

DARK, HARD AND VITREOUS (DHV) HRS WHEAT KERNEL CONTENT EFFECT  
ON FLOUR AND BAKING QUALITY

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## ABSTRACT

Kernel vitreousness is an important grading characteristics of Hard Red Spring (HRS) wheat in the U.S., as subclasses vary in the dark, hard and vitreous kernel (DHV) content. This research investigated different subclasses of HRS wheat on flour and baking quality characteristics. The U.S. Regional Crop Quality Survey samples from three consecutive years were used for subclass segregation. Samples were milled, and flour quality and bread baking characteristics were evaluated for both regional and protein composites. A significant ( $P < 0.05$ ) difference in the flour water absorption was found between vitreous kernel treatments, and high DHV content resulted in greater water absorption. An example further showed the importance of flour water absorption on potential economical incentives that can be gained with high DHV content. These results enable the flour milling and baking industry to choose between the different subclasses of HRS wheat with varying DHV content for their intended end-use applications.

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## TABLE OF CONTENTS

ABSTRACT .....	iii
ACKNOWLEDGEMENTS .....	iv
LIST OF TABLES .....	vii
LIST OF FIGURES .....	ix
LIST OF ABBREVIATIONS .....	x
LIST OF APPENDIX TABLES .....	xi
1. INTRODUCTION .....	1
2. LITERATURE REVIEW .....	2
2.1. Wheat .....	2
2.1.1. Wheat Growing Conditions and Trading .....	2
2.1.2. Wheat in the United States .....	3
2.2. Wheat Classification .....	4
2.3. Hard Red Spring Wheat .....	5
2.4. The Wheat Kernel .....	6
2.4.1. Bran .....	7
2.4.2. Germ .....	8
2.4.3. Endosperm .....	8
2.5. Wheat Kernel Characterization .....	9
2.6. Wheat Flour Proteins and Their Role on Dough Characteristics .....	12
2.7. Protein Quality and Its Effect on Bread Quality .....	15
2.8. Flour Quality Characteristics and End-Use Quality .....	16
2.9. Hard Red Spring Wheat Subclass Differentials on Wheat Pricing .....	18

3. OBJECTIVES AND NEED STATEMENT .....	20
3.1. Need Statement .....	20
3.2. Research Objectives .....	21
4. EXPERIMENTAL APPROACH .....	22
4.1. Sample Preparation and Analysis .....	23
4.2. Statistical Analysis .....	26
5. RESULTS AND DISCUSSION .....	27
5.1. Regional Composites .....	27
5.2. Protein Composites .....	41
5.3. Glenn Variety .....	55
5.4. Flour Water Absorption and Its Influence on Dough Weight.....	60
6. CONCLUSIONS .....	63
7. FUTURE RESEARCH DIRECTIONS .....	64
REFERENCES .....	66
APPENDIX.....	73

## LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Kernel quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel.....	27
2. Kernel quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel across growing years.....	28
3. Milling and flour quality characteristics of regional composites varying percentages of dark, hard and vitreous kernel.....	30
4. Milling and flour quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel across growing years.....	31
5. Dough properties and bread baking quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel.....	34
6. Correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-extractable and -unextractable proteins and flour protein content and vitreous kernel for regional composites.....	36
7. SDS-extractable and -unextractable protein fractions of regional composites varying different percentages of dark, hard and vitreous kernel.....	37
8. Analysis of variance of vitreous kernel content, test weight, 1000-kernel weight, flour extraction, and break flour yield for protein composites.....	41
9. Kernel and milling quality characteristics of protein composites varying different percentages of dark, hard and vitreous kernel across growing years.....	43
10. Analysis of variance of flour protein content, starch damage, wet gluten, farinograph water absorption, baking water absorption, and bread loaf volume for protein composites.....	47
11. Flour and bread quality characteristics of protein composites varying different percentages of dark, hard and vitreous kernel across growing years.....	50
12. Correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-extractable and -unextractable proteins and flour content and vitreous kernel for protein composites.....	51

13. Kernel quality characteristics and milling yield of Glenn variety varying different percentages of dark, hard and vitreous kernel .....	55
14. Flour and bread quality characteristics of Glenn variety varying different percentages of dark, hard and vitreous kernel .....	57
15. SDS-extractable and -unextractable protein fractions of Glenn variety varying different percentages of dark, hard and vitreous kernel.....	60



## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Wheat types and types of products varying in protein content.....	2
2. A longitudinal section of wheat kernel.....	6
3. A cross section of wheat kernel.....	7
4. Light microscopy images of cross cut sections of vitreous (left) and starchy kernels (right).....	10
5. Scanning electron micrographs of cross sections of hard wheat breakage at the cell wall (left) and soft wheat breakage through the cells (right).....	12
6. Wheat gluten-forming proteins.....	13
7. Physical dough properties of wheat gluten (left) and its components: gliadin (center) and glutenin (right).....	14
8. Spectrum of correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-extractable proteins and vitreous kernel (A) and bread loaf volume (B) over retention time for regional composites.....	38
9. Spectrum of correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-unextractable proteins and vitreous kernel (A), farinograph peak time (B), and bread loaf volume (C) over retention time for regional composites.....	40
10. Scanning electron microscopy (SEM) images of cross cut sections of vitreous kernel at different magnifications.....	44
11. Scanning electron microscopy (SEM) images of cross cut sections of starchy kernel at different magnifications.....	44
12. Spectrum of correlation of coefficients (r) between size-exclusion HPLC absorbance areas of SDS-extractable proteins and vitreous kernel (A), and bread loaf volume (B) over retention time for protein composites.....	52
13. Spectrum of correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-unextractable proteins and vitreous kernel (A), farinograph peak time (B), and loaf volume (C) over retention time for protein composites.....	54

