EFFECT OF GENOTYPE AND ENVIRONMENT ON HARD WHEAT WATER ABSORPTION TOLERANCE

by

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Abstract

Water absorption tolerance is an important parameter in commercial bread production. Hard winter wheat (HWW) flours have shown different water absorption tolerance behaviors in routine wheat quality analysis. Flours with high water absorption tolerance allow broader variation in water addition without affecting optimum dough conditions. Although studies have demonstrated that genotype and environmental factors affect optimum water absorption, mixing time and dough strength, there is no research defining or quantifying water absorption tolerance behavior or explaining reasons and factors affecting such behavior and its correlation with end product quality. Using the mixograph®, this study identified high and low absorption tolerance behaviors in five HRW varieties (Jagger, Jagalene, Fuller, 2137 and Overley) grown in six locations (Finney, Labette, Republic, Thomas, Riley and Sumner Counties) in Kansas, during crop year 2009. Milling, wheat and flour quality tests, Solvent Retention Capacity (SRC), damaged starch, protein composition and identification, flour and starch particle size distribution and bread baking tests were conducted and analyzed. Jagger grown at Finney County possessed the largest water absorption tolerance range while the smallest range was observed for Fuller grown at Riley and Sumner Counties. A positive high correlation was observed between water absorption tolerance and the following parameters: protein content, kernel hardness, extractable polymeric proteins, unextractable polymeric proteins, gliadins and flour particle size (41-300 μm). SDS PAGE and proteomic analyses determined that γ-gliadins were present in a significantly higher concentration in the high tolerance sample suggesting that these proteins play a primary role in water absorption tolerance behavior. The five wheat varieties grown at locations with high and low water absorption tolerance were blended together by location and test baked at three different absorption levels. Loaf volume varied between flours but did not vary between different water levels. It is unclear if this was an effect of the differing protein contents of the blends, water tolerance or both. However, a negative effect on crumb grain characteristics was observed when lower water levels were used.

Table of Contents

List of Figures

List of Tables

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Dedication

To my parents, for all the opportunities and love they have given me in my life. To my grandparents *(in memorium*), for all good moments we had together and for their example of honesty, kindness and hard work.

CHAPTER 1 - Introduction

Hard winter wheat (HWW) is widely used for breadmaking. It has been observed during routine wheat quality analysis that HWW flours exhibit different water absorption tolerance behaviors. Flours with low absorption tolerance show dramatic changes in mixogram curves with small changes in water addition. Doughs made with these flours would easily become too wet (slack and sticky) or too dry (stiff and bucky). This would, in turn, affect dough processing and bread quality parameters such as texture and volume. Mixogram curves from flours with high absorption tolerance show no or little change when water addition is varied. Because these changes are well tolerated, optimum dough handling properties and bread quality are more stable.

Water absorption tolerant varieties are more desirable in commercial bakeries as small variations in water addition would not affect end product quality. Commercial bakeries generally use water absorptions based on flour specifications and do not perform optimum absorption tests or water absorption tolerance tests for each flour received. Tolerant varieties would be of great interest and value to wheat breeders and the baking industry.

Broadly speaking, wheat flour is composed of starch (≈ 75 %), water (≈ 14 %), protein (≈ 10-12 %), arabinoxylans (≈ 2%) and lipids (≈ 2%) (Goesaert et al 2005). It is known that hardness and protein content are correlated with bread quality and optimum water absorption (Carson and Edwards 2009; Finney and Shogren 1972). However, the role of starch, flour particle size and arabinoxylans in controlling absorption are still not clear. Some research has been done investigating the effect of various ingredients on Mixograph®, Farinograph® and test baking characteristics (Ram et al 2005). However, there is no research defining water absorption tolerance behavior or explaining the factors affecting such behavior and its correlation with end product quality in HWW varieties. For the research in this thesis, a broad sample set composed of varieties grown in different locations in a single crop year was used for genotype and environmental effect determination. The Mixograph® was used to determine flour absorption tolerance ranges. Solvent Retention Capacity was employed as a tool to identify flour components possibly related with water absorption tolerance. Subsequent tests were selected based on the results obtained.

Objectives

The focus of this research work was to identify genotype and environmental effects on water absorption tolerance behavior in HRW wheat varieties grown in different locations within the state of Kansas.

The main objectives were:

1. To classify hard red winter wheat varieties grown in different locations in 2009 as tolerant or intolerant to changes in absorption.
2. To identify environmental and genetic effects on water absorption tolerance in these varieties.
3. To determine chemical and/or physical factors and mechanisms affecting water absorption tolerance.

CHAPTER 2 - Literature Review

2.1 - Wheat kernel and flour characteristics

2.11 The wheat kernel

The wheat kernel is a single seeded caryopsis (Cornell and Hoveling 1998; Delcour and Hoseney 2010; Wrigley 2009). The pericarp surrounds the seed and is composed of several layers. The inner pericarp is formed by intermediate, cross and tube cells. The seed coat is firmly joined to the tube cells on their side and consists of three layers as well. The endosperm is composed of the aleurone layer and the starchy endosperm. The aleurone layer, nucellar epidermis, the seed coat and the pericarp become the "bran" as isolated by the milling process. Thus, the bran is composed of multiple layers of crushed cells, high in cellulose, hemicelluloses, ash and the aleurone layer (Cornell and Hoveling 1998). The starchy endosperm cells produce the flour when reduced to appropriate particle size. They are packed with starch granules embedded in a protein matrix. The germ makes up 2.5-3.5 % of the kernel, is rich in protein and lipids (Cornell and Hoveling 1998; Eliasson and Larsson 1993) and consists of two parts: the embryonic axis and the scutellum (Delcour and Hoseney 2010). The starchy endosperm is composed of starch and proteins including albumins, globulins, glutenins and gliadins (Cornell and Hoveling 1998). As the grain develops, storage proteins present in protein bodies are deformed into a continuous matrix by the developing starch granules (Cornell and Hoveling 1998; MacMasters et al 1978). Large lenticular starch granules are synthesized first then smaller spherical granules are synthesized, filling the spaces between the large ones (Eliasson and Larsson 1993). The wheat kernel is generally classified as hard or soft, red or white, spring or winter (Cornell and Hoveling 1998). Grain color is determined by the intensity and amount of red pigmentation in the seed coat. Wheat endosperm hardness is controlled by presence or absence of friabilin protein on the starch granules' surfaces (Wrigley 2009).

2.12 Wheat flour composition

Wheat flour has unique properties which are essential to the manufacture of many wheat-based products such as bread, breakfast cereals, snacks and pasta (Puppo et al 2005). Wheat flour is the main ingredient obtained from the kernel and is mainly composed of starch (70-75%),

water (14%) and protein (10-12%). Other components, including arabinoxylans (2%) and lipids (2%), are also present in smaller amounts (Goesaert et al 2005).

Starch represents the most prevalent component in flour and contributes to dough viscoelasticity by acting as a filler. It is actively involved in the baking process when gelatinization occurs. Retrogradation (a starch phenomenon) affects bread quality (staling) after storage (Stauffer 1999). Up to 10% of hard wheat starch (flour weigh basis) can be damaged during the milling process. Damaged starch increases water absorption and is more susceptible to alpha amylase activity (Stauffer 1999).

Wheat storage proteins are composed of glutelins (glutenins) and prolamins (gliadins) that form gluten when hydrated. These are the proteins responsible for dough's viscoelastic properties. A small portion of the endosperm proteins are water soluble (albumins) and salt soluble (globulins) (Eliasson and Larsson 1993; Stauffer 1999).

Pentosans (arabinoxylans) make up ≈ 2-2.5% of the flour. They are very hydrophilic molecules that absorb ten times their weight of water (Roels et al 1993; Vanhamel et al 1993; Zhang et al 2007) and can form covalent linkages with gluten proteins (Stauffer 1999). They exist as water in extractable (WE) and unextractable (WU) forms. It has been reported that the addition of pentosans to dough results in stiffer and drier dough due to formula water immobilization resulting in negative effects on dough handling and bread quality (Kulp and Bechtel 1963; Kulp 1968). Poor quality flours had a higher level of water extractable pentosans (Roels et al 1993). An increase in loaf volume was observed when arabinoxylans in flour were first hydrolyzed by arabinoxylonase enzymes (Eliasson and Larsson 1993; Stauffer 1999).

Lipids comprise ≈ 2.5% of flour, with 1% of that being non-polar lipids and 1.5% being polar (galactosyl glycerides and phospholipids) lipids. Although they complex with gluten proteins during mixing and may affect bread quality, they have little or no effect on the mixograph® curve characteristics (Eliasson and Larsson 1993; Stauffer 1999).

2.13 Wheat flour proteins and MW distribution characterization

It is known that wheat flour functionality is directly related to gluten protein content (Goesaert et al 2005). Gluten is composed of two fractions, glutenins and gliadins. Glutenin is responsible for elastic properties while gliadins act as a plasticizer (Puppo et al. 2005; Southan and MacRitchie 1999; Verbruggen and Delcour 2003). Together these protein classes form a

continuous network and cohesive dough during mixing (Bean et al 1998). The role of non-gluten proteins such as enzymes, enzymes inhibitors, lipid binding proteins and triticins are not as well known (Goesaert et al 2005).

Protein molecular weight distribution (MWD) is one of the most important parameters determining flour functional properties. Although it is not a static property, it is the MWD of gluten protein in the mature grain that drives dough properties. These properties may change during storage, mixing and processing. MWD is affected by the number of cysteines and SH groups available. The availability of SH groups is proposed as a mechanism providing for dough strength (Wrigley 2004). In this model, a glutenin subunit needs to have two cysteine residues in order to participate in polymer growth. If some subunits are modified (gliadins for example) resulting in presence of a odd number of cysteine residues, the polymer molecular weight will be shifted to a lower value (MacRitchie and Lafiandra 2001).

Gliadin and glutenins are classified as monomeric and polymeric proteins, respectively (Panozzo and Eagles 2000; Southan and MacRitchie 1999). In this case, monomeric proteins are composed of single chain polypeptides with molecular weights ranging from 20,000 (albumins and globulins) to 30,000-40,000 (α, β and γ gliadins) and 60,000-80,000 (ω-gliadins). Gliadins tend to aggregate through noncovalent bonding (hydrophobic and/or ionic bonds) (Hussain and Lukow 1997). α, β and γ gliadins have intramolecular disulphide bonds (S-S) between the sulphydryl (SH) groups of cysteine residues. ω-gliadins do not posses cysteine residues. α - gliadins posses six cysteine residues in their structure while γ gliadins and LMW glutenin subunits contain eight cysteines (Clarke et al 2003; Zhao et al 1999).

Studies suggest that the gliadins can be classified according to their content of sulphur-containing amino acids and electrophoretic mobility. Ω-gliadins are sulphur-poor prolamins while α, β and γ gliadins are sulphur-rich prolamins (Clarke et al 2003; Uthayakumaran et al 2001). Under gel electrophoresis, α-gliadins migrate the fastest and ω-gliadins are the slowest (Eliasson and Larsson 1993). ω-gliadins do not contain α helix or β-sheet structures; instead they contains β-turns and, thus, are able to form hydrogen bonds readily. α, β and γ gliadins posses α-helix structures and the ability to form hydrogen bonds which stabilize the structure (Eliasson and Larsson 1993). Ω-gliadins are the most hydrophilic while γ-gliadins are the most hydrophobic (Eliasson and Larsson 1993). As gliadin hydrophobicity increases, dough mixing time, maximum resistance to extension and peak resistance decreased while dough extensibility

increased. Doughs supplemented with gliadins added to them showed shorter mixing time, lower maximum resistance to extension and decreased loaf volume. Specifically, γ-gliadins were found to have positive effects on loaf quality. γ-gliadins were also positively correlated with dough strength, dough resistance, mixing tolerance and breadmaking (Uthayakumaran et al 2001).

Polymeric proteins are composed of multiple chains consisting of individual polypeptides are linked intermolecularly by disulfide bonds and form α-helix structures (Carceller and Aussenac 2001; Gupta et al 1992; Delcour and Hoseney 2010; Southan and MacRitchie 1999; Stauffer 1999). Based on molecular weights estimated by SDS-PAGE, A type HMW glutenin subunits range from 80,000 to 120,000 kDa, while B type subunits LMW glutenin are smaller (40,000 to 55,000 kDa). C subunits range from 30,000 to 40,000 kDa and are sulphur-rich prolamins. Due to the ability of some subunits to polymerize, glutenin polymer molecular weight can have wide variability. The lower limit is around 100,000 but the upper limit is so large that is not easily identified. Some studies report the biggest glutenin fraction eluted by gel permeation chromatography at $20x10^6$ (Southan and MacRitchie 1999).

Polymeric proteins have been recently differentiated/classified as extractable or unextractable polymeric proteins. Difference in solubility (extractability) is attributed to differences in MW distributions. The largest MW polymers are present in the unextractable fraction (Bean et al. 1998; Carceller and Aussenac 2001). HMW glutenin proteins are insoluble, primarily because they are composed of a high proportion of hydrophobic amino acids such as glycine, proline, glutamine and leucine with small proportions of acidic or basic amino acids (Khan and Bushuk 1978). The relative amount of polymeric or monomeric proteins may differ between varieties. The molecular weight distribution of polymeric protein and the monomeric: polymeric ratio are genetically controlled but can be modified by environmental conditions. That said, the relationship between monomeric: polymeric ratio and flour quality are not well established (Singh et al. 1990a; Southan and MacRitchie 1999). Glutenins from different flours may have different strengths because of differences in their molecular weights. This leads to difficulty when making comparisons between samples. It is known that the contributions of ionic, hydrogen and hydrophobic bonds to aggregation of glutenin proteins is higher in good quality gluten than in poor quality gluten (Stauffer 1999).

