aid for estimating relevant absorption ranges and mix times more accurately, there

by reducing trials and time needed for dough preparation prior to commercial start up (Ingelin 1997).

Four flour components absorb water: protein, intact starch, damaged starch, and pentosans (Bushuk 1966, Stauffer 1999). See table 2.1 for each component's imbibing capacity (Stauffer 1999). Flour ash content, a minor flour constituent, indirectly affects water absorption due to inorganic salts decreasing gluten ionization, which encourages more hydrophobic molecular association and reduces the hydration level (Stauffer 1999). The same gluten network tightening effect is seen upon sodium chloride addition; therefore, salt addition is often delayed until after gluten proteins are hydrated in the initial dough development stage.

**Table 2.1 Influence of flour components on water absorption**

| Flour Component | Water per g of component (g) | Amount per 100g flour (g) | Absorption per 100g flour (g) |
|---|---|---|---|
| Protein | 1.3 | 12 | 15.6 |
| Intact Starch | 0.4 | 57 | 22.8 |
| Damaged Starch | 2.0 | 8 | 16.0 |
| Pentosans | 7 | 2 | 14.0 |

Intact starch has the highest contribution to absorption due to the sheer volume present in flour and the swelling effect of gelatinization during baking (Stauffer 1999). However, native starch granules are relatively impermeable to water without the introduction of mechanical energy (Stauffer 1999). With the addition of water, the exposed flour particles' surface is readily hydrated and water slowly penetrates the interior. The abrasive force impacted by moderately high shear rates during mixing removes the hydrated layer to expose a dry layer to expedite the diffusion process (Ingelin 1997, Delcour and Hoseney 2010). As the protein becomes fully hydrated, the dough system's resistance to extension increases due to a more intimate starch granule and protein fibril interaction in the developing dough (Delcour and Hoseney 2010).

The difference in degree of damaged starch observed between hard (6-12%) and soft wheat (2-4%) flours results from a greater pressure requirement on milling reduction rolls for hard endosperm disruption than for soft (Stauffer 1999). The greater force applied causes cracks and fissures in some granules and allows for amylolytic attack not possible with intact starch

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granules. Adjusting the differential roll speed, roll pressure, feed rate, and roll surface can control the level of damaged starch and resulting additional water-imbibing capacity (Stevens 1987). Additional water uptake by damaged starch during dough formation limits water available for gluten and a low final loaf volume results due to the rigid protein state not permitting dough expansion (Sanstead 1961, Pyler and Gorton 2008). However, a low level of starch damage (4.5-8% for hard winter or spring wheat flours, depending on protein content) is desirable for feeding yeast to initiate fermentation (Pyler and Gorton 2008, Ross and Bettge 2009). With excessively high starch damage a lower absorption must be applied because the water holding capacity of partially digested damaged starch lowers during resting periods (fermentation and proofing) causing the dough to become fluid and sticky (Tipples et al 1978, Kunerth and D'Appolonia 1985, Stauffer 1999). In a study by Tipples et al (1978) flour milling streams of Canadian hard red spring wheat were analyzed and starch damage was the most important estimator of Farinograph® absorption. Inclusion of protein content improved the prediction model, as seen by an increase in $r^2$. The most important factor for predicting optimum baking absorption in the remix and Chorleywood baking methods used was gluten quality, as calculated by wet gluten/protein content. Adding pentosan or starch damage content to the model had no significant impact on predicting baking absorption. Varying the proportion of gliadins, glutenin, or gluten at a constant total protein content was found to have no effect on water absorption while the inclusion of proteins with alternative hydrophobicity characteristics significantly altered absorption (Haraszi 2004). In a flour fractionation and reconstitution study conducted by Patil et al (1976), the water-soluble pentosan fraction had no effect on optimum absorption and minimal, variable effect on mixing times.

In theory, smaller flour particle size could offer a greater absorption due to a larger surface area; however, any additional water uptake could be due to starch damage from excessive grinding required for particle size reduction (Stevens 1987). In a scenario with finer flour particle size but no increase in starch damage, the absorption level did not change but hydration was achieved more quickly allowing for a lower overall mixing time (Pratt 1978).

## 2.7 Mixograph® Recording Mixer

The Mixograph® is an instrument used to measure resistance of wheat flour and water dough to mixing (Stauffer 1999). Four head vertical pins rotating around three stationary bowl

pins apply a high shear rate to hydrate the flour as well as stretch and fold the developing dough (Wikstrom and Bohlin 1996, Ingelin 1997). The mixing bowl is mounted on a swivel base and permitted to rotate up to 40° as torque is transmitted through the dough. The subsequent movement is transferred by pen to chart paper or recorded electronically with a potentiometer sensor. By starting the pen at one of the minute-spaced arced lines, comparing characteristics between mixograms and determining the required mix time becomes simpler. The optimum mixing time, tolerance to overmixing, and approximate bake (optimum) absorption can be determined from the resulting graph, termed a mixogram, (AACC Method 54-40A, Finney and Yamazaki 1967) and as a result create a more concise test baking assessment (Ingelin 1997).

The mixogram conveys changes in dough consistency throughout the four stages of mixing (hydration, blending, development, and breakdown) (Kunerth and D'Appolonia 1985, Stauffer 1999) and the same stages can be observed in a commercial mixer (Pyler and Gorton 2008) see Figure 2.2 below (Stauffer 1999). During hydration, proteins, damaged starch granules, and water insoluble pentosans swell with water enabling the proteins to polymerize (Stauffer 1999, Willis and Okos 2003). The development stage creates visco-elastic dough with a dry, silky appearance due to the formation of di-sulfide and hydrogen bonds between protein fibrils creating a continuous protein network (Bloksma 1964, Gras et al 2001). After peak development, the resistance to mixing decreases and the dough becomes highly extensible as a result of excessive gluten protein size reduction (Willis and Okos 2003) and water begins to become unbound (Gras et al 2001).
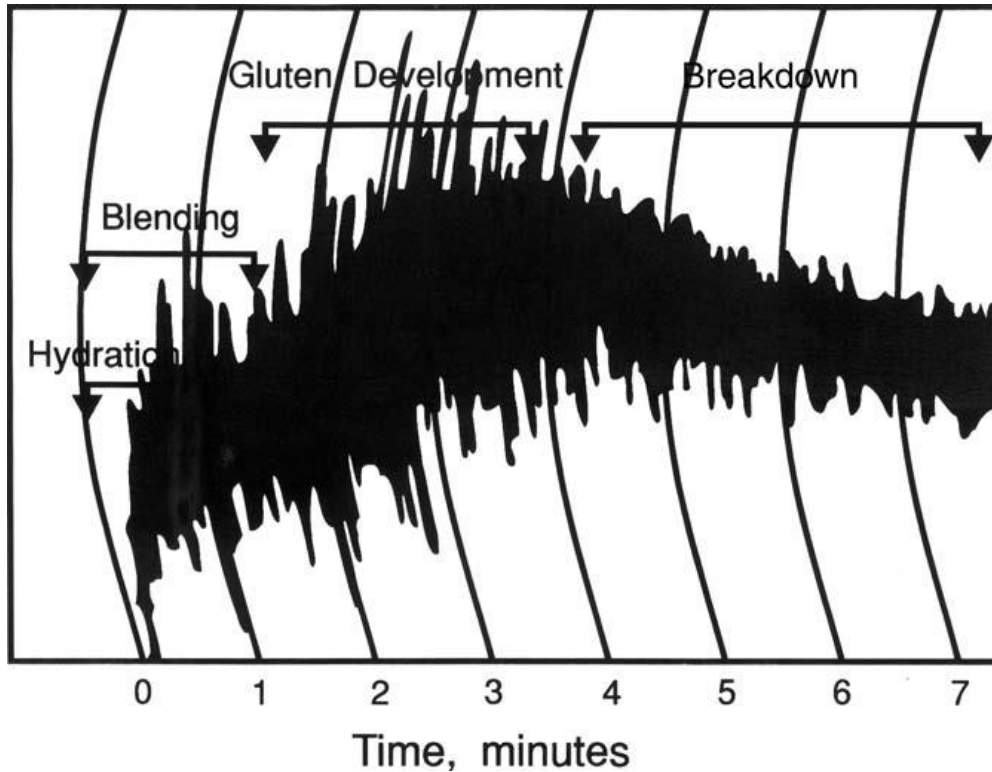
**Figure 2.2 A Mixograph indicating the four main stages of dough formation (Stauffer 1999).**

Insufficient water absorption produces dry stiff dough and a mixogram with wild swings (Hazelton et al 1997) while water absorption above optimum produces slack dough and a narrow mixogram (Finney and Shogren 1972). The degree of swayback during dough development before peak mix time and narrow bandwidth after indicates over hydration and rate of dough breakdown, respectively (Ingelin 1997). Kunerth and D'Appolonia (1985) found absorption to be positively correlated with peak time and area under the curve (r=0.935 and 0.860, respectively) and negatively correlated with peak height (r= -0.991). The larger the angle between the development and breakdown slopes the greater the mixing tolerance (Ross and Bettge 2009). Baig and Hoseney (1977) showed in an absorption series of four medium to long mix time flours that mixogram height decreases and the time to maximum resistance is more prolonged as the water absorption level increases. As protein content increases within a cultivar, water absorption increases (Finney and Shogren 1972) and peak height increases (Swanson 1941).

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The Mixograph® has been used to evaluate dough rheology, flour quality, influence of ingredient on mixing properties, and breadmaking potential during the wheat breeding process. The capability to predict dough processing characteristics and final product quality has made the Mixograph® useful for selecting flour for cookies (Labuschange et al 1996), bread (Knackstedt et al 1994), noodles (Baik et al 1994) and pasta (Kovacs et al 1994). In response to wheat breeders' need for a rapid method to evaluate flour functionality using fewer resources, Finney and Shogren (1972) reduced the flour requirement from 35g to 10g by developing a smaller mixing bowl and set up. Bruinsma et al (1978) proved mixograph data from sifted ground whole meal correlates highly with refined white flour thus, allowing a more simple and inexpensive approach to predicting dough characteristics of early generation wheat varieties and shortening the time required to select desirable breadmaking strains. The Mixograph® can also be used in a commercial setting to determine the suitability of a certain flour lot with specific mixer characteristics (scale, geometry, and mixing intensity) based on empirical rheological attributes observed (Ross and Bettge 2009).

Since the Mixograph® is not as standardized as other recording dough mixers, adherence to AACC method specifications is necessary and operators must be trained to interpret mixograms (Finney and Yamazaki 1967, Ingelin 1997, Hazelton et al 1997). In an attempt to make mixogram evaluation more objective and automated, the direct drive mixograph was developed in which the energy pull required to maintain a consistent mixing speed is recorded continuously and a computerized mixogram is generated. Current drawn at preset mixogram locations (mid-line peak mix time and height) and mixogram parameters (band width at multiple curve locations, ascending and descending slopes, area under the curve amongst many others) can be used to assess subtle changes in dough development. Several studies used this technical approach to develop a regression equation evaluating the predicted optimum water absorption level accuracy (Hazelton et al 1997, Ohm and Chung 1999), a bread volume prediction model (Wikstrom and Bohlin 1996), and a method to evaluate bread making potential as determined by the effect of certain flour components on mixogram parameters (Martinant et al 1998).

## 2.8 Solvent Retention Capacity (SRC)

The solvent retention capacity test assesses the functional contributions of individual flour components; whereas, most flour quality evaluation methods observe the combined effect

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of flour constituents (Kweon et al 2011). SRC is the amount of solvent retained in a hydrated flour sample after centrifugation and is reported as a percentage of flour weight corrected to a 14% moisture basis (Slade and Levine 1994). Each solvent evaluates chemical and physical flour aspects: the 5% lactic acid solution is linked to gluten proteins and predicts loaf volume in relation to protein quality, the 5% sodium carbonate solution swells damaged starch, the 50% sucrose solvent is associated with water soluble arabinoxylans (pentosans), and water displays the interworking of all polymer networks (Slade and Levine 1994, Guttieri et al 2001, Bettge et al 2002, Xiao et al 2006, Kweon et al 2011). Originally, SRC was developed as a soft wheat flour evaluation tool for predicting cookie quality (Slade and Levine 1994, Guttieri et al 2004); however, now the test has proven useful for determining flour functionality in hard wheat products as well (Xiao et al 2006, Kweon et al 2011). The four solvents provide a flour quality profile of baking and flour processing characteristics for proper final product application (Kweon et al 2011, Ram et al 2005).

## 2.9 Dough formation, processing, and bread quality

The formation of dough begins with mixing flour and water to homogeneously hydrate flour particles (Ross and Bettge 2009). Mixing provides the mechanical energy needed to develop the gluten structure through shear and tensile stresses in order to impart a viscoelastic nature to the dough (Hoseney 1985, Southan and MacRitchie 1999). Wheat storage proteins are unique in this capability as compared to other cereal storage proteins (Stauffer 1999). Mixing also evenly incorporates the ingredients and traps air cells (Bloksma 1964). Final loaf quality is highly dependent on the level of mixing (Eliasson and Larsson 1993). Dough is optimally developed at maximum resistance to mixing (MacRitchie 1984). The more glutenin molecules present and the higher their molecular weight the greater extent of mixing required, as more work is needed to stretch and extend the large molecules (MacRitchie 1986). Flour with a medium-long mix time (4-5 minutes) is best for baking performance; however, a medium dough development time (3-3.5 minutes) might be preferred by processors to minimize the energy input required to fully develop the dough (Finney and Shogren 1972, Wrigley et al 2009). Dough with a long stability to over mixing is less sensitive to mechanical treatment and increases flexibility in manufacture.

The straight dough process is the original method used for commercial bread production and the basis for alternative methods developed (Ross and Bettge 2009). In the straight dough method all ingredients are mixed together and fermented with optional punching steps. The no-time straight-dough process uses mixers with higher work input to speed up dough development and greatly reduces fermentation time by employing dough improvers (Ross and Bettge 2009). In the sponge and dough method, about half of the formulated flour and a fraction of the formulated water are fermented. The resulting sponge is mixed with the remaining ingredients for the final developed dough. The sponge and dough process is claimed to offer greater flexibility in processing, more product uniformity, higher loaf volume, better crumb grain, and softer texture (Kulp and Ponte 2000, Pyler 2008) due to a more extensible dough after remixing (Cauvain and Young 2007). It is the main commercial bread production method used today.

The aim of all dough processing methods is to improve gluten elasticity and gas retention capabilities. Bulk fermentation modifies the protein structure through a prolonged resting period while the energy intense approach expedites the process. Folding or punching the dough during bulk fermentation subdivides $CO_2$ inflated cells into smaller cells less likely to coalesce thus resulting in a finer and more uniform cell structure. The level of energy applied during mixing is directly related to loaf volume and a fine, uniform crumb texture. Optimum ranges of protein content also differ between processes.

Wheat cultivars and their subsequent flour differ by intrinsic composition and functionality factors (Ross and Bettge 2009). Such biochemical differences determine end uses of wheat varieties (Xiao et al 2006). Achieving optimum product quality depends on starting with a flour possessing suitable functionality properties for each product's processing requirements (Bergman et al 1998). Such factors are kernel texture, seed coat color, milling characteristics, enzymatic activity, wheat and flour protein contents, gluten protein composition, dough characteristics, and thermal processing behavior of starch (Ross and Bettge 2009). Requirements specific to dough for bread include consistent formation of cohesive, elastic, and relatively soft dough, which can be molded into a desired shape which resists deformation without excessive stickiness (Cauvain and Young 2007). The degree of elastic and viscous dough attributes must be balanced for dough strength to endure stress during processing (mixing, fermentation, dividing, molding) and retain fermentation gases in order to form the desired aerated breadcrumb and soft texture (Stauffer 1999).

# Chapter 3 - Materials and Methods

## 3.1 Wheat Samples

Twenty hard winter wheat varieties (Norwest 553, Boundary, Bauermeister, Farnum, Eddy, Paladin, Palomino, Whetstone, BC002-2-2, Agripro 79-5-1, Agripro 79-5-2, IDO 660, OR2070181H, OR2070174H, OR2080111H, OR2080156H, OR2080178H, OR2080227H, OR2080229H, OR2080236H) were grown as part of cooperative farmers' fields at two locations, Pendleton (latitude 45.737, longitude -118.52) and Arlington (latitude 45.522, longitude -120.165), in North-Central Oregon (Figure 3.1) in the 2011 crop year. The plots received agronomic treatment typical for the respective area and an additional fertilization with urea and sulfur. The Pendleton and Arlington plot elevations were 558 meters and 370 meters, respectively.  A total of 39 samples were provided. One variety (Agripro 79-5-1) was not available from the Arlington location.



**Figure 3.1 Oregon wheat sample growing locations**

## 3.2 Wheat Physicochemical Tests

A Carter Dockage tester (Carter-Day Company, Minneapolis, MN) was used to clean the wheat samples. The AACC approved method 55-10 was used to measure test weight (lb/bu). The mean kernel weight (mg), diameter (mm), and hardness index values were determined using the Single Kernel Characterization System (AACC Approved Method 55-31) (SKCS 4100, Perten Instruments, Springfield, IL).

## 3.3 Wheat Milling

Samples were tempered to 15% moisture content overnight (AACC approved method 26-95). In the Wheat Quality Laboratory at Kansas State University, roughly 1000g samples were milled to approximately 70% extraction on the Quadrumat Senior Experimental Mill (Brabender, Duisburg, Germany) using the modified AACC method 26-50. A 150g/min feed rate was applied. Flours produced were straight grade.