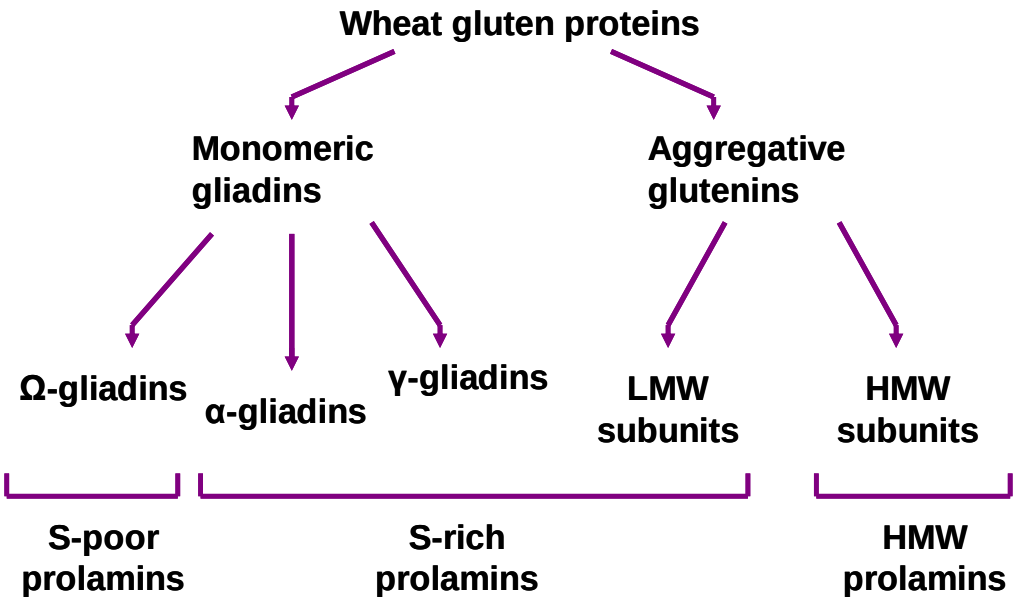

Figure 1 Classification of wheat gluten proteins (Source: Shewry et al 2009)

Generally, an increase in total protein content within a given class of wheat enhances the amount of gliadin and LMW glutenins (Carson and Edwards 2009). An increase in protein content also increases dough extensibility and loaf volume (Carson and Edwards 2009). Studies have shown that loaf volume is not only related to protein content but also effected by protein quality (Eliasson and Larsson 1993; Goesaert et al 2005). Dough physical properties depend on the continuous protein phase. Both molecular weight and molecular weight distribution affect protein physical properties (Southan and MacRitchie 1999).

Although studies have shown correlation between LMW glutenins, ω and γ-gliadins and flour quality (Johansson 1996), other studies have shown that HMW glutenins were strongly related to flour quality characteristics and baking (Bean et al. 1998; Carceller and Aussenac 2001; Gupta et al. 1993; Van Lonkhuijsen et al 1992). Dough mixing behavior is related to the polymeric character behavior because shear and tensile stress develop the dough into a continuous network, imparting viscoelastic properties to it (Gupta et al 1992; Southan and MacRitchie 1999). Larger glutenin polymer size requires more energy input to develop the dough (Southan and MacRitchie 1999). At the same time, as the subunit size increases, its ability to contribute to dough strength also increases (Panozzo and Eagles 2000; Wrigley 2004).

Approximately nine loci are known to code for gluten proteins. Three Glu-1 loci (Glu-A1, Glu-B1, Glu-D1) located on the long arms of chromosomes 1A, 1B and 1D, respectively,

code for HMW glutenins. The three complex Gli-1/Glu-3 loci (Gli-A1/Glu-A3, Gli-B1/Glu-B3, Gli-D1/Glu-D3) on the short arms of chromosomes 1A, 1B, 1D, respectively, code for ω and γ gliadins and LMW glutenins. Three Gli-2 loci (Gli-A2, Gli-B2, Gli-D2) code for α and β gliadins (MacRitchie and Lafiandra 2001).

2.131 Quality correlations

Studies have found a high correlation between dough strength and the highest molecular weight polymeric fraction which makes 60% of total polymeric proteins. A lower correlation was obtained when all polymeric fractions were considered (Bersted and Anderson 1990; Southan and MacRitchie 1999). Bean et al. (1998) reported high correlation between protein content and the level of insoluble polymeric protein, however, the relative amount of insoluble protein (insoluble protein/total flour protein) was weakly correlated with flour protein inferring that insoluble proteins are related to quality but not with the protein content itself. A high correlation between protein content and insoluble protein content with mixing tolerance and water absorption was also found. Flour water absorption is a linear function of protein content. Mixing tolerance requirements, dough handling characteristics and loaf volume are also related to protein content (Finney and Shogren 1972).

2.2 Techniques used to separate and identify flour proteins

2.21 Size Exclusion HPLC

Using Size Exclusion (SE) HPLC, proteins are separated by size based on the extent to which they enter the pores of the column matrix as a solvent carries proteins through a column of a chromatographic medium (Bietz 1984). The larger proteins elute rapidly at the column's void volume while smaller proteins penetrate through the pores freely and elute later. The method is rapid, has automatic sampling feature and requires small sample size (Singh et al 1990a; Southan and MacRitchie 1999). It has been widely used for isolation and comparisons of wheat proteins (Bietz 1984).

2.22 SDS PAGE

SDS-PAGE electrophoresis has been successfully used to characterize all gluten proteins (Cornell and Hoveling 1998). Sodium Dodecyl Sulfate (SDS) denatures proteins and forms random coil structures that carry a negative charge and allow electrophoretic separation based on MW. In addition, intermolecular disulfide bonds can be broken by reduction. High MW fractions are reduced to smaller subunits and thus become feasible to be separated by SDS-PAGE. By this technique, characterized glutenin subunits range from 30,000 to 140,000 kDa while gliadins range from 30,000 to 80,000 kDa (Cornell and Hoveling 1998).

2.23 Proteomics

Proteomics is the study of the full protein complex of a tissue or cell. It requires the ability to conduct high resolution protein fraction separation. The technique uses the availability of a library of genome sequences to identify proteins based on the mass spectrometric determination of masses of peptide fragments resulting from tryptic digestion of small amounts of proteins previously separated by electrophoresis. Proteomics is effective in studying changes in gene expression that can occur during grain development for instance. Environmental effects on grain composition and functional properties can be detected and correlated with bread making quality (Shewry et al 2009).

2.3 - Genotype and environment role in wheat quality

The development of the wheat kernel can be affected by both genotype and environment (Finlay et al 2007). Several factors including light, temperature, fertility, CO_2 and water stress influence grain maturation (McMaster 2009). Studies using both winter and spring wheats have shown that environment, genotype and genotype x environment interaction affect grain development and quality. Most of these studies attributed the main quality variation factor to environment (Finlay et al 2007). Environmental effects were observed when parameters such as flour yield, test weight and protein were evaluated while flour pigment, mixograph® development time and kernel hardness had smaller influences. Non-significant effects were found for genotype-location and genotype-year interactions (Finlay et al 2007). Bequette (1989) reported genotype and environmental effects on wheat kernel size, flour ash content and bake absorption. The formation of polymeric proteins during kernel development is not completely understood but it appears to be controlled by genotype (Southan and MacRitchie 1999).

Guttieri and Souza (2003) found that genotype significantly affected values for all four SRC solvents while genotype vs environment effects were insignificant on soft wheat flours. Studies conducted by Lukow and McVetty (1991) found that genotype, environment and genotype x environment interactions were significant for grain, flour, dough and bread loaf characteristics whereas, environment and genotype x environment interaction were not significant for flour yield and farinograph® dough development time. Wrigley (2009) reported that grain hardness is influenced by genotype while other factors that are mainly responsible for flour quality such as rainfall, fertility and temperature during growing season, harvest and storage are affected by growth conditions.

2.31 Hard Red Winter (HRW) wheat varieties

Wheat is classified as hard or soft based on the physical strength of its endosperm. Grain exposed to warm dry weather during fruiting and filling periods will tend to have a harder texture and higher protein content (Bergman et al 1998). Five HRW varieties were used in this study. Fuller, named after the botany professor Herbert Fuller Roberts, was developed by the Kansas Agriculture Experiment Station and Agricultural Research Services (KAES-ARS) in 2006. It comes from an unknown pedigree population, but it is believed that Jagger and leaf rust resistant lines from USDA are included in its pedigree. Fuller is a bronze-chaffed, semi-dwarf hard red winter wheat resembling Jagger. Fuller has shown good milling and baking performances, protein content equivalent to Jagger, higher flour extraction rates and 1 percent lower baker's percent water absorption than Jagger (Fritz et al 2007). Jagger is a variety developed in 1994 by the KAES-ARS. It was named after Joe Jagger, a long-time wheat producer and leader in wheat agriculture. Jagger showed significant improvements in baking, disease resistance and grain yield compared to other varieties popular at the time. Jagger is an awned, semi-dwarf with an upright grown habit (Sears et al 1995). Overley was developed in 2003 by KAES-ARS. It is named for Carl Overley, a KSU professor of Agronomy. It is a cross between Jagger, Heyne (a hard white winter released by Kansas Agricultural Station) and Tam-107. Overley shows outstanding milling and baking properties and higher loaf volumes than Jagger. Protein content and water absorption have been similar to Jagger (Fritz et al 2004). Jagalene, a cross between Abilene and Jagger varieties, was released in 2001. It shows exceptional baking and milling

characteristics. 2137, a cross between 2163 and experimental lines, was released in 1995. It has acceptable milling and baking performances but is susceptible to stripe rust (Watson 2007).

2.32 Environment effects

The understanding of and ability to control growth conditions would help to predict cultivar performances (Blumenthal et al 1993). Some factors such as the influence of day length or extreme temperatures are beyond farmers' control (Johnson et al 1975). Dough properties can vary substantially due to the environmental influence in addition to the effects of protein content and genotype. Various factors such as grain moisture, presence of weed seeds and pesticide residues depend on growth, harvest and storage (Blumenthal et al 1993). Soil type, fertilizer levels (particularly nitrogen and sulphur), rainfall and late season frosts during grain filling have an effect on wheat quality and protein content (Ciaffi et al 1996; Wrigley 2009).

Sulphur fertilizer levels affect the frequency of S-S bonds in protein. If this fertilizer becomes limiting, S-poor proteins are predominant so the proportion of HMW glutenins have a higher proportion of S-poor proteins compared to LMW glutenins (Southan and MacRitchie 1999). Generally, a higher level of nitrogen fertilizer increases the level of protein and grain yield (Johnson et al 1975). The level of nitrogen and time of application have a strong effect on both gliadin and glutenin agglomeration and gliadin/glutenin ratio (Jia et al 1996). According to Pomeranz (1978), the synthesis of proteins contributing to gluten formation and loaf volume starts three weeks before wheat is ripe. Those proteins reach optimum contribution to volume, crumb grain and mixing attributes as early as two weeks before wheat physiological maturity. Gluten forming capacity improves dramatically during this period.

Few studies address growth temperature effects on grain quality. Wheat protein content is determined by rates and duration of protein and starch synthesis during grain filling. When temperature increases, the proportion of protein to starch also increases. When temperature increases beyond 30 ºC, synthesis of both starch and protein seem to be suppressed. High temperatures may also alter the synthesis of proteins in the grain leading to changes in protein composition and could explain effects on dough quality. Studies conducted at temperatures above 35ºC showed changes in glutenin:gliadin ratio. In most cases, there was an inverse relationship between grain filling and grain yield and a direct relationship between grain filling

and protein content. It was also observed that, in general, gliadin to glutenin proportion increased in heat stressed samples (Blumenthal et al 1993; Ciaffi et al 1996).

In general, flour from wheat grown under higher temperature stress produced lower loaf volumes (Blumenthal et al 1993). Environment affected dough strength when the same variety containing similar protein content was grown in two different locations. High average temperatures were experienced at the site that produced weaker dough (Blumenthal et al 1993; Ciaffi et al 1996). The studies concluded that temperature during grain filling is the factor most likely affecting grain quality. Increases in temperature up to 30 ºC during grain maturation increased dough strength (measured by Extensigraph) while temperatures above 30 ºC tended to decrease dough strength (Blumenthal et al 1993; Ciaffi et al 1996; Panozzo and Eagles 2000).

2.4 Flour granulation

Water absorption is thought to be related to flour particle size, in the sense that flours possessing finer particles hydrate at a faster rate and form a continuous dough mass sooner when work is applied because of the greater surface area available. Flour bulk density is also affected by particle size. Both particle size and shape influence product flow (Pratt 1978).

2.5 Flour water absorption

Four flour components are believed to be involved in water absorption: protein, damaged starch, pentosans (Morgan et al 2000, Stauffer 1999) and native starch (Stauffer 1999). Their influence is shown in Table 1.

Table 1 Effect of flour components on water absorption

Flour component	Water per g of component (g)	Amount per 100g flour (g)	Absorption per 100 g flour
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

Source: (Stauffer 1999)

Hard wheat flour particles (≈150 μm) are much larger than starch granules and protein components. When water and flour come in contact, particle surfaces hydrate rapidly. As particle

surface area is limited, the water is present is excess which gives mobility to the system (Faubion and Hoseney 1990). Part of the water is present is tightly absorbed by flour particles and constituents and is thus unavailable for solvent action. Multiple hydration layers of water are formed until the last is held with less strength and can interact with damaged starches and other components (Pyler and Gorton 2008). In the beginning, the action of the mixer converts the protein matrix into a soft, plasticized state (Stauffer 1999), and allows the formation of fibrils that extend and interact to form a continuous network (Faubion and Hoseney 1990; Stauffer 1999). Mechanical energy input plays a significant role in dough formation. Flour particles are agglomerates of starch granules immersed in a network of protein. As hydration proceeds the network is softened (plasticized) by hydration and agitated by mixing. Starch becomes less firmly attached with protein and all ingredients are blended giving a homogeneous dough (Stauffer 1999). The development stage of mixing occurs when the flour-water mixture becomes a viscoelastic dough, which when mixed to peak consistency gives the maximum loaf volume compared to over and under mixed doughs.

The water absorption capacity of different wheat flours is of great importance to a bread baker as it is related to bread texture and yield. Absorption capacity has been shown to depend on protein and damaged starch content. The amount of damaged starch can be controlled to some extent during milling, being affected by the severity of grinding and reduction of coarse stocks. Additional factors thought to be involved in water absorption are; protein quality, mineral content and pentosans (Bergman et al 1998; Meredith 1966).