## 3.4 Flour Characteristics

### 3.41 Flour Physiochemical Tests

A DA 7200 NIR (Perten Instruments, Springfield, IL) was used to measure flour protein and moisture content. The Megazyme® Starch Damage Assay (Wicklow, Ireland) kit was used in accordance with AACC approved method 76-31 to evaluate flour starch damage for all samples in triplicate. Flour ash was determined in duplicate using AACC approved method 8-01.

### 3.42 Water absorption tolerance recorded by Mixograph®

Optimum water absorption was estimated based on the equation $Y = 1.5x + 43$, in which $x$ is flour protein content on a 14% moisture basis, and tested using the 10-g Mixograph® (National Manufacturing Co, Lincoln, Nebraska) according to AACC approved method 54-40. For each flour sample a series of mixograms with 2% incremental lower and higher water absorption intervals from the predicted optimum water absorption level were conducted to determine dry, optimum, and wet dough conditions. Dry dough conditions were determined based on wild pen strokes; while, a swayback characteristic during development and narrow band after peak height established wet dough conditions (Figure 3.2). The water absorption tolerance range was calculated in milliliters of water as the interval between dry and wet absorption levels. Flour samples were determined to have a high, medium, or low tolerance by splitting the total range of tolerance range values (0.6 to 1.2 ml) into three 0.4 ml wide intervals and categorizing the flours accordingly.

|  Dry  |  Optimum  |  Wet  |



| 57% abs, 2.75 min mix time | 65% abs, 3.25 min mix time | 73% abs, 4.25 min mix time |
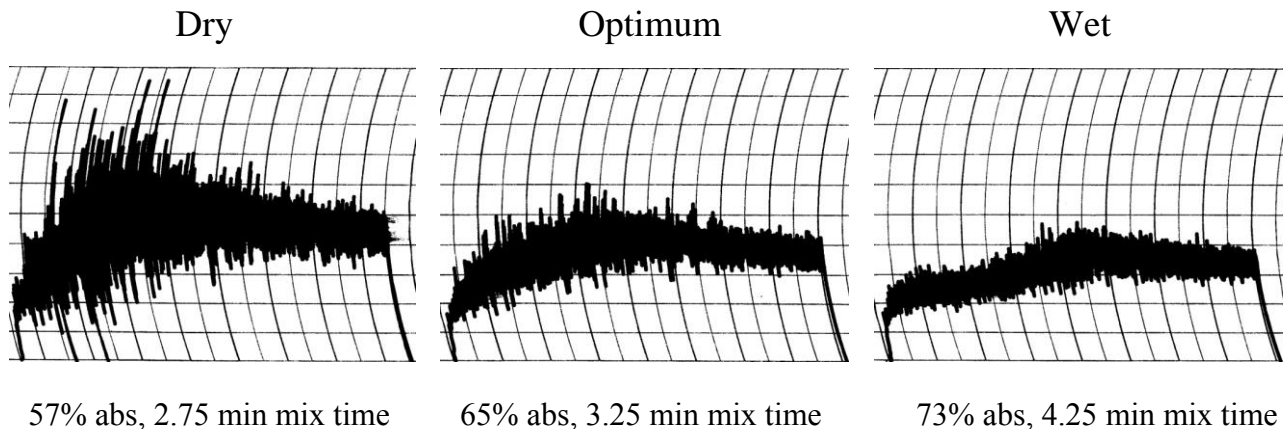
**Figure 3.2 Example of mixogram series containing dry, optimum, and wet water levels used to determine water absorption tolerance range**

Water absorption tolerance interval (mL) = Wet absorption level - dry absorption level

### *3.43 Solvent Retention Capacity*

A reduced scale version (1g flour with 5 ml solvent) of the solvent retention capacity test (AACC Approved Method 56-11.02) was applied to all flour samples in triplicate. The analysis was used to correlate which flour constituents potentially cause flour water absorption tolerance.

## 3.5 Wheat Protein Extraction and Identification

### *3.5.1 Wheat Protein Extraction using Sonication*

The wheat protein extraction method used by Singh et al (1990), Batey et al (1991), and Wesley et al (2001) was modified and employed for all samples. Samples were weighed in a microfuge tubes as close to 10mg as reliably possible. The appropriate quantity of 0.05M NaPhos + 0.5% SDS buffer at pH 6.9 was added to each weighed sample to maintain a 10mg flour/1000μL SDS buffer solution ratio ie) 9.8mg sample would receive 980μL SDS buffer. For total protein analysis, the flour and buffer mixture was vortex mixed (Daigger Vortex Genie 2, Vernon Hills, IL, USA) for 5 minutes at setting 5 to thoroughly suspend the flour particles. The mechanical shear of sonication (XL-2000 Misonix sonicator, Qsonica LLC, Newtown, CT, USA) for 15 seconds at 6 watts output solubilized the largest protein fraction (glutenin polymers). Microfuge tubes were placed in ice during sonication. The sonication probe tip was submerged one-third the distance from the bottom in the tube center. Centrifugation (centrifuge

5424, Eppendorf, Hauppauge, NY, USA) for 20 minutes at 12,000 x g separated the supernatant and residue. The supernatant was decanted into a 0.45µm nylon filter microfuge and centrifuged for 10 minutes at 12,000 x g to remove any particulates. The strained supernatant was transferred to a vial and sealed. To prevent possible proteolytic activity and stabilize the samples, the sample vials were partially submersed in an 85°C water bath for 10 minutes. Sample vials equilibrated to room temperature for at least 30 minutes before Size Exclusion-High Pressure Liquid Chromatography (SE-HPLC) analysis.

The same procedure was conducted for extracting the soluble proteins but without sonication. After centrifugation and removal of the extractable protein fraction, the isolated unextractable protein fraction remained as a residue. The same volume of buffer solution was added to the pellet as for when extracting the soluble protein fraction. Vigorous vortex mixing for 10 minutes at setting 7 dispersed the protein within the buffer and sonication for 25 seconds at 6 watts output followed to reduce polymeric protein molecular weight. The centrifugation, filtration and heat treatment steps previously applied to the total protein analysis sample were followed for the extractable and unextractable protein fractions.

### 3.5.2 Wheat Protein Fractionation by Size Exclusion-High Pressure Liquid Chromatography (SE-HPLC)

A Biosep SEC-400 column (Phenomenex, Torrance, CA, USA) in a HPLC (Agilent 1100, Agilent Technologies, USA) system with a binary pump and automated sample injector were used to fractionate 20µL aliquots of each extracted protein sample. The mobile phase consisted of a 50/50 ratio of deionized water + 0.1% trifluoroacetic acid (TFA) and acetonitrile + 0.1% TFA (Sigma Aldrich, St. Louis, MO, USA). The solvent flow rate was 0.5mL/min. The analysis conditions included a 30-minute run at 24°C and the protein sub-fractions were detected using a UV variable wavelength detector at 214nm. Samples were extracted and analyzed in duplicate. ChemStation software (Agilent Technologies, USA) was used to regulate pump control, accumulate sample data and integrate chromatograms. Peak areas were determined as the area under the curve between the lowest points (valleys) and convey the proportion of protein constituents within the total protein content (Figure 3.3). The peak 1 area of total protein extraction/ total area of total protein extraction x 100 indicated the proportion of polymeric glutenin proteins in a wheat flour sample. For the same formula, peak 2 and peak 3 show the