Flour water absorption is crucial in baking. The maximum amount of water tolerated by flour in forming a dough that possesses acceptable handling properties is called the optimum absorption. The Farinograph® and Mixograph® are recording mixers used to determine optimum absorption. The Farinograph® mixes using a gentle pressing and shearing action on the dough. The optimum absorption is defined as the water addition required to center the curve peak at 500 BU. The Mixograph® develops the dough by a more intense folding and stretching planetary action and optimum water absorption is determined by experienced subjective analysis of the curve. Optimum Mixograph® water absorption is estimated based on flour protein content. However, it is often necessary to conduct trial adjustments to find the true optimum due to the influence of other flour components (Ross and Bettge 2009). Optimum water determination is essential for precise rheological testing as well as product quality (Ram et al 2005). In an

experiment considering different varieties in which protein content was adjusted to a constant level, those requiring higher water addition and longer mixing times produced higher loaf volumes, suggesting that the breadmaking potential can be attributed to factors governing mixing time and water absorption (Roels et al 1993).

2.6 The mixograph®

The mixograph® is an instrument used to evaluate empirical rheological attributes of wheat flour doughs during mixing. It is used as a guide to predict flour mixing time and water absorption (Bergman et al 1998). The mixograph® has been used to evaluate mixing characteristics (Pratt 1978) and to predict dough processing properties and baking quality. This technique is also used for testing the suitability of flours for cookie, cracker and pasta technology (Vergnes et al 2003). The resulting curves, called mixograms, are widely used for screening in breeding programs. They have played an important role in predicting dough properties of early generation wheats, being fundamental in variety selection (Bruinsma et al 1978; Finney 1989; Gra and O'Brien 1992; Walker et al 1997). In commercial bakeries, they are used to detect suitability of incoming flour lots for a specific manufacturing facility through empirical relationships observed between mixograph® and specific industrial mixer outcomes (Ross and Bettge 2009).

The mixograph records and measures the resistance to mixing affected by the dough pins while the mixing head rotates. The mixograph® measures energy input (torque) required when flour particles are being hydrated and mixed. Torque measurements are recorded using a pen in a chart paper or via potentiometer. The stages (hydration, blending, gluten development and breakdown) of dough formation can be seen graphically in Figure 2 (Stauffer 1999). The curve length accounts for the time the dough has been mixed. The curve width is related to the cohesiveness and elasticity of the dough and indicates mixing tolerance. Curve peak time and height are determined, to a certain extent, by flour quality and protein content and also by water absorption.

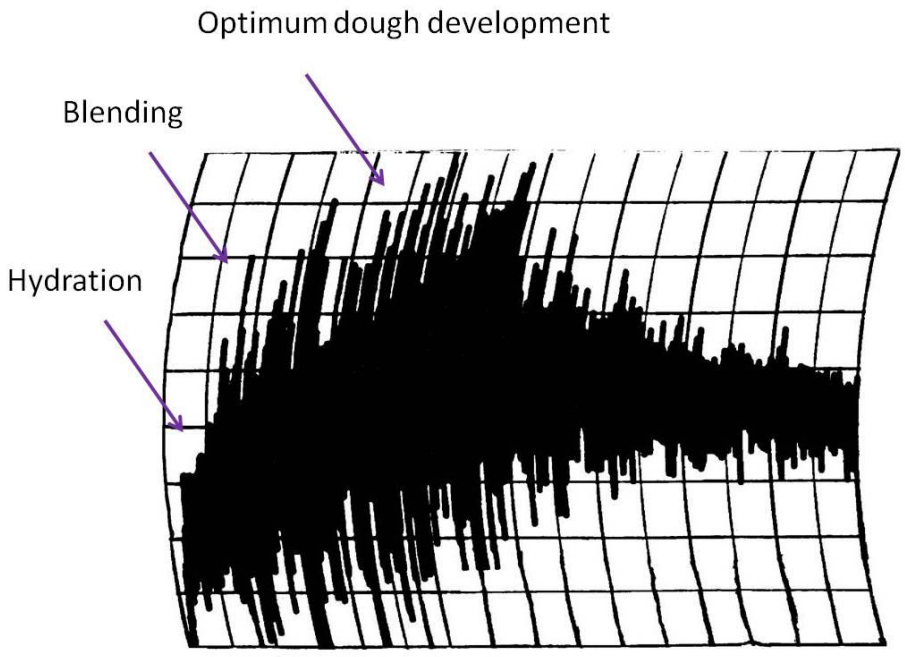

Figure 2: Mixogram showing the main phenomena taking place during mixing

The first step in the mixing process is moistening flour particles. High adhesion forces between particles are induced by particle surface absorption (Dendy and Dobraszczyk 2001; Hoseney 1985; Vergnes et al 2003). As the water is in excess, the system is not viscous and there is little resistance during mixing (low curve) (Hoseney 1985). Then, solubilization and swelling of albumins, globulins, arabinoxylans and damaged starch granules occurs as well as plasticization and interaction between gliadins and glutenins (Dendy and Dobraszczyk 2001; Vergnes et al 2003). Resistance to mixing increases (higher curve) until a peak is formed (optimally mixed dough). Wet and slack overmixed dough is produced when dough is mixed beyond the optimum (Hoseney 1985).

The interpretation of the mixogram curve is subjective based on training and the experience of the operator. Flours can also produce atypical mixogram patterns making them difficult to analyze. Without success, several workers have attempted to take objective measurements by fixing readings at bandwidths and areas under the curve to develop correlations with bread making performance using statistical multiple regression analysis (Dendy and Dobraszczyk 2001).

2.7 Solvent Retention Capacity (SRC)

The solvent retention capacity test (SRC) measures the weight of solvent retained by flour as percentage of flour weight after centrifugation (Pasha et al 2008). The SRC test is based on the swelling behavior of polymer networks in the presence of specific solvents (Kweon et al 2009). Each solvent determines a specific flour component's contribution. Retention of sucrose relates to water soluble pentosans or arabinoxylan content, lactic acid retention indicates glutenin network and dough strength, sodium carbonate retention reflects damaged starch contribution and pure water relates to general absorption (all components) (Bettge et al 2002; Guttieri and Souza 2002; Kweon et al 2009; Pasha et al 2009; Xiao et al 2006; Zhang et al 2007). SRC profiles can assist in determining flour processing and baking characteristics (Bettge et al 2002). The SRC test is widely used in soft wheat flours but it has been successfully used to evaluate HRW wheat varieties for breadmaking (Kweon et al 2009; Xiao et al 2006).

2.8 Dough processing and bread characteristics

Traditionally dough is created from water and flour (Puppo et al 2005; Singh and MacRitchie 2001; Stauffer 1999) by the use of mechanical energy (Stauffer 1999). Flour particle dissolution and storage protein interaction during mixing allows the formation of a coherent, continuous protein matrix surrounding the starch granules (Puppo et al 2005).

The forces applied during mixing cause gluten proteins to interact and form a continuous network throughout the dough. The dough's resistance to extension increases during mixing. Viscoelastic properties are developed and become optimum at peak consistency (Faubion and Hoseney 1990; Goesaert et al 2005; Singh and MacRitchie 2001). Only after dough is fully developed is the full bread making potential is achieved. Dough mixing properties can be used to evaluate flour quality (Bergman et al 1998). Strong flours are more elastic and require longer mixing time and energy to develop while weak flours have lower elasticity, strength and mixing requirements (Carson and Edwards 2009). Variability in dough mixing properties between varieties is related primarily to glutenin quality. Specifically the proteins produced by the Glu-1 loci have been highly associated with mixing properties. A (2 + 12) subunit composition results in weak mixing and a 5 + 10 composition to strong mixing (Bergman et al 1998). Modern bread-baking processes require flours possessing high water absorption, medium to medium-long mixing requirements, good loaf volume potential, good color and good crumb grain (Pyler and

Gorton 2008). The end use quality of a hard wheat variety is directly related to the properties and interactions of the wheat kernel's biochemical components that contribute to processing quality (Li et al 2009; Xiao et al 2006). Studies conducted by Eliasson and Larsson (1993) showed that gliadins are not crucial in baking performance while glutenins showed better correlation with bread quality. Glutenin influence is based on three characteristics: gliadin/glutenin ratio, MW distribution of glutenins and the presence or absence of certain HMW glutenin subunits. Specific HMW subunits were found to increase mixograph® development time and loaf volume in test baking.

CHAPTER 3 - Materials and methods

3.1 - Wheat samples

Five commercial varieties of hard red winter wheat (Fuller, Jagger, Jagalene, Overley and 2137) were grown in six locations (Thomas, Republic, Riley, Labette, Finney and Sumner Counties) in Kansas in crop year 2009 (Figure 3). Samples were gown in a split plot randomized design. Each plot was 6.1 meters in length divided into three rows. Each sample was collected randomly in four replicates with a plot combine. The samples were combined and reduced to approximately 1 kilogram weight. A total of 30 samples were obtained. For descriptive details of the varieties refer to section 2.31 on page 10.

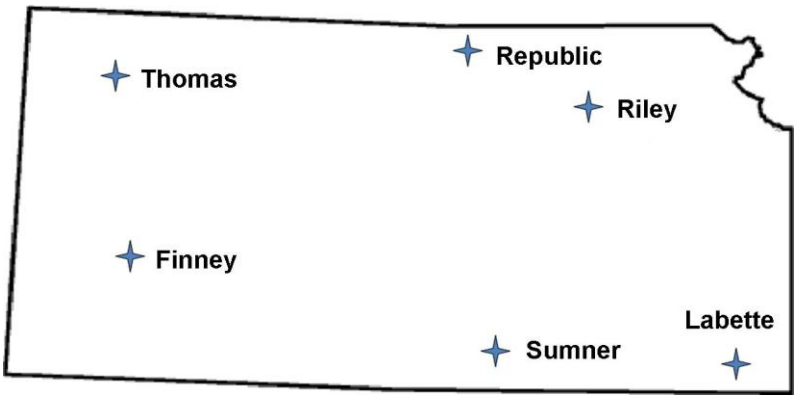

Figure 3: Kansas map showing locations identified by county name for crop year 2009

3.2 - Wheat physiochemical tests

Wheat samples were cleaned using a Carter Dockage tester (Carter-Day Company, Minneapolis, MN). Test weight (lb/bu) was measured by AACC approved method 55-10. Single Kernel Characterization System (SKCS 4100, Perten Instrument, Springfield, IL) was used to determine the mean values of kernel weight (mg), diameter (mm), moisture content (%) and hardness index following AACC approved method 55-31. A RO-Tap sieving system (The Tyler Company, Cleveland, Ohio) was used to measure kernel size fractions (large, medium and small). One hundred grams of wheat was tapped for 1 min through a sieve stack consisting of a #7 sieve, #9 sieve and pan. Wheat protein and moisture content were determined by NIR (DA 7200 Perten). Ash was determined by AACC method 08-01.

3.4 - Wheat milling

Samples were tempered to 15% moisture content overnight according to AACC 26-95. Approximately 1000g samples were milled on the Quadrumat Senior Experimental Mill (Brabender, Duisburg, Germany) in the Wheat Quality Laboratory at Kansas State University using modified AACC method 26-50. The feed rate was adjusted to 150g/min.

3.5 - Flour characteristics

3.51 Flour physiochemical tests

Flour moisture and protein content were determined using NIR (DA 7200 Perten). Flour color was determined using a Minolta CR-310 color meter in conjunction with a CR-A50 granular materials attachment. Color was reported as Hunter *L a b* values. *L* is a measure of the brightness from black (0) to white (100); *a* describes red-green color with positive values indicating redness and negative values indicating greenness; *b* describes yellow-blue color with positive values indicating yellowness and negative values indicating blueness. Flour particle size was determined using a laser diffraction particle size analyzer LS 13320 (Beckman/ Coulter, Miami, FL) and dry module. Flour particle size ranges used in this study were: < 10μm, 10-41 μm and 41-300 μm according to Hareland (1994). Falling number tests were conducted in duplicate according to AACC method 56-81 to detect presence of α-amylase activity in the flour indicating sprout damage. Flour damaged starch level was determined in four replicates for all samples using the Megazyme® kit method (AACC approved method 76-31).

3.52 Water absorption tolerance by Mixograph®

Optimum water absorption was estimated based on the equation Y = 1.5x + 43, where x is flour protein content on 14% moisture basis tested with a 10-g Mixograph® (National Manufacturing Co, Lincoln, Nebraska) according to AACC approved method 54-40. For each flour sample, the specific water absorption tolerance was determined by running dry, optimum and wet conditions (Figure 4). From this analysis, the tolerance range was calculated in milliliters of water by subtracting dry absorption from the wet. Samples were identified as having high tolerance, medium tolerance and low tolerance according to subsequent statistical analysis.

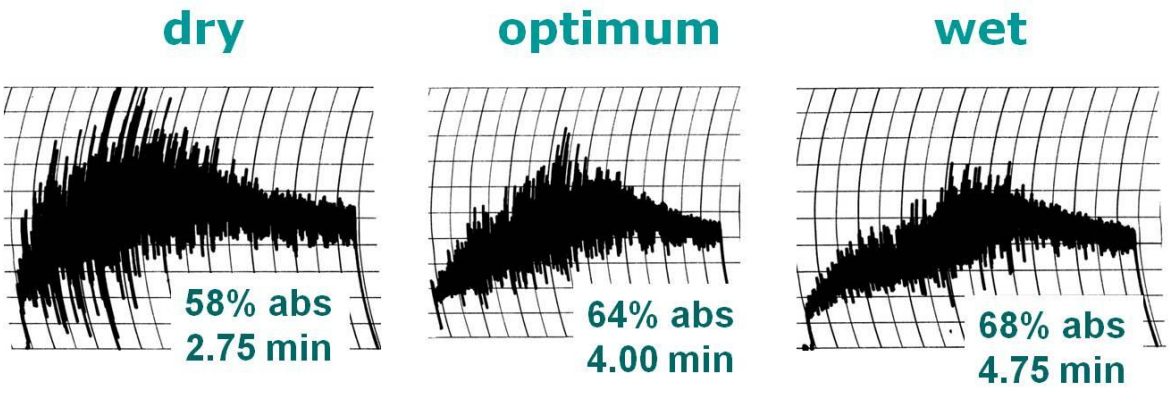

Figure 4 Example of a mixogram series used to determine water absorption tolerance range showing optimum, dry and wet water levels

Water absorption tolerance interval (mL) = Wet absorption – Dry absorption

3.53 Solvent Retention Capacity

The solvent retention capacity profile of each sample was run in four replicates according to AACC approved method 56-11. SRC was used to determine potential components related to flour water absorption tolerance.

3.6 Starch extraction and particle size distribution

Starch was isolated by an adaptation of Park et al. (2006) where wheat flour (2.5g) was mixed with buffer (50mL) in a 1:20 ratio. The buffer was composed of 0.1M sodium phosphate, 0.5% sodium dodecyl sulfate (SDS) and 0.5% sodium metabisulfite diluted in distilled water to pH 7. After gentle mixing, the solution was sonicated for 100 seconds (using an ultrasonic processor VCF-1500, Sonic & Materials, Newton, CT) equipped with a 25.4 mm probe positioned 5mm from beaker bottom. The glass beaker was immersed in ice water to prevent sample heating. The sonication amplitude was 75 %. The solution was transferred to 50 mL plastic tubes, centrifuged for 10 min at 4000 rpm and decanted. Approximately 40 mL of distilled water was used to wash the precipitated starch through a 62 µm mesh screen and the resulting solution was centrifuged for 5 min at 4000 rpm. The precipitate was resuspended in 40 mL of distilled water using a vortex mixer and decanted two more times. The extracted starch was freeze-dried. Dry starch was ground with a mortar and pestle and suspended in 1 % sodium azide solution for particle size distribution determination using a laser diffraction particle size analyzer LS 13320 (Beckman/ Coulter, Miami, FL) using the Universal Liquid Module. The granule size distributions were evaluated in the three ranges described by Park et al. (2009), where A granules were > 15µm, B granules were 5-15 µm and C granules were 0-5 µm.

3.7 Wheat Protein extraction and identification

3.71 Size-Exclusion High Performance Liquid Chromatography (SE-HPLC)

Wheat flour proteins were extracted by a modification of the method used by Singh et al., (1990b) and Batey et al (1991). 10mg ± 0.1 of flour was weighed into a microfuge followed by the addition of 1mL of 50mM NaPhos + 0.5% SDS buffer at pH 6.9 and solubilized by vortex agitation for 5 min. This isolated total and extractable polymeric protein fractions. To solubilize the largest molecular size fraction, the total protein fractions samples were sonicated (60 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA, USA) at room temperature at an output of 6 watts for 15 s. The sonicator probe was placed 1/3 distance from the bottom of the microfuge tube without touching the tube walls. After sonication, the tubes were then centrifuged at 12000 x g for 20 min. The supernatants of all samples were filtered twice for 5 min thru 0.45 µm filter tubes then transferred to HPLC vials and sealed. To ensure stability of prepared samples, the vials were heated in a water bath at 85^0C for 10 min to inhibit any intrinsic proteolytic activity.

After heat treatment, vials were cooled with crushed ice for 10 min and kept at room temperature for 30 min before being analyzed by Size-Exclusion High Performance Liquid Chromatography (SE-HPLC). Unextractable proteins, or obtained as the residue of extractable fraction using the same procedure described above except that sonication was done for 25 s at 6 watts output. 20 µl aliquots of each sample were fractionated in a Biosep SEC-4000 column (Phenomenex, Torrance, CA) in a SE-HPLC (Hewlett-Packard 1100) system comprised of a model 510 pump, a model 481 variable wave length detector and a model 712 WISP automated sample injector. The mobile phase was composed of deionized water + 0.05% trifluoroacetic acid (TAF) and acetonitrile + 0.05% TAF. Solvent flow rate was 0.5 mL/min. Pump control and data acquisition were achieved using ChemStation software (Agilent Technologies, USA). Peak areas were automatically calculated to quantify specific wheat protein groups according to figure 4. In measuring the size of each peak, the lowest points on the troughs (valleys) were used as cutoff points between peaks. Samples were run in duplicate. Areas of the peaks were calculated and expressed in arbitrary units according to Gupta et al (1993). The amount of glutenins from Total Extractable Polymeric protein was determined as (Peak 1 area %/Total area %) x 100, amount of gliadins was determined as (Peak 2 area %/Total area %) x 100 and the amount of albumin/globulins was determined as peak 3 area %/total area %) x 100. The percentages of Extractable and Unextractable Polymeric Protein were determined as peak 2 area (extractable)/Peak 1 area (total) x 100 and peak 3 area (unextractable)/Peak 1 area (total)x100, respectively.

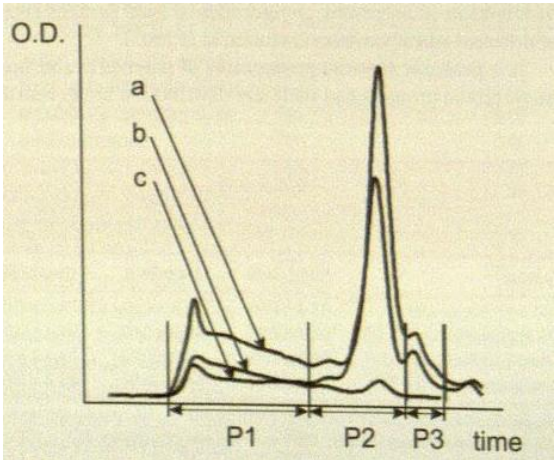

Figure 5: Size-exclusion high-performance liquid chromatography profiles of a) Total polymeric proteins. P1 refers to glutenin, P2, gliadins and P3 albumin/globulins fractions.

b) Soluble fraction and c) Unextractable fraction. O.D. is optical density. (Source: Shewry et al 2009).

3.72 SDS PAGE electrophoresis

SDS PAGE electrophoresis was used to characterize extractable subunit composition by separation on NuPAGE Mini Gels 4-12% Bis-Tris one dimension using Xcell sure lock mini cell IM 8042. In a microtube, the extractable protein (see above for isolation procedure) obtained from 10mg of flour were dried and re-suspended in 100 µl of water. 10µl of the sample suspension was added to 5µl of sample LDS 4x (lithium dodecyl sulfate with comassie dye), 2µl of reducing agent and 7µl distilled water. The tubes were agited in a microfuge (Fisher Scientific) for proper homogenation then placed in a 95°C heater for 5min. 10 µl sample aliquots and molecular markers were slowly added to the mini gels. Voltage was adjusted to 150 V and samples were eluted for 60 min. Gels were washed with distilled water for 10 min and left overnight in Coomassie Brilliant Blue R250 (BIO-RAD, Hercules, CA, catalog# 161-0436), and de-stained in 30% methanol and 10% acetic acid until clear. Images were taken with an EpsonTM Twain 5 scanner and band density was analyzed with Image QuantTM TL Software (GE Healthcare Bio-science Corp., Sweden). Known concentrations of bovine serum albumine (BSA, fraction V, 2.0mg/mL in a 0.9% aqueous NaCl solution containing sodium azide) were used as standards for band density. A standard curve of pixels versus BSA concentration was prepared and band concentration was calculated by using the linear equation for the standard curve. The gel bands were cut and isolated for mass spectroscopic identification.

3.73 Proteomic analysis

3.11.31 Sample preparation

Stained gel slices were de-stained and the SDS removed by washing in 100 µL 1:1 acetonitrile/water at 30 °C for 1 h (three times). Gel pieces were then dried for 10 min under vacuum and subjected to reduction and alkylation using 50 mM Tris (2-carboxyethyl) phosphine (TCEP) at 55°C for 10 min followed by 100 mM iodoacetamide in the dark at 30°C for 1 h. The carboxymethylated gels were thoroughly washed, re-dried under vacuum, then rehydrated with 100ng of sequencing grade trypsin (Trypsin Gold, Promega, Madison, WI) in 20 µL of 40 mM ammonium bicarbonate, pH 8.0. Hydrolysis was at 30 °C for 17 h. The digestion solution was transferred to 1.5 mL microcentrifuge tubes and tryptic peptides remaining within the gel plugs

were recovered by another extraction with 50 µL of 50% acetonitrile in water and 2% trifluoroacetic acid (TFA) at 30 °C for 2 h. Fractions were combined and dried by speed vacuum concentration and tryptic peptides were resuspended in 10 µL of 33% acetonitrile/0.1% TFA.

3.11.32 1D nano-HPLC and Electrospray Ionization Tandem Mass Spectrometry (ESI−MS/MS)

For 1D nano-HPLC, tryptic digested solution (9 µL) was mixed with 100 µL of 2% acetonitrile in 0.1% formic acid. 30 µL of the solution was injected automatically in line into a C18 reverse-phase micro column (300 µm ID × 1 cm, PepMap: LC Packings) to remove salts. Peptides were separated with a C18 reversed-phase nanocolumn (75 µm ID × 15 cm, PepMap: LC Packings) by a nanoflow linear acetonitrile/formic acid gradient. The 1D-nanoLC was performed automatically using a microcolumn switching device (Switchos; LC Packings) coupled to an autosampler (Famos: LC Packings) and a nanogradient generator (UltiMate Nano HPLC; LC Packings). The system control software, Hystar 3.2, was used to control the entire process. The eluted peptides were injected into an HCT Ultra Ion Trap Mass Spectrometer (Bruker Daltronics). The mass spectrometer was set up in the data dependent MS/MS mode to alternatively acquire full scans (*m*/*z* acquisition range from 300 to 1500 Da/e). The four most intense peaks in any full scan were selected as precursor ions and fragmented by collision energy. MS/MS spectra were interpreted and peak lists were generated by DataAnalysis 3.4 and Biotools 3.0 software (Bruker Daltronics).

3.11.33 Bioinformatics

Peptide masses were compared to NCBInr.2010. using MASCOT (http://www.matrixscience.com). The following parameters were used in all searches: the maximum number of missed cleavages allowed was 1; the mass tolerance was 0.5 Da and the monoisotopic masses of observation were used to match the calculated monoisotopic fragment mass for protein identification. Fixed modification was set on cysteine with carbamidomethylation. Variable modification was done on methionine with oxidation. Wheat *(Triticum aestivum L.)* was selected in the taxonomy selection of database.

3.12 Bread baking

Bread was baked as 100 g pup loaves using AACC method 10-10.03 straight dough procedure with 90 min fermentation (AACC international 2000). The bread formula consisted of flour (100 g, 14% mb), shortening (3 g), instant active dry yeast (2 g), sugar (6 g), salt (1.5 g) and ascorbic acid (50 ppm). Water absorption and mix time were optimized for each flour. Loaf volume was measured by rapeseed displacement. Crumb grain was evaluated subjectively on a scale of 1 (poor) to 8 (excellent). All flours were baked in triplicate.

3.13 - Statistical Analysis

The experiment was analyzed by proc mixed by SAS (v.9.1, Institute, Cary, NC) using a completely randomized design. Analysis of variance (ANOVA) was applied to samples using proc GLM and means were compared using Tukey HSD. Parameters were correlated using Pearsons correlation coefficient.

CHAPTER 4 - Results and discussion

4.1 Water absorption tolerance classification

A series of mixograms was run for each of the 30 flour samples from wheat grown in 2009. Optimum water absorption was estimated initially based on flour protein content. Water addition was varied until wet and dry extremes were observed in the curves. Water absorption tolerance interval (in mL) was calculated for each sample by subtracting the observed dry absorption from the observed wet absorption. The mixogram series of Jagalene grown in Finney county is illustrated below as an example (Figure 6). This sample had 12.24 % protein content (14 % mb). The estimated optimum water absorption based on protein content was 64 %. Mixogram curves were typical of excessive water at a level of 68 % water while 58 % absorption was too dry.

Water absorption intervals were calculated in mLs of water (Table 2). Those ranges varied from 0.2 mL for the shortest range (Fuller grown at Riley) to 1.1 mL for the highest absorption range (Jagger grown at Finney). These water absorption ranges (tolerance) are classified as shown in Table 3. In general, varieties grown at Finney County showed the highest tolerances to water variation while Sumner and Riley County locations showed the lowest absorption tolerances (Figure 7). Other locations (Thomas, Labette and Republic Counties) showed an intermediate water absorption tolerance trend. Jagger had the highest water absorption tolerance interval across all locations except when grown at Riley. Jagger was a parent of all varieties tested except 2137.

4.2 Effect of genotype and environment on water absorption tolerance

Table 4 presents the ANOVA analysis of the effects of genotype and environment. Genotype showed a significant effect on water absorption tolerance (Table 4). Jagger showed the highest absorption tolerance interval. 2137 and Jagalene were intermediate and Overley and Fuller had the lowest absorption tolerance (F-value= 4.23 and P-value 0.01). Environment also showed a significant effect on water absorption tolerance. Samples grown in Finney and Labette Counties exhibited the highest tolerance followed by Republic and Thomas Counties at the intermediate level and Riley and Sumner possessing low water absorption tolerances (F-value=

10.76 and P-value < 0.0001). Growth environment had a larger effect on water absorption tolerance variability than did genotype. There are a number of factors affecting plant growth and grain maturation such as nitrogen and sulphur fertilizer levels, temperature, rainfall rate, crop rotation, etc (Blumenthal et al 1993; Ciaffi et al 1996; Jia et al 1996; Johnson et al 1975; Southan and MacRitchie 1999; Wrigley 2009). These factors may have affected synthesis of grain components and thus, affected flour functionality including absorption tolerance. This work was not focused on determining the effect of specific environment factors on water absorption tolerance but some available data was collected and is given in Tables 11, 12 and 13 of Appendix A.

4.3 Correlations of parameters tested with water absorption tolerance

Correlation between water absorption tolerance and various wheat and flour parameters is listed in Table 5. Water absorption tolerance was high and positively correlated with Solvent Retention Capacity of SRC-deionized water (R= 0.69 and P-value = < 0.0001). This correlation shows that water absorption tolerance may be influenced by all hydrophilic flour components. A lower but still positive correlation was also observed for SRC-lactic acid (R= 0.49 and P-value= < 0.0001). This solvent reflects absorption by gluten protein polymers, showing that polymeric proteins play a role in water absorption tolerance. In agreement with what was found from the SRC test, flour protein content was positive strongly correlated with water absorption tolerance (R= 0.72 and P-value = < 0.0001). Additionally, a positive high correlation was observed for specific protein fractions: gliadins (R= 0.59 and P-value = < 0.0001), extractable polymeric proteins (EPP) (R= 0.82 and P-value= < 0.0001) and unextractable polymeric proteins (UPP) (R= 0.60 and P-value = < 0.0001). Kernel hardness (R= 0.68 and P-value = < 0.0001) and flour particle size (41-300 µm) also showed positive correlations (R= 0.42 and P-value = 0.0009). A weak positive correlation was observed for starch A-granules (> 15µm) (R= 0.33 and P-value= 0.01). D'Appolonia and Gilles (1971) were unable to correlate starch particle size distribution with either dough water absorption and bread properties. On the other hand, Sahlstrom (1998) associated high water absorption with a greater proportion of A-type granules.