proportion of monomeric gliadins and monomeric albumin/globulin fractions, respectively. The percentage of extractable polymeric protein (EPP) was determined as peak 1 area of the extractable protein sample chromatogram / (area of peak 1 from the extractable protein chromatogram + area of peak 1 from the unextractable protein chromatogram) x 100 (Wesley et al 2001). The unextractable polymeric protein (UPP) percentage was calculated similarly only with the numerator as peak 1 area from the unextractable protein chromatogram. The sum of the two percentages should equal 100 since the formula is a ratio between EPP and UPP and conveys polymeric protein molecular weight distribution.
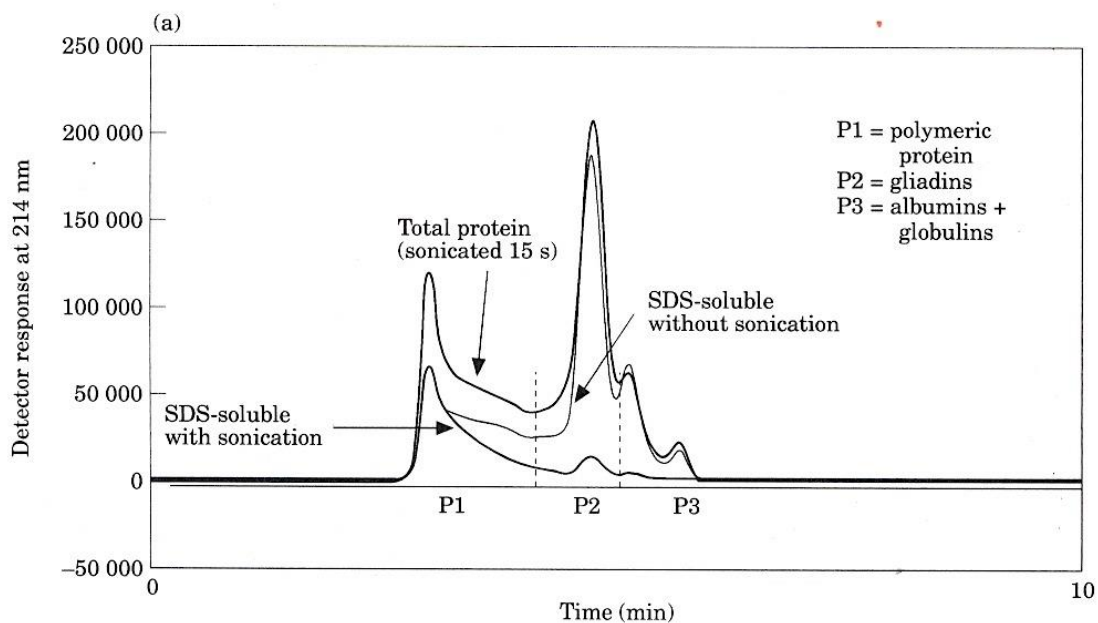


**Figure 3.3 Chromatograms of total protein, EPP and UPP extractions (Wesley et al 2001).**

### 3.5.3 Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis  (SDS-Page)

For select variety pairs showing interesting water absorption tolerance ranges for different locations, the extractable protein composition was evaluated. Separation of the extractable protein subunits was achieved in one dimension using SDS-PAGE electrophoresis on NuPAGE 4-12% Bis-Tris Mini Gels using Xcell SureLock® mini-cell (Life Technologies, Carlsbad, CA, USA). The extraction procedure used previously for HLPC analysis was employed and the samples were frozen before freeze-drying. The samples were rehydrated in 100μL of water. 10μL of the sample suspension, 5μL LDS 4x (lithium dodecyl sulfate with comassie dye), 2μL BME, and 7μL distilled water were combined in a 0.5mL vial. The samples

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were agitated to incorporate the solutions and heated in a 95°C water bath for 5 min. 10μL of molecular markers (Mark 12 Invitrogen) and sample aliquots were slowly loaded into different gel wells. Voltage was set at 150V. The electrophoresis unit ran until the dye reached the end of the separating gel bed. The plastic frame was split open and the gel end was cut to release the gel. Gels were fixed and stained overnight using the Novex colloidal coomassie blue G-250 staining kit (Invitrogen). Gels were soaked in distilled water until the background turned clear and scanned using an Epson Perfection V700 photo scanner.

## 3.6 Environmental Data

Maximum and minimum temperatures and precipitation data was acquired from the Applied Climate Information System (ACIS) database of national climate information. Data from the Arlington (latitude 45.721, longitude -120.206) and Pendleton (latitude 45.691, longitude -118.852) recordings sites closest to each farm was obtained to understand climate conditions and determine if growing sites' climates differed significantly.

## 3.7 Data Analysis

Since only one sample from each of two locations was provided, the environmental by genetics influence cannot be quantified because there is nothing to measure variability between within a location. However, a Randomized Complete Block design with location as a block and variety as a treatment is able to decipher varietal and location influences. The GLM procedure by SAS was used for ANOVA analysis. The location x variety interaction was used as an estimate for experimental error. Relevant pairwise comparisons were conducted using the Tukey method to avoid inflation of type I error rate due to multiple comparisons. Correlations between tested parameters and water absorption tolerance range were determined.

# Chapter 4 - Results and Discussion

## 4.1 Flour classification by water absorption tolerance

For each flour sample, a mixograph series was conducted by increasing and decreasing water absorption levels by 2% from the estimated optimum water absorption determined using the Finney absorption calculation until excessively wet and dry conditions were observed in the mixogram curves. The difference between the water addition levels for wet and dry doughs (in mL) defined the water absorption tolerance range. Figure 4.1 exhibits a water absorption mixograph series of the Farnum flour sample from Arlington. The flour protein, as determined by NIR, was 9.58 (14% mb). The estimated optimum water absorption level (fwb) based on the Finney absorption equation (water absorption=1.5x flour protein % on 14% mb + 43) was 57%; however, after evaluating the mixogram curves in the water absorption series, 65% was identified as the optimum due to ideal curve shape (smooth development and little narrowing after the peak). An excessively wet dough formed at 73% absorption as indicated by the swaybacked slump before the mixogram curve peak. The wild jagged swings on the mixograph curve appearing at the 57% absorption level as compared to the previous 59% absorption curve indicated the dough was too dry.
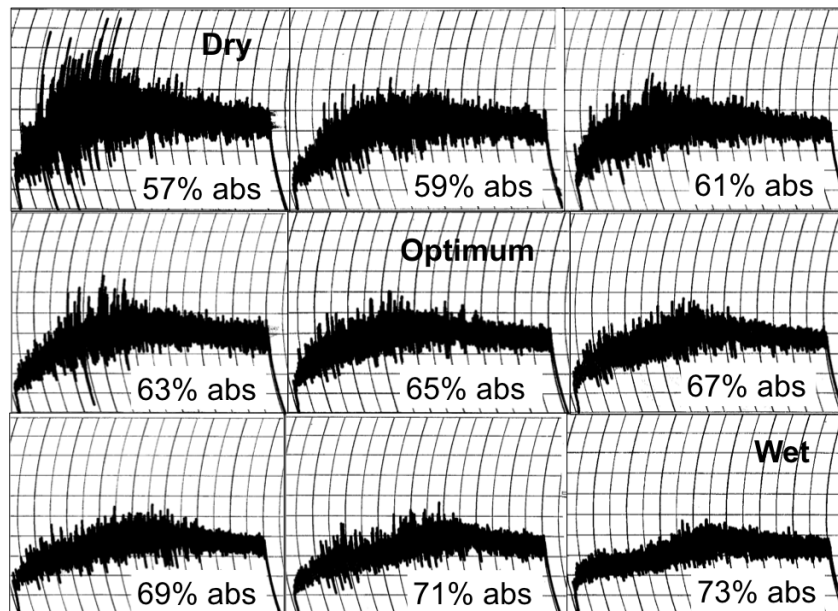


**Figure 4.1 An example mixograph water absorption series showing dough hydration and mixing characteristics for wet to dry water absorption levels in a Farnum flour sample grown at Arlington.**

The calculated water absorption tolerance values and classifications for all the flour samples are in Table 4.1 and Figure 4.2. Farnum and OR2080156H grown at Arlington had the highest water absorption tolerance range (1.6mL) while Farnum, Eddy, Paladin and OR2080227H grown at Pendleton exhibited the lowest interval (0.4mL). Just over half of the samples grown at Arlington possessed a higher water absorption tolerance than their Pendleton counterpart; however, the opposite occurred for 5 varieties (Bauermeister, BC002-2-2, OR2070174H, OR2070178H, and Palomino). Three varieties (Agripro 79-5-2, Norwest553, and Whetstone) had the same water absorption tolerance for both locations.

**Table 4.1 Water absorption tolerance range (mL) and classification identified by evaluation of mixograph water absorption series**

| Variety | Arlington | | Pendleton | |
|---|---|---|---|---|
| | Range | Class | Range | Class |
| Agripro 79-5-1 | NA | NA | 1.2 | Medium |
| Agripro 79-5-2 | 1.2 | Medium | 1.2 | Medium |
| Bauermeister | 0.6 | Low | 1.0 | Medium |
| BC002-2-2 | 1.0 | Medium | 1.3 | High |
| Boundary | 1.0 | Medium | 0.6 | Low |
| EDDY | 1.0 | Medium | 0.4 | Low |
| Farnum | 1.6 | High | 0.4 | Low |
| IDO660 | 1.0 | Medium | 0.8 | Low |
| Norwest553 | 0.6 | Low | 0.6 | Low |
| OR2070174H | 0.6 | Low | 1.2 | Medium |
| OR2070181H | 1.2 | Medium | 1.0 | Medium |
| OR2080111H | 1.4 | High | 1.0 | Medium |
| OR2080156H | 1.6 | High | 1.0 | Medium |
| OR2080178H | 1.0 | Medium | 1.2 | Medium |
| OR2080227H | 1.2 | Medium | 0.4 | Low |
| OR2080229H | 1.4 | High | 1.2 | Medium |
| OR2080236H | 1.4 | High | 1.0 | Medium |
| Paladin | 1.0 | Medium | 0.4 | Low |
| Palomino | 0.6 | Low | 1.0 | Medium |
| Whetstone | 0.6 | Low | 0.6 | Low |

## Water Absorption Tolerance
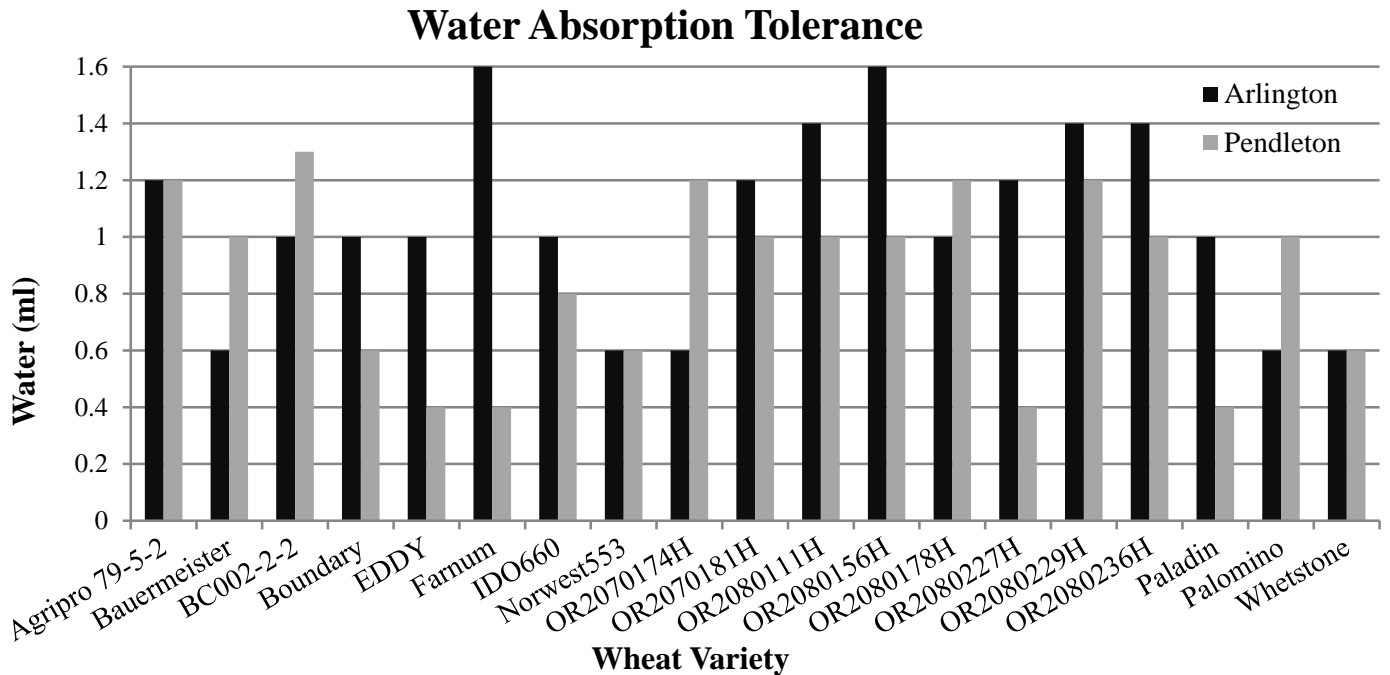


**Figure 4.2 Water absorption tolerance range (mL) of crop year 2011 PNW sample flours**

## 4.2 Correlations between tested parameters and water absorption tolerance

Correlation coefficients and p-values for all tested parameters and water absorption tolerance are reported in Table 4.2. Average kernel weight ($r = 0.389$, p-value = 0.014) and average kernel diameter ($r = 0.37$, p-value = 0.021) had a statistically significant but low correlation with water absorption tolerance while other grain characteristics including test weight (p-value = 0.403) and flour yield (p-value = 0.347) did not. The lack of correlation between protein content and kernel hardness (p-value = 0.336) seen in this study was also found by Pomeranz et al (1985); while, Peterson et al (1992) found a moderate negative genotypic correlation ($r = -0.43$) and insignificant small phenotypic correlation ($r = 0.06$). The insignificance of NIR flour moisture (p-value = 0.683) and protein (p-value = 0.219) revealed inherent moisture and protein quantities are not major factors affecting water absorption tolerance.

Flour components such as damaged starch, pentosans, gluten proteins, and ash are well known to affect flour water absorption. The Solvent Retention Capacity (SRC) test measures flour constituents' imbibing capacity by using different solvents. The SRC profile including the flour absorption level in each solvent indicates adequacy of flour for certain baked product applications. For bread production, high water absorption, good gluten strength, and sufficient

damaged starch and arabinoxylan contents are required (Kweon et al 2011). A lack of correlation between all SRC solvents and water absorption tolerance (sucrose p-value = 0.772, lactic acid p-value = 0.191, sodium carbonate p-value = 0.709, and water p-value = 0.393) indicates pentosans, gluten protein content, starch damage level, and the combined flour components were not causes for the water absorption tolerance effect observed. Since pentosan content decreases relative to the endosperm as grain filling occurs, the moderate negative correlations observed between SRC sucrose (a test evaluating pentosan content) and test weight (r= -0.47, p-value=0.003) and flour yield (r= -0.398, p-value= 0.012) is logical. Theoretically, the SRC sodium carbonate test should have correlated with water absorption tolerance since the test evaluates the effect of starch damage and the damaged starch content of the flours, as found by enzymatic analysis, positively and significantly correlated (r = 0.33, p-value = 0.04) to water absorption tolerance. The lack of significant correlation could be attributed to the indirect manner used by the SRC sodium carbonate test to evaluate starch damage. The sodium carbonate solvent targets and swells amylopectin prematurely exposed from starch granule degradation (Kweon et al 2011); whereas, the enzymatic evaluation method hydrolyses all free dextrose to glucose for direct starch damage quantification. As expected for hard wheat flour, the damaged starch damage content correlated significantly with average kernel hardness (r = 0.454, p-value = 0.004); however, average kernel hardness did not significantly correlate with water absorption tolerance (p-value = 0.708). The inorganic material content of the flours did not greatly impact water absorption tolerance as seen by an insignificant correlation with flour ash (p-value = 0.564).

A nonsignificant correlation with water absorption tolerance was found for the glutenin (p-value = 0.328), gliadin (p-value = 0.332), and albumin/globulin (p-value = 0.752) protein fractions; while, significant but low correlations with water absorption tolerance were seen for the extractable polymeric protein (EPP) (r = 0.317, p-value = 0.049) and unextractable polymeric protein UPP (r= -0.317, p-value = 0.049) fractions. Rattin (2011) also found a significant low correlation with water absorption tolerance for EPP (r= 0.289, p-value= 0.025) and UPP (r= -0.289, p-value= 0.025). A strong negative correlation was found between glutenin and gliadin (r = -0.836, p-value = <0.0001) due to the two fractions constituting the majority of wheat protein (87%, by variety). Gupta et al (1992) and Panozzo and Eagles (2000) observed the same proportionality and interaction. For such compositional data a negative correlation is statistically inevitable (Aitchison 1986).

**Table 4.2 Correlation coefficients and p-values for water absorption tolerance and tested parameters on 2011 crop year wheat and flour samples**