There was no evidence of significant correlation between water absorption tolerance and SRC-sodium carbonate and SRC-sucrose retention. Although damaged starch and pentosans are known to play a role in water absorption, in this study these components were not correlated with

water absorption tolerance behavior. This may be explained by the facts that; both components are present in relatively low amounts (pentosans 2 %; damaged starch 5-7 %) in HRW wheat flours and that all samples belong to the same class of wheat. There is, thus, less variability in content than between different classes of wheat. Unexpectedly kernel hardness and damaged starch were not correlated (data not shown). Even though deionized water retention reflects all flour components' absorption, these results suggests that the flour protein fractions were driving the effect.

Lack of correlation was observed for flour ash, wheat ash, wheat test weight, kernel weight, kernel diameter and damaged starch. A negative correlation was found between water absorption tolerance and albumin/globulin proteins (R = -0.57 and P-value = < 0.0001), soluble glutenins (TPP) (R=-0.46 and P-value= 0.0002), flour particle size (< 10 µm) (R= -0.30 and P-value= 0.02), flour particle size (10-41 µm) (R= -0.43 and P-value= 0.0007), B-granules (5-15 µm) (R= -0.25 and P-value= 0.06) and C-granules (0-5 µm) (R= -0.32 and P-value= 0.01).

4.31 Relationship between protein content and specificsub- fractions

As described above, flour protein content and specific protein fractions (gliadins, unextractable (insoluble) glutenins and extractable soluble polymeric proteins) showed higher correlation with water absorption tolerance. In this study, a high positive correlation was observed between protein content and gliadins (R= 0.83 and P-value= < 0.0001). Panozzo and Eagles (2000) reported that protein content was positively correlated with proportion of gliadins in flour protein and negatively correlated with proportion of soluble glutenin. High positive correlation was also observed between protein content and extractable polymeric proteins (EPP) (R= 0.75 and P-value= < 0.0001), which is in accordance with results obtained by Dachkevitch and Autran (1989). Protein content and unextractable polymeric proteins were also positively correlated (R= 0.85 and P-value= < 0.0001). Zhu and Khan (2001) also reported a correlation between protein content and monomeric proteins and insoluble glutenins. A negative correlation was observed for protein content and albumins/globulins (R= -0.73 and P-value= < 0.0001) and soluble glutenins (R= -0.75 and P-value= < 0.0001).

4.4 Effect of genotype and environment on factors showing a significant effect on water absorption tolerance

The effect of genotype and environment was determined for all parameters that showed significant correlation with water absorption tolerance. Results are presented in Tables 6 and 7. Genotype did not have a significant effect on SRC-deionized water. Jagger, 2137, Jagalene, Overley and Fuller had SRC retentions within the range of 59-62 %. Environment showed a significant effect on SRC deionized water. Samples grown in Finney (65.42 %) and Labette (64.82 %) Counties exhibited the highest absorptions while Republic, Riley and Sumner Counties showed the lowest (58.96 % 58.08 % and 57.14 %) respectively. Genotype showed a significant effect on SRC-lactic acid. Overley had the highest retention, (147.77 %) while Jagger showed the lowest retention (133.83 %). Environment also showed a significant effect on SRC-lactic acid. Finney (177.52 %) County exhibited the highest retention while Riley and Sumner Counties had the lowest (124.43 % and 122.26 %). Lactic acid retention reflects absorption by glutenin polymers. This shows that genotype and environment had a significant effect on gluten polymeric proteins.

Genotype also had a significant effect on flour particle size range (41-300 μm). Jagalene and Jagger had the highest proportion (apparent volume %) of large flour particles; 82.12 % and 80.96 %, respectively, while Fuller (76.75 %) had the lowest (Table 6). Environment had a significant effect on large flour particle size. Samples grown in Finney, Republic and Thomas Counties contained ≈ 81 % large particles while Sumner County had the lowest proportion (75.61 %). A typical flour particle size distribution plot is shown in Figure 8, where flour particle size profile of Fuller grown at Finney was shown in duplicate.

In previous studies, hydration properties have been reported to be related to flour particle size distribution with smaller particles hydrating at a faster rate because of their higher specific surface area (Pratt 1978). Wheat flour protein particles are generally larger than 35 μm and remain attached to other endosperm components (Hareland 1994) being part of the 41-300 μm range interval while A-type starch granules are present in 10-50 μm range and B-type starch granules appear in 1-10μm range (Hareland 1994).

In this study, only large flour particles were in some way correlated with water absorption tolerance, suggesting again that the protein fractions were the main component responsible for this behavior.

The ANOVA results did not show significant evidence that genotype affected protein content (F-value= 1.13 and P-value= 0.37). However, a significant effect was found for environment (F-value= 45.65 and P-value= < 0.0001) (Table 7). Several studies have reported an environmental effect on wheat flour protein content of bread making varieties (Gupta et al 1994; Ciaffi et al 1996 and Zhu and Khan 2001). Samples grown in Finney County had the highest protein content (12.50 %) while Sumner County samples had the lowest (8.98 %) (Table 7). This supports Shewry et al.'s (2009) conclusion that protein content is very sensitive to growing conditions. Fertilizers and weather conditions contribute in a range of 7 % to 20 % of the variability. Genotype showed a significant effect on kernel hardness (F-value= 17.23 and P-value= < 0.0001). Jagger had the highest hardness index (74.93) and Fuller had the lowest (68.58). Environment also showed a significant effect on kernel hardness (F-value= 42.54 and P-value= < 0.0001). Finney (79.50) , Labette (76.30) and Thomas (79.04) Counties possessed the highest average hardness index while varieties grown in Sumner (57.90) and Riley (61.00) Counties had the lowest average hardness index. These results agree with the results Bergman et al (1998) showing that hardness was significantly affected by environment. Gliadins were significantly affected by genotype (F-value= 234.11 and P-value= < 0.0001), Fuller showed the highest gliadin peak area (47.19%) and 2137 showed the lowest (44.02%). Environment also showed a significant effect on the gliadin fraction (F-value= 941.92 and P-value= < 0.0001). Finney County showed the highest peak area (50.34 %) and Sumner County showed the lowest (42.31 %). As mentioned earlier, gliadin peak areas were highly correlated with water absorption tolerance. These results are in agreement with Singh et al (1990a) who reported a positive high correlation between absolute quantities of gliadin and water absorption.

Genotype had a significant effect on extractable polymeric protein content (F-value = 220.17 and P-value= < 0.0001). Jagger showed the highest peak area (9724.11 %) while Overley showed the lowest (8175.96 %). Environment also had a significant effect on extractable polymeric proteins (F-value= 485.69 and P-value= < 0.0001). Finney County samples showed the highest peak area (10351.03%) and Sumner County the lowest (7750.18 %). According to Eliasson and Larsson (1993), extractable polymeric proteins play a role in wheat hardness. In this study the correlation between hardness index and extractable polymeric proteins was (R= 0.7 and P-value= < 0.0001) supported their conclusion. Genotype had a significant effect on unextractable polymeric proteins as well (F-value= 109.35 and P-value= < 0.0001). Overley

showed the highest peak area (10232.05 %) and 2137 showed the lowest (8890.15 %). Environment showed a significant effect on unextractable polymeric proteins (F-value= 418.69 and P-value= < 0.0001). Finney County had the highest peak area (11661.35 %) and Sumner County had the lowest (8448.49 %).

Environment has shown a larger effect on protein content and protein composition than has genetics. Although it was not the focus of this research to determine the effect of specific environmental factors, it is known that higher nitrogen availability later in the growth cycle increases the amount of protein in wheat flour (Delcour and Hoseney 2010). Gupta et al (1992) and Daniel and Triboi (2000) reported an increase in gliadin synthesis compared to other protein fractions when nitrogen availability was increased. Such a correlation was also observed in this study where as protein content increased, gliadin concentration increased (R= 0.83 and P-value= < 0.0001) and water absorption tolerance also increased. Environmental factors such as nitrogen availability appear to have an effect on water absorption tolerance. Experiments considering different levels of nitrogen fertilizer availability at different periods of wheat growth could be conducted in a greenhouse where other conditions could be kept consistent. Such a study could be useful to further understand correlations between nitrogen (N) and specific protein fractions.

Sulphur (S) availability can also affect wheat protein synthesis and quality. Zhao et al (1999) reported that bread making wheat varieties generally possess both (≈ 10 %) S in the grain and higher protein content than do non-bread making varieties. Further, Wrigley et al (1984) showed that if the level of sulphur as a fertilizer is limiting, changes in protein molecular weight distribution may occur. S-poor proteins increase compared to S-rich proteins. ω-gliadins and HMW glutenins are S-poor proteins while α, β and γ gliadins and LMW glutenins are S-rich. According to results found in this study, S-rich proteins were positively correlated with water absorption tolerance. Decreasing grain sulphur concentration is thought to result in a decrease in disulphide links in wheat flour, thereby affecting flour functional properties by decreasing the intrinsic viscosity of gluten dispersions (Zhao et al 1999). The functional consequence is shown in studies conducted by Moss et al (1981) which found a positive correlation between sulphur concentration and loaf volume. Thus, sulphur availability appears to play a role in protein synthesis and the resulting molecular weight distribution of wheat proteins. This would affect both flour end use functionality and water absorption tolerance as the results reported here found

that some protein fractions showed higher correlation with water absorption tolerance than did others.

An increase in protein content and gliadin synthesis (especially γ-gliadins) was observed when growth temperature was increased (Daniel and Triboi 2000). Although specific temperature effects were not researched in this study, there is evidence from other studies showing that temperature variation may play a role in both total protein content and the synthesis of specific protein fractions such as gliadins, which were shown here to be highly correlated with water absorption tolerance.

4.5 SDS PAGE and proteomic analysis

Total, extractable and unextractable protein fractions of Jagger grown at Finney County, (JF; high water absorption tolerance) and Jagger grown at Sumner County (JS; low water absorption tolerance) were extracted and eluted in duplicate by SDS PAGE electrophoresis. The resulting gel is shown in Figure 9.

The high tolerance sample (JF) had greater stain intensity in spot 14 than did the corresponding spot for the low tolerance sample (JS) (Figure 10). Spots 14 and 23 correspond to γ-gliadin as identified by mass spectrometry (Table 9). A significantly higher concentration of γ-gliadins was present in Jagger grown at Finney County (20.16 µg/µL) than in Jagger grown at Sumner County (5.96 µg/µL) (Table 8). However, there was not enough evidence to identify differences between HMW glutenins located at spot 3. This suggests that γ-gliadins may play a critical role in water absorption tolerance. According to those results, it also appears that the proportion of γ-gliadins in the gliadin fraction increases as protein content increases. γ-gliadins are known to be S-rich proteins and posses a hydrophobic surface.

Thus, γ-gliadins appear to have a high number of points to interact covalently with HMW and LMW glutenins during mixing due to their higher number of cysteine residues. Gliadins may interact with themselves or they may interact with glutenins through specific non-covalent interactions, hydrogen bonding or covalently via S-S linkages during dough mixing (Kuktaite et al 2004). Gliadins also possess the ability to unfold and form a more or less voluminous network in proportion to their concentration (Van Lonkhuijsen et al 1992). There is evidence for this phenomenon from studies conducted by Lee et al (2002) who found a decrease in extractable γ-gliadins during dough development. It is likely that γ-gliadins will have stronger chemical

interactions with HMW and LMW glutenins and contribute to protein matrix formation because of the number of cysteine residues they contain. Such protein network entanglements appear to allow a broader range of water to be present without affecting the optimum mixogram characteristics. It is possible that a larger number of interspaces were available between the protein fibrils network formed through S-S bonds to interact with water molecules while low absorption tolerance flours had less intermolecular spaces between network polymers (Figure 10 and 11).

A limitation of this study is that only one pair of the same variety grown in different locations representing a high and a low tolerance flour were tested in this experiment, further studies using a broader sample set is recommended to confirm those findings.

4.6 Bread baking

A blend of all of the varieties grown at Finney County (12.8 % protein content) was created to represent a composite high water absorption tolerant flour. A blend of all varieties grown at Sumner County (9.47 % protein content) represented flour with low tolerance to water addition. All flours were baked at three levels of dough water content (high tolerance, optimum and low tolerance judged by mixogram analysis), extreme wet and extreme dry absorptions were not used. Loaf volume did not differ significantly within each flour blend as water level was changed even when the formula water was increased by 5% from the optimum for Finney blend (high tolerance flour) (Table 10). Dough handling properties were not affected either. These results show that flour with high water absorption tolerance offer great advantages to bread production allowing both the addition of higher levels of water and a broader range of water content without significantly affecting product quality. Average loaf volumes of two flours (high and low tolerance) were significantly different from each other, Finney had 986 cc while Sumner had 831 cc. Because the protein content of the flour blends differed significantly, it is unclear whether the difference in loaf volume was caused by flour protein content, water absorption tolerance or both. Protein content plays a critical role in water absorption tolerance (Table 5 and 7) and loaf volume (Carson and Edwards 2009). Protein content and quality were highly correlated with loaf volume (Eliasson and Larsson 1993). Bread crumb score was satisfactory (5) for all Finney absorption levels. Sumner flour showed questionable (3) crumb grain score for high and optimum absorptions while unsatisfactory (2) crumb was obtained for low water

absorption level. Crumb grain scores decreased for low water absorption flour and low water addition level. It appears that lower water content had a negative effect on crumb grain score.

CHAPTER 5 - Conclusions

● Different wheat samples could be differentiated according to their water absorption tolerance behavior. Both genotype and environment showed significant effects on water absorption tolerance. A higher water absorption tolerance trend was observed for all varieties grown at Finney County in crop year 2009.

● The main kernel components that showed positive correlation with water absorption tolerance were flour protein content, kernel hardness, extractable polymeric proteins, unextractable polymeric proteins and gliadins. Among the parameters analyzed, only flour particle size (41-300μm) was correlated with water absorption tolerance. Flour proteins is the main component present in this particle size range.

● SDS PAGE and proteomics results showed that γ-gliadins (present in extractable polymeric protein fraction) appear to be the main protein fraction involved in water absorption tolerance behavior. The fact that γ-gliadins possess 8 cysteine residues/monomer allow greater interaction with HMW and LMW glutenin subunits during dough development facilitate water entrapment in the polymer network.

● When the 5 wheat varieties grown at locations with high, medium, and low water absorption tolerance were blended together by location and test baked, loaf volume varied between flours. It is unclear if this was an effect of the differing protein levels of the blends or the water absorption tolerance. There was no difference in loaf volumes of flours from each blend baked at high, optimum and low water levels showing that the tolerance behavior allowed broader variation in water addition without affecting product quality attributes. However, a negative effect was noticed on crumb grain when lower water levels were used.

CHAPTER 6 – Future studies

- Carry out research utilizing additional wheat classes and varieties grown over multiple crop years in order to further clarify/ define components controlling water absorption tolerance
- Isolate specific proteins of interest using modern biochemical technology such as insertion of a gene of interest into a foreign host (yeast, insect cell lines or *Escherichia coli*) called heterologous expression. This technique allows purification and production of proteins in large quantities. Experiments using reconstituted flours could be conducted to test protein fractions functionality.
- Use genetic engineering techniques to produce proteins (gliadins) with different number of cysteine residues to determinate its functional role.
- Study relationship between water absorption tolerance vs protein content and quality. It may be the case that presence of certain protein fraction has higher contribution than total protein content and this would allow lower protein flours to have the same or higher absorption tolerance. This would have impact in commercial bakeries.
- Evaluate water absorption tolerance behavior between different wheat classes. Observe which components effect water absorption tolerance.

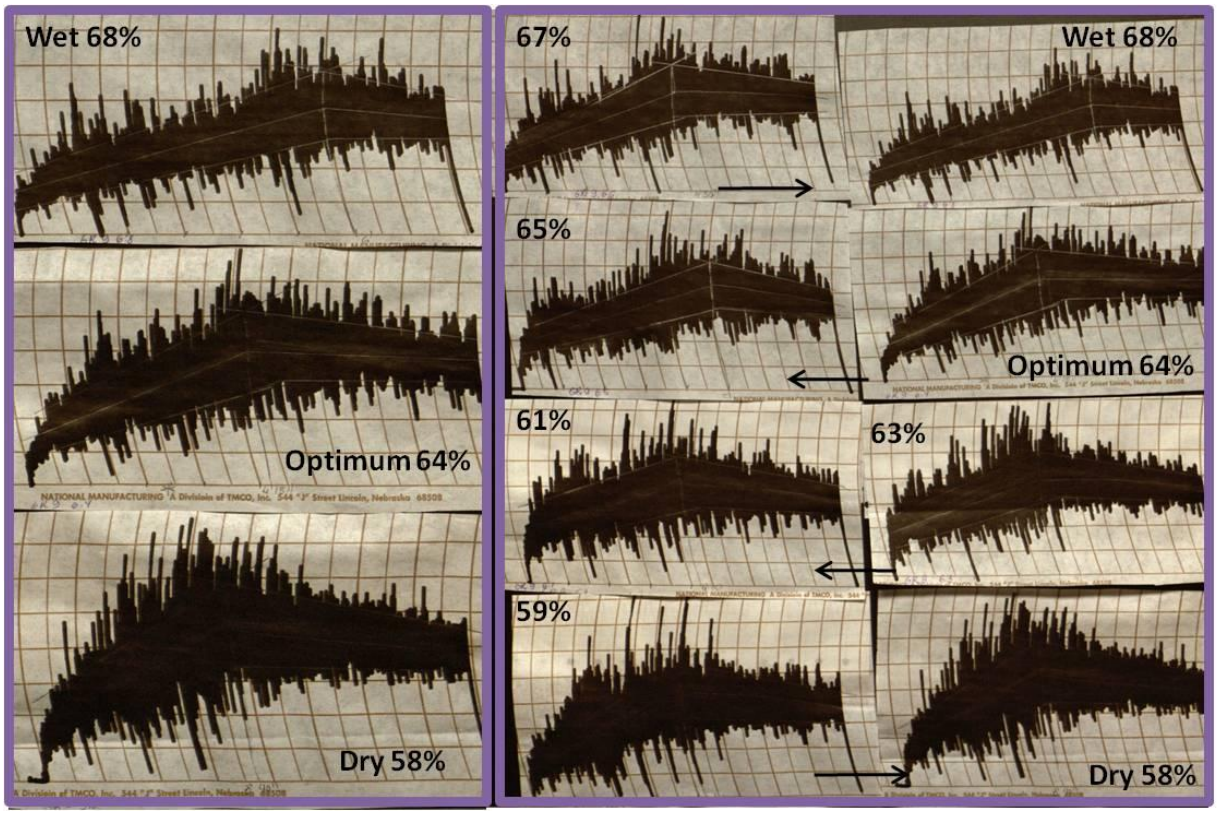

Figure 6 Example mixograms of a series representing dry to wet absorption extremes used to calculate water absorption range (mL H_2O) for Jagalene grown at Finney County.

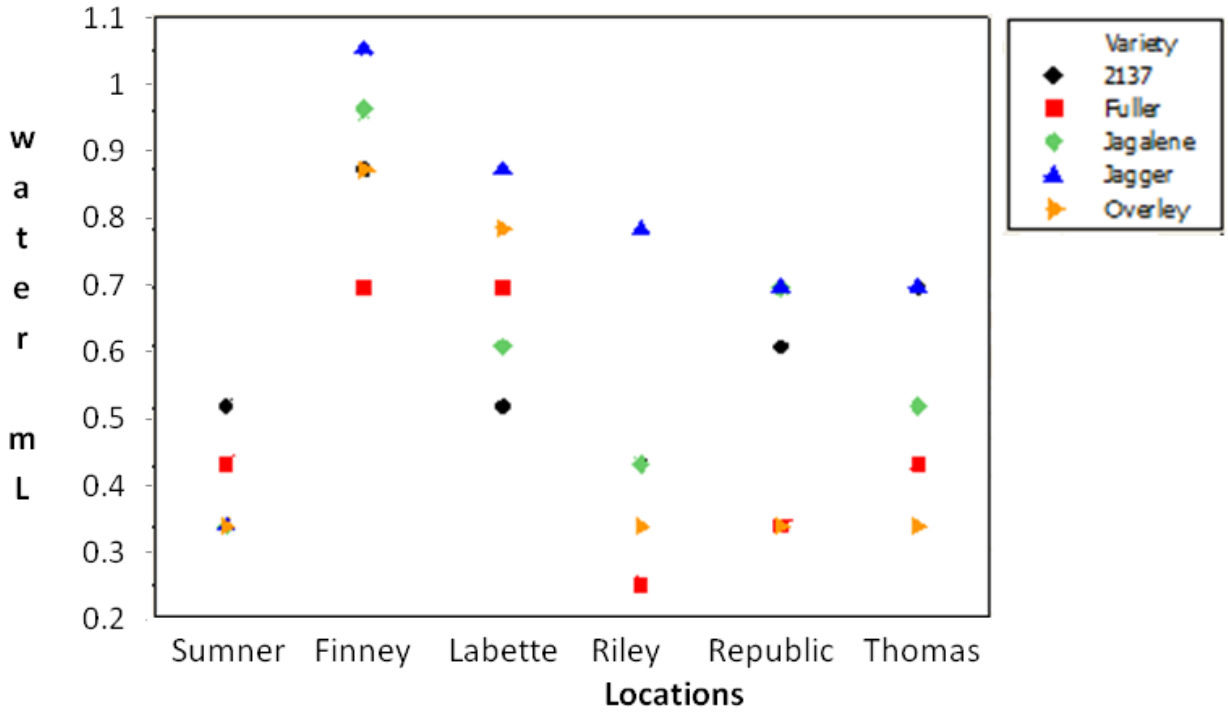

Figure 7 Water absorption tolerance behavior.

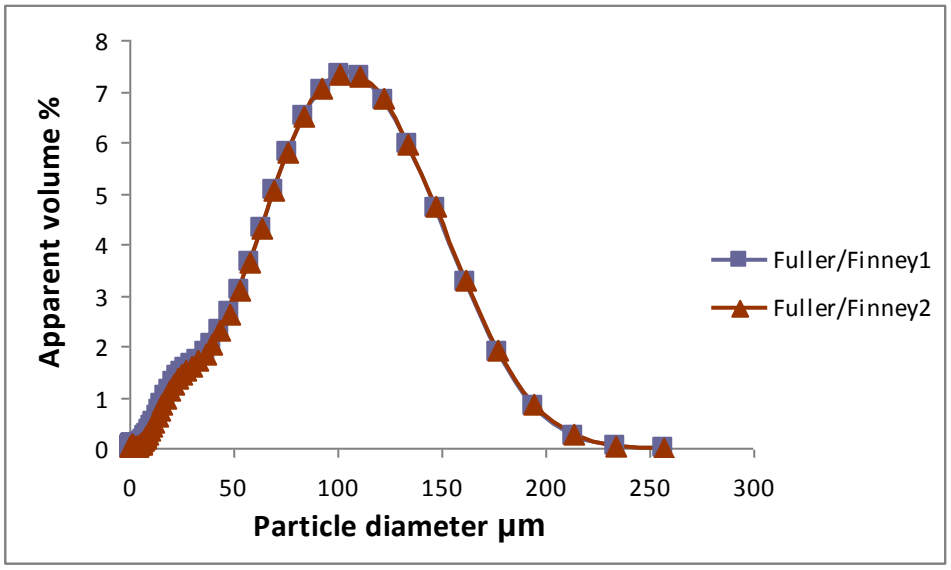

Figure 8 Example of flour particle size distribution plot obtained via laser diffraction particle size analyzer. Two replicates of Fuller grown at Finney are shown.

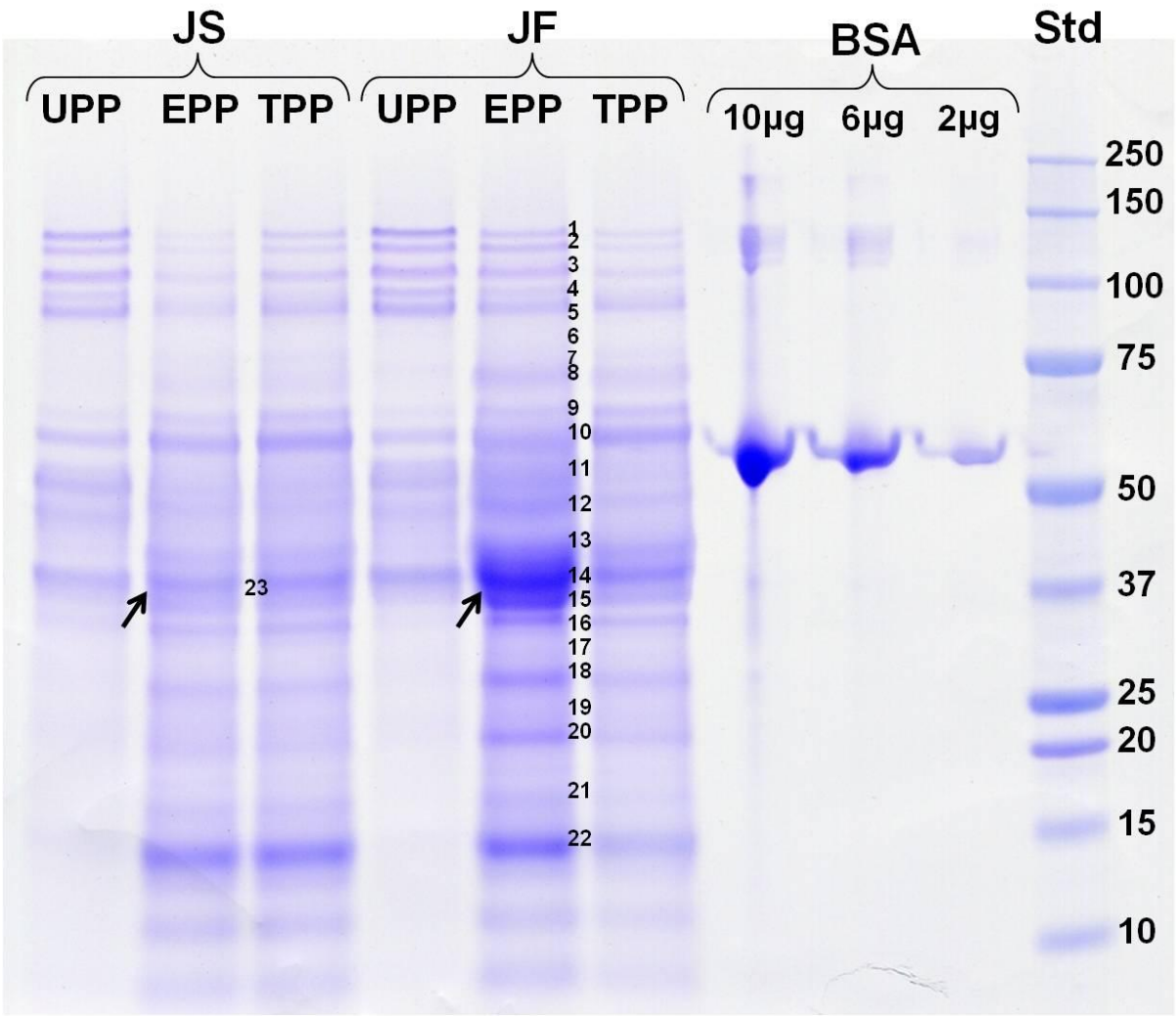

Figure 9 SDS PAGE electrophoresis gel showing; Total Polymeric Proteins (TPP), Extractable Polymeric Proteins (EPP) and Unextractable Polymeric Proteins (UPP) of Jagger grown at Finney (JF; high tolerance) and Sumner (JS; low tolerance) counties. Bovine Serum albumin (BSA) was applied in known concentrations to obtain a calibration curve.