| | Water Abs Tol | Test Weight | Flour Yield | Flour Moisture | Flour Protein | Kernel Weight | Kernel Diameter | Kernel Hard | Sucrose | Lactic Acid | Water | Na$_2$Co$_3$ | Starch Damage | Flour Ash | Glutenin | Gliadin | Albumin/Globulin | EPP | UPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Water Abs Tol** | 1.000 | 0.138<br>0.403 | -0.155<br>0.347 | 0.068<br>0.683 | -0.201<br>0.219 | **0.389**<br>**0.014** | **0.370**<br>**0.021** | -0.062<br>0.708 | 0.048<br>0.772 | -0.214<br>0.191 | 0.141<br>0.393 | 0.062<br>0.709 | **0.330**<br>**0.040** | -0.095<br>0.564 | -0.161<br>0.328 | 0.159<br>0.332 | -0.052<br>0.752 | **0.317**<br>**0.049** | **-0.317**<br>**0.049** |
| **Test Weight** | | 1.000 | 0.241<br>0.140 | 0.288<br>0.075 | **-0.470**<br>**0.003** | **0.754**<br>**<0.0001** | **0.786**<br>**<0.0001** | **-0.366**<br>**0.022** | **-0.411**<br>**0.009** | -0.186<br>0.257 | -0.221<br>0.177 | **-0.393**<br>**0.013** | 0.252<br>0.122 | **-0.692**<br>**<0.000** | -0.143<br>0.386 | 0.174<br>0.290 | -0.105<br>0.524 | -0.069<br>0.675 | 0.069<br>0.675 |
| **Flour Yield** | | | 1.000 | -0.267<br>0.101 | **-0.398**<br>**0.012** | **0.342**<br>**0.033** | 0.267<br>0.100 | **-0.368**<br>**0.021** | **-0.551**<br>**0.000** | **-0.391**<br>**0.014** | **-0.400**<br>**0.012** | -0.288<br>0.076 | 0.108<br>0.514 | -0.215<br>0.189 | 0.059<br>0.723 | -0.189<br>0.249 | 0.258<br>0.113 | 0.142<br>0.387 | -0.142<br>0.387 |
| **Flour Moisture** | | | | 1.000 | 0.177<br>0.281 | 0.232<br>0.155 | 0.288<br>0.076 | -0.292<br>0.072 | -0.136<br>0.408 | 0.141<br>0.392 | **-0.341**<br>**0.034** | **-0.485**<br>**0.002** | -0.235<br>0.150 | **-0.470**<br>**0.003** | -0.005<br>0.977 | 0.137<br>0.406 | -0.243<br>0.137 | -0.203<br>0.216 | 0.203<br>0.216 |
| **Flour Protein** | | | | | 1.000 | **-0.600**<br>**<0.0001** | **-0.556**<br>**0.000** | 0.158<br>0.336 | **0.497**<br>**0.001** | **0.489**<br>**0.002** | -0.064<br>0.701 | -0.086<br>0.603 | **-0.601**<br>**<0.0001** | 0.306<br>0.059 | -0.055<br>0.742 | 0.305<br>0.059 | **-0.475**<br>**0.002** | -0.232<br>0.155 | 0.232<br>0.155 |
| **Kernel Weight** | | | | | | 1.000 | **0.975**<br>**<0.0001** | **-0.570**<br>**0.000** | **-0.350**<br>**0.029** | **-0.477**<br>**0.002** | -0.129<br>0.434 | -0.307<br>0.057 | **0.364**<br>**0.023** | **-0.559**<br>**0.000** | -0.153<br>0.352 | 0.160<br>0.330 | -0.065<br>0.692 | 0.238<br>0.144 | -0.238<br>0.144 |
| **Kernel Diam** | | | | | | | 1.000 | **-0.568**<br>**0.000** | **-0.326**<br>**0.043** | **-0.416**<br>**0.009** | -0.156<br>0.344 | **-0.342**<br>**0.033** | **0.339**<br>**0.035** | **-0.555**<br>**0.000** | -0.124<br>0.452 | 0.156<br>0.343 | -0.101<br>0.542 | 0.156<br>0.342 | -0.156<br>0.342 |
| **Kernel Hard** | | | | | | | | 1.000 | **0.400**<br>**0.012** | 0.226<br>0.168 | **0.619**<br>**<0.0001** | **0.708**<br>**<0.0001** | **0.454**<br>**0.004** | **0.466**<br>**0.003** | 0.291<br>0.072 | **-0.353**<br>**0.028** | 0.212<br>0.195 | 0.071<br>0.668 | -0.071<br>0.668 |
| **Sucrose** | | | | | | | | | 1.000 | **0.425**<br>**0.007** | **0.655**<br>**<0.0001** | **0.609**<br>**<0.0001** | -0.022<br>0.894 | **0.396**<br>**0.013** | -0.154<br>0.350 | 0.223<br>0.173 | -0.178<br>0.279 | -0.036<br>0.830 | 0.036<br>0.830 |
| **Lactic Acid** | | | | | | | | | | 1.000 | 0.167<br>0.311 | 0.128<br>0.438 | **-0.401**<br>**0.011** | -0.078<br>0.636 | 0.092<br>0.579 | 0.012<br>0.941 | -0.158<br>0.337 | **-0.694**<br>**<0.000** | **0.694**<br>**<0.000** |
| **Water** | | | | | | | | | | | 1.000 | **0.919**<br>**<0.0001** | **0.580**<br>**0.000** | **0.362**<br>**0.024** | 0.049<br>0.768 | -0.091<br>0.583 | 0.093<br>0.574 | 0.108<br>0.513 | -0.108<br>0.513 |
| **Na$_2$Co$_3$** | | | | | | | | | | | | 1.000 | **0.541**<br>**0.000** | **0.455**<br>**0.004** | 0.090<br>0.588 | -0.188<br>0.252 | 0.210<br>0.199 | 0.178<br>0.279 | -0.178<br>0.279 |
| **Starch Damage** | | | | | | | | | | | | | 1.000 | 0.077<br>0.640 | 0.200<br>0.221 | -0.296<br>0.068 | 0.242<br>0.137 | **0.370**<br>**0.020** | **-0.370**<br>**0.020** |
| **Flour Ash** | | | | | | | | | | | | | | 1.000 | 0.195<br>0.234 | -0.233<br>0.153 | 0.137<br>0.407 | 0.060<br>0.718 | -0.060<br>0.718 |
| **Glutenin** | | | | | | | | | | | | | | | 1.000 | **-0.836**<br>**<0.0001** | 0.045<br>0.785 | -0.285<br>0.079 | 0.285<br>0.079 |
| **Gliadin** | | | | | | | | | | | | | | | | 1.000 | **-0.585**<br>**<0.0001** | 0.140<br>0.397 | -0.140<br>0.397 |
| **Albumin/Globulin** | | | | | | | | | | | | | | | | | 1.000 | 0.167<br>0.308 | -0.167<br>0.308 |
| **EPP** | | | | | | | | | | | | | | | | | | 1.000 | **-1.000**<br>**<0.000** |

Within each cell the correlation coefficient (r) is listed first and the p-value is below. Bolded values are significant at a p < 0.05 level.

## 4.3 Effect of genotype and environment on water absorption tolerance

Table 4.3 displays the environmental and varietal effects on all parameters tested. Neither a varietal nor environment influence on water absorption tolerance range was shown to be significant. This could be due to the phenomenon not being easily quantifiable despite its known existence from experienced bakers' observations. The large residual error resulting from using the genetic by environment interaction as the error term, out of necessity since only two locations were available to compare between for each variety, decreased the F-value and caused null hypothesis acceptance. This indicates water absorption tolerance did not differ between varieties or locations. Substituting the location by variety interaction for the "real" error term (which would be seen if another location were available) produced an elevated error term and decreased the test's precision for differentiating location and variety causal effects.

Significant varietal difference in kernel hardness and perhaps in milling characteristics (bran clean up, reduction, and sifting properties) would account for the unexpected lack of correlation between flour yield and test weight (Bequette 1989). The varietal significance of kernel hardness (p-value = <0.0001), SRC sodium carbonate (p-value = <0.0001), and percent of damaged starch (p-value = <0.0001) is logical because the SRC sodium carbonate test evaluates starch damage, which is directly related to the varietal trait kernel hardness (Bergman et al 1998, Blumenthal et al 1993, Bequette 1989).

The observed significant varietal and environmental influences seen on test weight (p-value = 0.0053) and flour ash (p-value = (<0.0281) content are in agreement with established wheat breeding research (Bequette 1989, Blumenthal et al 1993). Protein content is also considered a varietal trait influenced by environment (Bequette 1989, Blumenthal et al 1993); however, only the environmental influence on protein content (p-value = 0.0021) proved significant in this flour sample set. Graybosch et al (1996) also reported a greater environmental influence on flour protein for hard winter wheat grown on the USA Great Plains. A significant environmental influence on kernel hardness (p-value = <0.0001) further indicates a difference in environment between growing sites (Symes 1965, Pomeranz et al 1985) despite no statistical difference in climate data. Pomeranz et al (1985) examined kernel hardness of 15 varieties varying in hardness classification grown under 11 different climatic conditions. Even though

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genotypic influence had the greatest impact, a small environmental influence was also significant.

Climate data collected from the collaborative farms in Arlington and Pendleton, OR for the 2010-2011 growing season were analyzed (Appendix A). No statistically significant difference between locations was found for average monthly minimum and maximum temperatures or precipitation (Table A.5). While no significant difference between seasonal periods occurred for precipitation, the minimum and maximum temperatures for period 4 (Jun.-Aug.) (12.5 and 26.7°C, respectively) were significantly higher than during the rest of the year (Table A.6). The maximum temperatures for period 3 (Mar.- May) and period 1 (Sept. – Nov.) (14.8 and 14.9°C, respectively) were statistically the same and significantly higher than period 2 (Dec.-Feb.) (6.5°C) as would be expected during the winter months. This temperature and precipitation profile of the Arlington and Pendleton growing sites indicates the growing climate is relatively mild and temperate.

Of the three prominent wheat protein fractions (glutenin, gliadin, and albulin/globulin), no environmental impact was observed statistically and only glutenin exhibited a significant varietal influence (p-value = 0.031). Graybosch et al (1996) and Panozzo and Eagles (2000) found varietal variation in glutenin to be greater than environmental variation for hard winter wheat grown on the USA Great Plains and wheat of various classifications grown in southern Australia, respectively. For both studies, a smaller varietal variation in gliadin (relative to glutenin) occurred; however, in the present study the p-value for gliadin increased above significance (0.090). This difference could be due to the capability of precisely dividing the variance observed between environmental, varietal, and environment by variety interaction factors in the previous studies due to more locations for each variety. Whereas, for the present study the interaction term was used for experimental error, out of necessity, and ANOVA analysis was used to test for environmental or genetic influence. The cultivar means for glutenin ranged from 36.0 to 40.1% while for gliadin 46.9 to 51.6%.

EPP and UPP contents were significantly influenced by variety (p-value = <0.0001) and environment (p-value = 0.039) as was also seen in a similar study using 5 Kansas wheat varieties in 6 locations (Rattin 2011). The cultivar means for EPP ranged from 58.53 to 48.30% and varieties OR2080227H and Palomino exhibited the highest and lowest levels, respectively. The UPP cultivar means varied from 41.47 to 51.70%. A reversal in varietal ranking occurred

(Palomino and OR2080227H had the highest and lowest levels, respectively) because UPP constitutes the remainder of polymeric protein not accounted for by EPP.

**Table 4.3 Location and varietal influence on tested parameters**

| Response Measured | Location Influence (p value) | Varietal Influence (p value) |
|---|---|---|
| Water absorption tolerance range | 0.0830 | 0.4811 |
| Test weight | **<0.0001** | **0.0053** |
| Flour yield | 0.1102 | 0.0695 |
| Flour Moisture | 0.1848 | 0.6688 |
| Flour Protein | **0.0021** | 0.4807 |
| Kernel weight | **<0.0001** | **0.0006** |
| Kernel diameter | **<0.0001** | **0.0002** |
| Kernel hardness | **<0.0001** | **<0.0001** |
| SRC Sucrose | **0.0070** | 0.0899 |
| SRC Lactic acid | **<0.0001** | **<0.0001** |
| SRC Water | **0.0134** | **<0.0001** |
| SRC Sodium carbonate | **<0.0001** | **<0.0001** |
| Starch damage | **0.0001** | **<0.0001** |
| Flour ash | **0.0015** | **0.0281** |
| Glutenin | 0.4042 | **0.0309** |
| Gliadin | 0.1327 | 0.0903 |
| Albumin/Globulin | 0.1806 | 0.5003 |
| Extractable Polymeric Protein (EPP) | **0.0392** | **<0.0001** |
| Unextractable Polymeric Protein (UPP) | **0.0392** | **<0.0001** |

Note: Bolded p-values are significant at a $p < 0.05$ level

## 4.4 SDS-PAGE analysis

SDS-PAGE analysis was conducted on the sample pairs of 8 varieties (Farnum, Norwest 553, OR2080178H, OR2080227H, IDO 660, Whetstone, Agripro 79-5-2, OR2070181H) exhibiting an intriguing water absorption tolerance trend between sample locations in order to separate the EPP, observe differences in composition, and possibly link the absence or abundance of a certain fraction to the water absorption tolerance phenomenon. A previous study using Kansas wheat varieties (Rattin 2011) found a significantly higher concentration of γ-gliadins in high water absorption tolerant flour and theorized that the abundance of γ-gliadins rich in sulfur (17-22 cysteine residues/100,000g of protein) (Tatham and Shewry 1985, Charbonnier et al 1980) permitted more disulfide bonding with HMW and LMW glutenin which

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enabled more water incorporation in the protein network without weakening the dough. However, for the current study no distinctive differences were found in protein separation pattern or concentration (as appraised visually by band color intensity) within the same flour variety grown at different locations or between high and low water tolerant varieties (Figures 4.3 and 4.4). This indicates another unidentified factor is responsible for the water absorption tolerance phenomenon observed in this sample set.
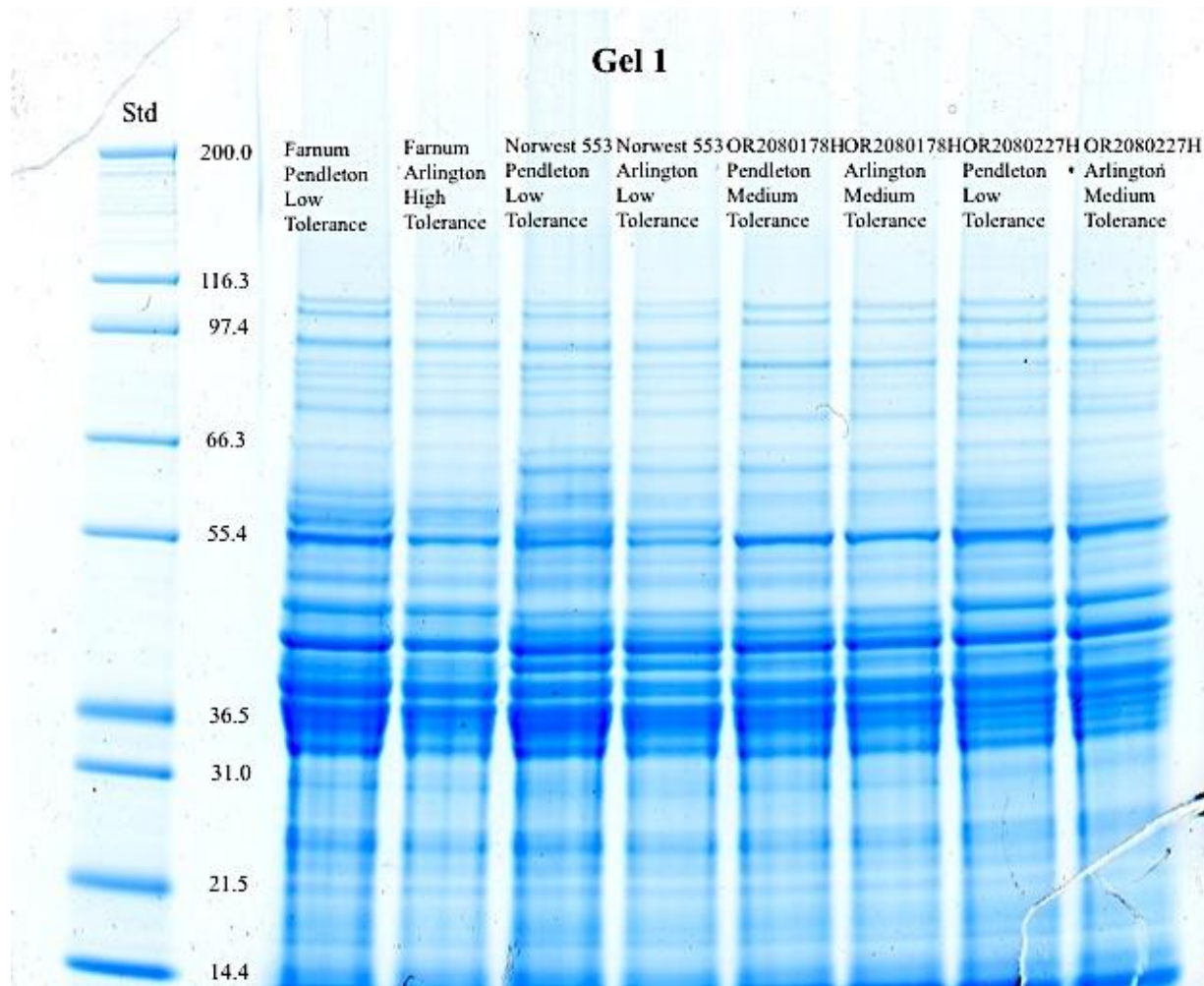