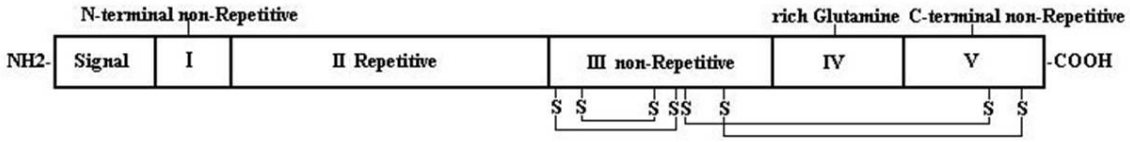

Figure 10 γ-gliadin model structure (Source: Qi et al 2009).

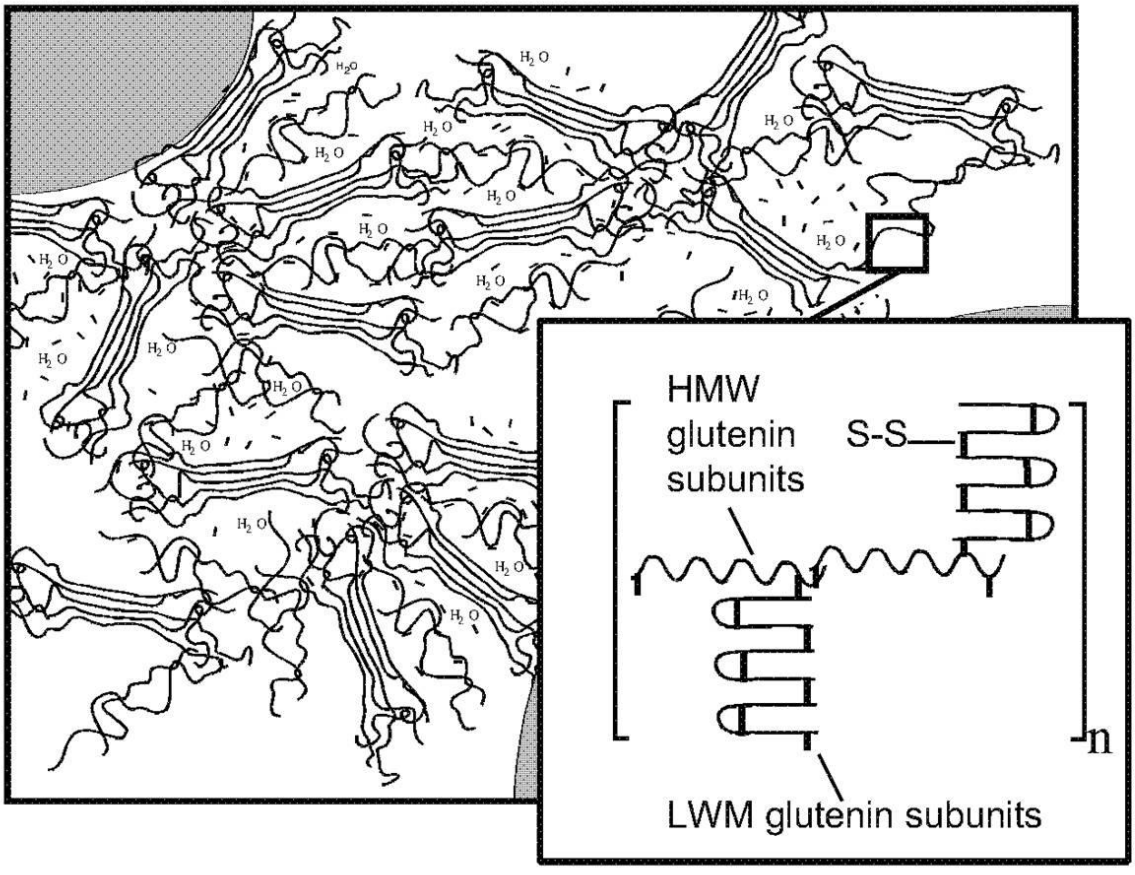

Figure 11 Possible entanglements mechanisms between LMW and HMW glutenins and water in dough (Gras et al 2001).

Table 2 Water absorption tolerance intervals (mL H_2O) determined via mixogram absorption series analyses

	Variety					
Location	**2137**	**Fuller**	**Jagalene**	**Jagger**	**Overley**	**Average**
Sumner	0.5	0.4	0.3	0.3	0.3	0.4
Finney	0.9	0.7	1.0	1.1	0.9	0.9
Labette	0.5	0.7	0.6	0.9	0.8	0.7
Riley	0.4	0.2	0.4	0.8	0.3	0.4
Republic	0.6	0.3	0.7	0.7	0.3	0.5
Thomas	0.7	0.4	0.5	0.7	0.3	0.5
Average	0.6	0.5	0.6	0.8	0.5	

Table 3 Water absorption tolerance classification

	Variety					
Location	**2137**	**Fuller**	**Jagalene**	**Jagger**	**Overley**	**Average**
Sumner	Interm	Low	Low	Low	Low	Low
Finney	High	High	High	High	High	High
Labette	Interm	High	Interm	High	High	High
Riley	Low	Low	Low	High	Low	Low
Republic	Interm	Low	High	High	Low	Interm
Thomas	High	Low	Interm	High	Low	Interm
Average	Interm	Interm	Interm	High	Interm	

Table 4 Effect of genotype and environment on water absorption tolerance range; ANOVA and Tukey analysis

Variety	Water absorption tolerance range (mL)	Water absorption tolerance classification
	F-value= 4.23	p-value=0.01
Jagger	0.75 a	High
2137	0.60 ab	Intermediate
Jagalene	0.58 ab	Intermediate
Overley	0.48 b	Low
Fuller	0.45 b	Low
Location	**Water absorption tolerance range (mL)**	**Water absorption tolerance classification**
	F-value= 10.76	p-value= <.0001
Finney	0.92 a	High
Labette	0.70 ab	High
Republic	0.52 bc	Intermediate
Thomas	0.52 bc	Intermediate
Riley	0.42 c	Low
Sumner	0.36 c	Low

Note: Values are average ranges obtained from mixogram series between locations for a certain variety and between varieties for a certain location. Same letters within a column indicate no significant difference, P-value ≥ 0.05. F-values and P-values were obtained from type III sum of squares. Absorption classification was determined considering intervals: 0.7-0.9 mL (High tolerance), 0.5-0.69 mL (Intermediate tolerance), 0.2-0.49 mL (Low tolerance).

Table 5 Correlation and p-value between water absorption tolerance and other parameters evaluated for crop 2009 samples.

	Water absorption tolerance	Wheat ash	Kernel weight	Kernel diam	Kernel hard ness	Kernel test weight	Flour ash	Damag ed starch %	Gliadin TPP	Glutenin TPP	Alb/glb TPP	EPP	UPP
Water absorption tolerance		R=-0.13 p=0.32	R=-0.25 p=0.05	R=-0.22 p=0.08	**R=0.68 p=<.0001**	R=0.12 p=0.34	R=0.26 p=0.05	R=0.02 p=0.85	**R=0.59 p=<.0001**	R=-0.46 p=0.0002	R=-0.57 p=<.0001	**R=0.82 p=<.0001**	**R=0.60 p=<.0001**
Sucrose%	R=0.18 p=0.17	R=0.33 p=0.01	R=-0.29 p=0.03	R=-0.22 p=0.10	R=0.54 p=<.0001	R=-0.12 p= 0.34	R=0.25 p=0.05	R=0.02 p=0.79	R=0.12 p=0.36	R=-0.10 p= 0.44	R=-0.11 p=0.40	R=0.39 p=0.002	R=0.12 p=0.38
Deionised water %	**R=0.69 p=<.0001**	R=0.05 p=0.72	R=-0.31 p=0.02	R=-0.28 p=0.03	R=0.75 p=<.0001	R=0.07 p=0.6	R=0.26 p=0.04	R=-0.01 p=0.89	R=0.44 p=0.0004	R=-0.37 p=0.003	R=-0.40 p=0.001	R=0.68 p=<.0001	R=0.38 p=0.003
Lactic acid %	**R=0.49 p=<.0001**	R=-0.50 p=<.0001	R=0.14 p=0.30	R=0.22 p=0.08	R=0.55 p=<.0001	R=0.51 p=<.0001	R=-0.23 p=0.08	R=-0.33 p=0.0004	R=0.77 p=<.0001	R=-0.70 p=<.0001	R=-0.66 p=<.0001	R=0.45 p=0.0003	R=0.68 p=<.0001
Sodium carb%	R=0.16 p=0.24	R=0.48 p=0.001	R=-0.39 p=0.002	R=-0.36 p=0.004	R=0.58 p=<.0001	R=-0.20 p=0.13	R=0.40 p=0.001	R=0.23 p=0.01	R=-0.08 p=0.56	R=0.14 p=0.28	R=0.001 p=0.99	R=0.36 p=0.005	R=-0.06 p=0.64
Flour prot	**R=0.72 p=<.0001**	R=-0.26 p=0.04	R=-0.19 p=0.14	R=-0.08 p=0.53	R=0.48 p=<.0001	R=-0.17 p=0.19	R=0.009 p=0.94	R=-0.19 p=0.15	R=0.83 p=<.0001	R=-0.75 p=<.0001	R=-0.73 p=<.0001	R=0.75 p=<.0001	R=0.85 p=<.0001
F41-300µm	**R=0.42 p=0.0009**	R=-0.37 p=0.004	R=-0.10 p=0.45	R=-0.04 p=0.77	R=0.65 p=<.0001	R=0.55 p=<.0001	R=-0.05 p=0.68	R=0.33 p=0.01	R=0.13 p=0.32	R=0.28 p=0.03	R=-0.57 p=<.0001	R=0.35 p=0.006	R=0.25 p=0.06
F10-41µm	R=-0.43 p=0.0007	R=0.40 p=0.002	R=-0.08 p=0.56	R=0.07 p=0.59	R=-0.65 p=<.0001	R=-0.55 p=<.0001	R=0.06 p=0.63	R=-0.34 p=0.007	R=-0.28 p=0.03	R=-0.13 p=0.32	R=0.58 p=<.0001	R=-0.37 p=0.004	R=-0.25 p=0.06
F< 10µm	R=-0.30 p=0.02	R=0.19 p=0.20	R=-0.16 p=0.20	R=-0.09 p=0.48	R=-0.50 p=<.0001	R=-0.45 p=0.0003	R= 0.007 p=0.96	R=-0.22 p=0.08	R=-0.22 p=0.09	R=-0.10 p=0.44	R=0.44 p=0.0004	R=-0.24 p=0.07	R=-0.20 p=0.12
Agranules	R=0.33 p=0.01	R=0.18 p=0.17	R=-0.30 p=0.02	R=-0.30 p=0.02	R=0.48 p=<.0001	R=-0.001 p=0.99	R=0.11 p=0.40	R=0.13 p=0.15	R=0.26 p=0.05	R=-0.21 p=0.10	R=-0.24 p=0.07	R=0.51 p=<.0001	R=0.16 p=0.22
Bgranules	R=-0.25 p=0.06	R=-0.01 p=0.93	R=0.16 p=0.23	R=0.16 p=0.20	R=-0.56 p=<.0001	R=-0.23 p=0.08	R=0.04 p=0.79	R=-0.20 p=0.03	R=-0.28 p=0.03	R=0.19 p=0.14	R=0.29 p=0.02	R=-0.34 p=0.009	R=-0.08 p=0.54
Cgranules	R=-0.32 p=0.01	R=-0.26 p=0.04	R=0.35 p=0.006	R=0.34 p=0.007	R=-0.34 p=0.007	R=0.16 p=0.22	R=-0.19 p=0.14	R=-0.57 p=0.54	R=-0.19 p=0.15	R=0.18 p=0.16	R=0.15 p=0.24	R=-0.54 p=<.0001	R=-0.18 p=0.18

Note: TPP: total polymeric protein, EPP: extractable polymeric protein, UPP: unextractable polymeric protein. F41-300µm: flour particle size range from 41 to 300 µm, F10-41 µm: flour particle size range from 10 to 41 µm, F< 10 µm: flour particle size range smaller than 10 µm. Agranules: starch granules type A particle size range bigger than 15 µm, Bgranules: starch granules type B particle size range from 5 to 15 µm, Cgranules: starch granules type C particle size range from 0 to 5 µm. Sodium carb: sodium carbonate absorption % - SRC.

Table 6 Effect of genotype and environment on Solvent Retention Capacity (SRC) and flour particle size; ANOVA and Tukey analysis.

Variety	SRC-Deionized water[1]	SRC-Lactic acid[1]	Fpart (41-300μm)[2]
	F-value= 7.58 p-value= <0.0001	F-value= 119.15 p-value= <0.0001	F-value= 33.95 p-value= <0.0001
Jagger	61.01 a	133.83 b	80.96 ab
2137	61.45 a	137.39 ab	79.97 b
Jagalene	61.37 a	140.69 ab	82.12 a
Overley	59.58 a	147.77 ab	78.32 c
Fuller	62.00 a	147.99 a	76.75 d
Location	F-value= 74.17 p-value= <0.0001	F-value= 721.40 p-value= <0.0001	F-value= 40.86 p-value= <0.0001
Finney	65.42 a	177.52 a	81.53 a
Labette	64.82 a	142.76 b	77.85 b
Republic	58.97 b	136.47 bc	81.18 a
Thomas	62.07 ab	145.79 b	82.32 a
Riley	58.08 b	124.43 c	79.25 b
Sumner	57.14 b	122.26 c	75.61 c

Note: [1] values refers to averages of four replicates measured as absorptions %. [2] Refers to average of two replicates measured as apparent volume % for flour particle size distribution range from 41 to 300 μm. Same letters within a column indicate no significant difference, P-values ≥ 0.05. F-values and P-values were obtained from type III sum of squares.

Table 7 Effect of genotype and environment on parameters showing high correlation with water absorption tolerance behavior; ANOVA and Tukey analysis.