**Figure 4.3 SDS-PAGE electrophoresis gel showing the extractable polymeric protein fraction of flour samples (labeled by variety, location, and water absorption tolerance classification). The protein standards units are kDa.**
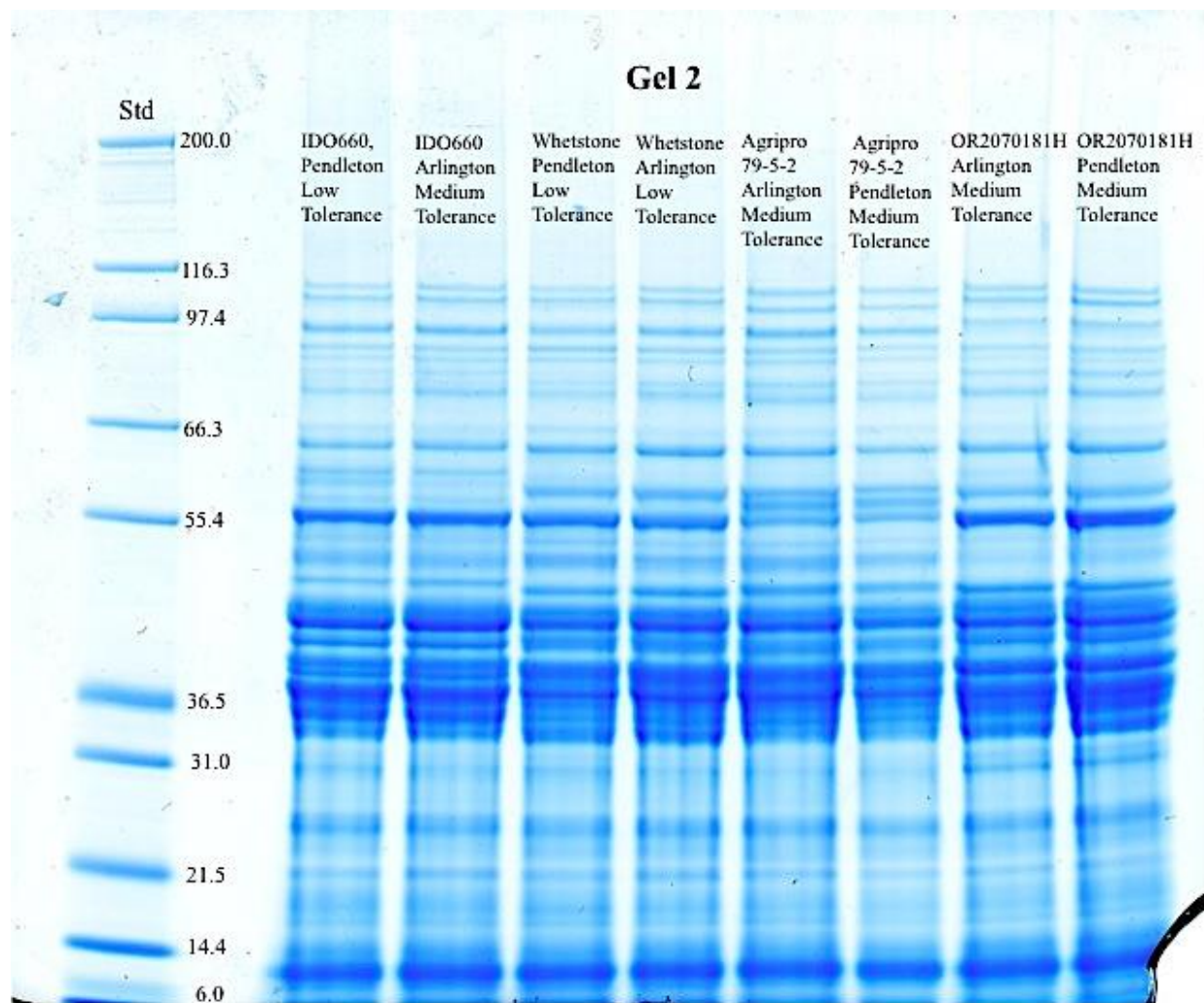
**Figure 4.4 SDS-PAGE electrophoresis gel showing the extractable polymeric protein fraction of flour samples (labeled by variety, location, and water absorption tolerance classification). The standard units are kDa.**

# Chapter 5 - Conclusions

- Differences in level of water absorption tolerance were observed between the 2011 crop year Oregon wheat flour samples; however, neither location nor varietal influences on the behavior proved statistically significant.

- Average kernel weight, average kernel diameter, percent damaged starch, EPP and UPP exhibited a statistically significant but low correlation with water absorption tolerance.

- Protein quantity, flour moisture, and pentosan content were not factors affecting water absorption tolerance.

- EPP and UPP content varied significantly between varieties and locations.

- This study confirmed a correlation between EPP and water absorption tolerance as observed in a previous Kansas wheat study (Rattin 2011).

# Areas for Future Research

- Further research is needed to identify the influential factor (i.e. a sub-protein fraction) causing the water absorption tolerance phenomenon. In order to more precisely test the exceptional water holding capacity of some unique wheat flours and attribute the behavior to environmental, genetic, and/or genetic x environmental interaction influences, the experimental statistical residual error must be reduced. This could be achieved by conducting the same water absorption tolerance evaluation and biochemical characterization analysis carried out in this study on flours from wheat varieties with known high or low water absorption tolerance cultivated under specified conditions at several locations with distinctly different climates. Conducting a study using the same wheat varieties over multiple crop years and in different environments would help identify varieties that resiliently exhibit high water absorption tolerance behavior despite unpredictable climactic changes.

- Additionally with adequate sample size, test baking could be performed to potentially correlate water absorption tolerance with protein quality via loaf volume assessment. Given the insignificance of protein content in this study and the theorized functional role of γ-gliadins, researching protein quality would be beneficial for determining the underlying cause of water absorption tolerance.

- Examining other wheat classifications for the water absorption tolerance behavior could prove useful in determining the flour components involved.

- Once the influential factor is identified, a model could be developed which predicts the water absorption tolerance level in any wheat variety, given the factor content is quantified from the flour. This would increase the wheat variety's marketability and add value to the wheat commodity processing chain.

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# Appendix A - Environmental Data Summary

## A.1 Growing Conditions

**Table A.1 Soil Conditions**

| Farm Location | Soil Type | Plant and Harvest Dates |
|---|---|---|
| Arlington, OR | Ritzville silt loam 2-7% slope | 9/24/10 - 7/27/11 |
| Pendleton, OR | Athena silt loam 1-7% slope | 10/11/10 - 8/18/11 |

**Table A.2 Precipitation level (in)**

| 2011 Crop Year | Arlington, OR | Pendleton, OR |
|---|---|---|
| September | 2.47 | N/A |
| October | 1.26 | 1.79 |
| November | 1.33 | 1.43 |
| December | 3.05 | 3.43 |
| January | 0.6 | 1.4 |
| February | 0.38 | 1 |
| March | 1.99 | 2.04 |
| April | 0.7 | 1.18 |
| May | 1.34 | 2.88 |
| June | 0.95 | 1.62 |
| July | 0.08 | 0.24 |
| August | N/A | 0.03 |

**Table A.3 Maximum Temperature** (°C)

| 2011 Crop Year | Arlington, OR | Pendleton, OR |
|---|---|---|
| September | 25.2 | N/A |
| October | 18.7 | 16.9 |
| November | 9.0 | 7.3 |
| December | 4.4 | 5.9 |
| January | 6.6 | 6.6 |
| February | 7.8 | 7.4 |
| March | 12.1 | 12.7 |
| April | 14.7 | 12.6 |
| May | 19.5 | 17.2 |
| June | 24.1 | 22.4 |
| July | 28.8 | 27.5 |
| August | N/A | 30.8 |

**Table A.4 Minimum Temperature** (°C)

| 2011 Crop Year | Arlington, OR | Pendleton, OR |
|---|---|---|
| September | 12.8 | N/A |
| October | 6.6 | 5.7 |
| November | 1.0 | -1.9 |
| December | -0.6 | -1.9 |
| January | -0.5 | -1.6 |
| February | -1.6 | -1.8 |
| March | 1.6 | 2.1 |
| April | 3.3 | 1.4 |
| May | 7.9 | 5.4 |
| June | 12.6 | 8.9 |
| July | 15.0 | 11.1 |
| August | N/A | 13.3 |

*Climate ANOVA and LSM analysis*

**Table A.5 Climate analysis by ANOVA**

| Location | Average Monthly Minimum Temperature (°F)<br>F-value= 3.11<br>p-value= 0.10 | Average Monthly Maximum Temperature (°F)<br>F-value= 0.61<br>p-value= 0.45 | Average Monthly Precipitation (in)<br>F-value= 0.64<br>p-value= 0.44 |
|---|---|---|---|
| Arlington | 5.3 a | 15.5 a | 1.29 a |
| Pendleton | 3.7 a | 15.2 a | 1.55 a |

Note: Values with the same letter indicate no significant difference.

**Table A.6 Period comparison of climate by LSM analysis**

| Period | Average Minimum Temperature (°F)<br>F-value= 16.68<br>p-value= <0.0001 | Average Maximum Temperature (°F)<br>F-value= 18.56<br>p-value= <0.0001 | Average Precipitation (in)<br>F-value= 1.60<br>p-value= 0.23 |
|---|---|---|---|
| 1 Sept. - Nov. | 4.3 a | 14.9 b | 1.64 a |
| 2 Dec. – Feb. | -1.3 a | 6.5 a | 1.64 a |
| 3 Mar. – May | 3.6 a | 14.8 b | 1.69 a |
| 4 Jun. – Aug. | 12.5 b | 26.7 c | 0.57 a |

Note: Values with the same letter indicate no significant difference.