Variety	Fprotct 14%mb	KHardness	GliadinTPP[1]	EPP[1]	UPP[1]
	F-value=1.13 p-value=0.37	F-value=17.23 p-value=<.0001	F-value=234.11 p-value=<.0001	F-value=220.17 p-value=<.0001	F-value= 109.35 p-value=<.0001
Jagger	10.49 a	74.93 a	46.13 b	9724.11 a	9794.95 c
2137	10.14 a	68.75 b	44.02 d	9354.45 b	8890.15 d
Jagalene	10.24 a	76.68 a	44.86 c	9023.63 c	10045.37 ab
Overley	10.12 a	63.13 c	45.81 b	8175.96 e	10232.05 a
Fuller	10.50 a	68.58 bc	47.19 a	8445.14 d	9988.52 bc
Location	F-value=45.65 p-value=<.0001	F-value=42.54 p-value=<.0001	F-value=941.92 p-value=<.0001	F-value=485.69 p-value=<.0001	F-value=418.69 p-value=<.0001
Finney	12.50 a	79.50 a	50.34 a	10351.03 a	11661.35 a
Labette	10.93 b	76.30 a	45.04 c	10092.36 b	10142.45 b
Republic	10.10 bc	68.80 b	46.34 b	8353.14 d	9903.27 c
Thomas	9.22 de	79.04 a	44.64 d	8579.15 c	8823.26 d
Riley	10.03 cd	61.00 c	44.92 cd	8542.10 cd	9762.43 c
Sumner	8.98 e	57.90 c	42.31 e	7750.18 e	8448.49 e

Note: [1] values reported are specific peak area /Peak 1 area (total) x 100. Correlation values between water absorption tolerance and parameters shown are: Fprotc R=0.72; Khardness R=0.68; GliadinTP R= 0.59; EPP R=0.82; UPP R=0.60. All P-values were <.0001. Values were obtained as averages for flour protein content (Fprotct) and kernel hardness (khardness). Values were obtained in duplicate for GliadinTPP, EPP and UPP. Same letters within a column indicate no significant difference, P-values ≥ 0.05. F-values and p-values were obtained from type III sum of squares. Fprotct: refers to flour protein content, khardness: refers to kernel hardness. TPP: refers to total polymeric protein, EPP: refers to extractable polymeric protein and UPP: refers to unextractable polymeric protein.

Table 8 HMW glu and γ-gliadin concentrations from SDS page analysis. Each protein was analyzed independently using ANOVA and Tukey analysis.

Water absorption tolerance	Sample	Spot	Protein name	Concentration µg/µL	MW KDa
High	**Jagger/Finney**	3	HMW glu	1.55 a [1]	88.6
Low	**Jagger/Sumner**	3	HMW glu	0.93 a	88.6
High	**Jagger/Finney**	14	γ-gliadin	20.16 a [2]	34.7
Low	**Jagger/Sumner**	23	γ-gliadin	5.96 b	24.4

Note: [1] F-value= 16.96 and P-value= 0.05. [2] F-value= 47.75 and P-value= 0.02. Same letters within a column portion indicate no significant difference, P-values ≥ 0.05. Values are averages of two replicates.

Table 9 Proteomics results for proteins present in extractable polymeric fraction after tryptic digestion.

Spot	*Protein name*	*Acc N°*	*pI/MW*	*MS/MS score*	*Peptides*	*Coverage %*
1	HMW glu Ax2*	gi|170743	6.15/88.6	454	9	16.1
2	HMW glu Ax2*	gi|170743	6.15/88.6	532	15	16.31
2	Phospoenolpyruvate carboxylase	gi|3341490	5.61/110.7	363	8	10.49
3	HMW glu Ax2*	gi|170743	6.15/88.6	888	38	28.71
3	HMW glu	gi|117650782	6.54/88.6	436	18	15.7
3	HMW glu 1By9	gi|22090	8.64/76.0	229	4	10.4
4	Sucrose synthase type 2	gi|3393044	6.17/93.0	820	16	23.19
4	Y-type HMW glu	gi|110341796	8.64/77.6	604	19	17.22
4	HMW glu 1By9	gi|22090	8.64/77.6	576	19	15.65
5	Sucrose synthase type I	gi|3393067	5.83/92.8	858	17	20.91
5	Sucrose synthase type 2	gi|3393044	6.17/93.0	668	14	19.5
5	HMW glu Dy10	gi|121449	7.64/69.9	530	22	22.06
6	HMW glu 1By9	gi|22090	8.64/76.0	491	11	15.55
6	HMW glu Dy10	gi|121449	7.64/69.9	393	11	15.88
7	HSP70	gi|2827002	5.14/71.3	763	11	22.06
7	HMW glu 1By9	gi|22090	8.64/76.0	315	8	14.46
8	Embryo globulin	gi|167004	6.8/72.5	187	9	10.83
9	Beta amylase	gi|32400764	8.6/31.0	567	23	54.64
9	Beta amylase	gi|3334120	5.24/56.8	384	17	13.32
10	Beta amylase	gi|32400764	8.6/31.1	630	33	46.09
10	Beta amylase	gi|3334120	5.24/56.8	376	17	15.90
11	LMW group3 type 2	gi|17425168	8.73/44.5	307	42	13.99
11	LMW group3 type 2	gi|17425170	8.22/42.4	191	19	20.16
12	Triticin	gi|171027813	6.37/65.2	336	10	15.07
12	LMW group3 type 2	gi|17425168	8.73/44.5	221	4	14.28
13	Triticin	gi|171027813	6.37/65.2	198	3	13.34
13	Globulin 1	gi|110341795	8.57/25.1	152	2	23.11
14	Gamma-gliadin	gi|121104	7.6/34.7	196	6	25.82
14	Gamma-gliadin	gi|15148400	6.96/24.4	187	6	38.42
15	Gamma-gliadin	gi|15148391	8.64/33.0	394	19	34.03
15	Cytosolic malate dehydrogenase	gi|49343245	5.75/35.8	180	3	14.71
16	Tritin	gi|391929	9.7/29.5	715	27	46.18
16	Xylanase inhibitor protein I	gi|20804336	8.66/33.47	343	8	27.30
17	Globulin 1	gi|110341801	8.05/25.1	382	14	44.69
17	Globulin 1	gi|110341790	8.72/25.5	375	12	39.73
18	19 kDa globulin	gi|32400820	7.66/26.6	206	3	23.58
18	Globulin 1	gi|110341801	8.05/25.1	205	3	22.40
19	Globulin 1	gi|110341795	8.57/25.1	206	4	31.55
20	Globulin 1	gi|110341795	8.57/25.1	199	3	23.11
20	19 kDa globulin	gi|32400820	7.66/26.6	194	3	22.40
21	Globulin 1	gi|110341801	8.05/25.1	133	2	18.58
21	19 kDa globulin	gi|32400820	7.66/26.6	129	2	18.25
22	Alpha amylase	gi|123958	5.31/16.3	103	4	24.47
23	Gamma-gliadin	gi|15148400	6.96/24.4	162	4	22.1
23	Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH	gi|7579064	7.83/25.4	265	5	33.2

Table 8 Bread baking results using a blend of varieties representing Finney, Republic and Sumner locations. Data was evaluated using ANOVA and Tukey analysis.

Sample ID	Water absorption level	Bread loaf volume cc	Crumb grain score
Finney High	70 %	980 a	Satisfactory 5
Finney Optimum	65 %	982 a	Satisfactory 5
Finney Low	63 %	997 a	Satisfactory 5
Sumner High	61 %	827 b	Questionable 3
Sumner Optimum	60 %	830 b	Unsatisfactory 2 DB
Sumner Low	59 %	837 b	Unsatisfactory 2

Values are average of three replicates at highest, optimum and lowest water absorption tolerances. Same letters within a column indicate no significant difference, P-value ≥ 0.05. F-values and P-values were obtained from type III sum of squares. Crumb grain was evaluated according to a scale from 1(poor) to 8 (excellent) based on appearance attributes. DB means double break. Highest and lowest absorptions refers to maximum and minimum water absorption tolerance. Wet and dry absorptions were not included.

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Appendix A - Summary of environmental data

A.1 Soil profile

Table 9 Soil profile

Locations	Soil type in previous crop	Pounds of N P_2O_5 K_2O	Plant harvest seed rate
Riley	Reading silt loam fallow	28 -- --	10/21/08-06/26/09 75lb/a
Labette	Parsons silt loam soybean	70 40 30	10/20/08-06/22/09 75lb/a
Sumner	Sandy loam wheat	110 40 --	10/28/08-06/25/09 60lb/a
Thomas	Keith silt loam fallow	60 40 --	09/19/08-06/30/09 60lb/a
Finney	Keith silt loam wheat	30 -- --	09/26/08-06/26/09 65lb/a
Republic	Crete silt loam fallow	100 30 --	10/01/08-07/02/09 90lb/a

A.2 Rainfall rate

Table 10 Rainfall rate in inches

Month 08-09	Riley	Labette	Sumner	Thomas	Finney	Republic
8-Oct	2.06	1.81	N/A	2.8	4.37	N/A
8-Nov	1.34	3.44	1.51	0.74	0.34	5.76
8-Dec	0.64	2.18	1.23	0.65	0.03	0.43
9-Jan	0.04	0.03	0.16	0.15	0.06	0.23
9-Feb	0.65	1.7	0.66	0.49	0.07	0.01
9-Mar	3.01	4.1	3.94	0.1	1.15	0.11
9-Apr	5.25	9.95	4.25	3.44	4.36	0.17
9-May	0.98	6.17	2.35	5.53	1.84	1.77
9-Jun	8.48	4.67	4.68	3.69	3.7	0.61
Total	22.4	34.0	18.7	17.6	15.9	9.09

A.3 Rainfall rate ANOVA and Tukey analysis

Table 11 Rainfall rate ANOVA analysis

Locations	Average rainfall rate (inches) F-value= 1.42 P-value= 0.30
Labette	3.78 a
Riley	2.49 a
Sumner	2.24 a
Thomas	1.95 a
Finney	1.77 a
Republic	1.35 a

Note: Rainfall rate data was divided in three periods:1, 2 and 3. The third period (apr, may and jun) had significantly higher rainfall rate (3.99) when compared with first (Oct, nov, dec) (1.87) and second (jan, feb, march) (0.92) periods with a F-value= 9.23 and P-value= 0.0042. All F-values and P-values were obtained from type III sum of squares. No significant differences were found between locations.

A.4 Temperature profile

Table 12 Temperature profile ºF

Months 08-09	Riley	Labette	Sumner	Thomas	Finney	Republic
8-Oct	42-68	45-69	45-68	39-66	41-69	42-67
8-Nov	33-60	40-62	34-56	29-59	30-60	30-53
8-Dec	25-50	30-50	21-44	20-45	22-50	13-37
9-Jan	18-40	20-40	19-44	12-40	15-50	15-42
9-Feb	19-41	22-42	26-55	12-42	15-45	19-49
9-Mar	25-52	30-52	33-58	20-50	25-58	26-56
9-Apr	35-62	40-62	41-65	30-60	30-65	37-65
9-May	38-71	50-70	53-75	40-70	42-72	52-77
9-Jun	55-80	60-85	66-88	50-82	58-88	62-84

A.5 Temperature ANOVA and Tukey analysis

Table 13 Temperature ANOVA and Tukey analysis

Locations	Average temperature ºF F-value= 1.68 P-value= 0.23
Labette	48.27 a
Sumner	47.66 a
Finney	46.37 a
Republic	45.89 a
Riley	45.20 a
Thomas	42.54 a

Note: Temperature data was divided in three periods: 1, 2 and 3. The three grouping periods had significant differences. Third period (apr, may and jun 59.08 ºF) > first period (Oct, nov, dec 44.81 ºF) > second period (jan, feb, mar 34.07 ºF) with F-value= 128.22 and P-value= <0.0001. All F-values and P-values were obtained from type III sum of squares. No significant differences were found between locations.

Appendix B - Particle size distribution curve for starch samples obtained using Laser diffraction liquid module system

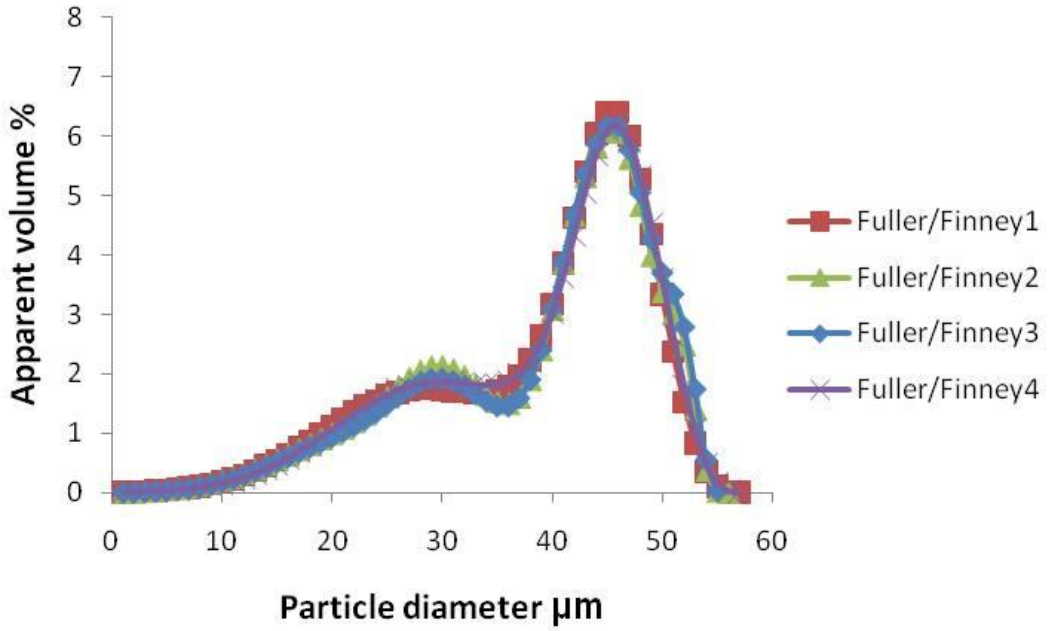

Figure 8 Example of starch particle size distribution plot for Fuller grown at Finney. Four replicates of Fuller grown at Finney are shown.