## LIST OF ABBREVIATIONS

HRSW .....	Hard Red Spring Wheat
HRWW .....	Hard Red Winter Wheat
DHV .....	Dark, Hard and Vitreous
LMW-G.....	Low Molecular Weight Glutenin
HMW-G .....	High Molecular Weight Glutenin
UPP .....	Unextractable Polymeric Protein
SE-HPLC .....	Size Exclusion High Performance Liquid Chromatography
KWT .....	Kernel Weight
SEM .....	Scanning Electron Micrograph
LSD .....	Least Significant Difference
SDS .....	Sodium Dodecyl Sulfate
ANOVA .....	Analysis of Variance

## LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
A 1. Analysis of variance for kernel quality and milling characteristics of regional composites.....	73
A 2. Analysis of variance for flour quality characteristics of regional composites .....	74
A 3. Analysis of variance for bread baking characteristics of regional composites .....	75
A 4. Analysis of variance for kernel quality characteristics for protein composites .....	76
A 5. Analysis of variance for milling characteristics of protein composites .....	77
A 6. Analysis of variance for flour quality characteristics for protein composites .....	78
A 7. Analysis of variance for farinograph quality parameters of protein composites .....	79
A 8. Analysis of variance for bread baking characteristics of protein composites.....	80
A 9. Analysis of variance for kernel quality and milling characteristics of Glenn samples .....	81
A 10. Analysis of variance for flour quality and farinograph parameters of Glenn samples .....	82
A 11. Analysis of variance for bread baking characteristics of Glenn samples .....	82
A 12. Kernel and flour quality, and bread baking characteristics of composites from 2010 .....	83
A 13. Kernel and flour quality, and bread baking characteristics for composites from 2011 .....	84
A 14. Kernel and flour quality, and bread baking characteristics of composites from 2012.....	85

## 1. INTRODUCTION

Hard Red Spring (HRS) wheat, grown in the United States, is divided into three subclasses based on the percentage of dark, hard and vitreous (DHV) kernels present. Differences in DHV content of these subclasses reflect variation in protein content (Dexter and Edwards, 1998). The DHV content is a widely used quality factor in grading and marketing of HRS wheat (Wang et al., 2002). DHV percentage is a primary marketing factor used for HRS wheat in the United States; thus, protein premiums are reflected in prices depending upon protein level (Wilson, 1989; Nielsen et al., 2003).

Kernel vitreousness is one of the most important grading characteristics affecting milling performance and end-use quality of wheat (Simmonds, 1974). In general, vitreous kernels are associated with high protein content, high water-absorption capacity, and also loaf volume potential in bread making (Carson and Edwards, 2009; Wang et al., 2002; Dexter and Edwards, 1998; Nielsen et al., 2003; Dexter et al., 1989). Therefore, in the production of bread, it is desirable for hard wheat to contain a high percentage of vitreous kernels (Carson and Edwards, 2009; Dexter and Edwards, 1998).

The current research is aimed at determining the effect of DHV kernel content on HRS wheat milling and baking quality by analyzing U.S. Regional Crop Quality Survey samples from three consecutive growing years, and also by analyzing a HRS wheat variety (Glenn) from two locations (Mergoum et al., 2006). In addition to determining the effect of DHV kernel content on HRS wheat milling and baking quality, the research is also aimed at determining the effect of DHV kernel content in the differences in flour and baking water absorption, and how water flour absorption could be economically beneficial in terms of total dough weight.

## 2. LITERATURE REVIEW

### 2.1. Wheat

Wheat is an important crop in many countries, including the United States and Canada. Wheat and bread are integral to human life as well as human food (Wrigley, 2009); and wheat is among the oldest and most extensively grown of all grain crops. Wheat is a member of the grass family (Gramineae), which includes the cereal grains (Delcour and Hosney, 2010). Wheat-based food products are of importance, and are considered staples, in many countries throughout the world. There are various types of products made from wheat flour depending on the desired end-use (Fig. 1).

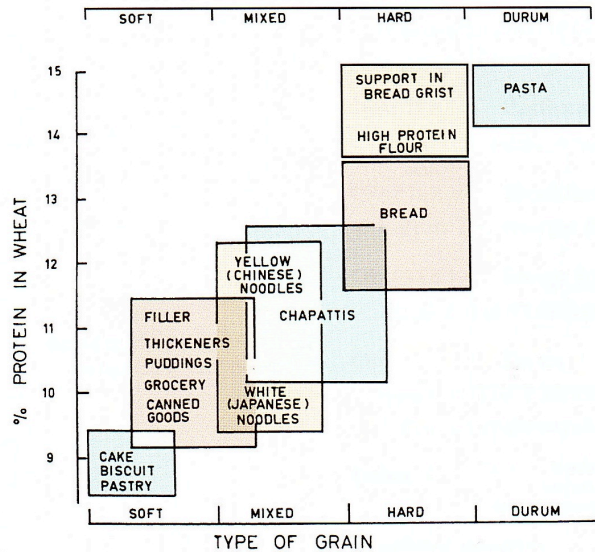


Figure 1. Wheat types and types of products varying in protein content (Reprinted from Delcour, J.A. and Hosney, R.C. 2010)

#### 2.1.1. Wheat Growing Conditions and Trading

Wheat can be grown as either a winter or a spring crop (Wrigley, 2009). Because the wheat plant is quite hardy, it can grow under a wide variety of environmental and soil conditions (Delcour and Hosney, 2010). Therefore, the wheat plants are grown annually

on all continents except Antarctica, producing well over 600 million tons of grain from about 220 million hectares with an average yield of nearly 3 tons/ha (Wrigley, 2009). Wheat is grown on more land than any other food crop and is harvested globally throughout the year (Posner and Hibbs, 2005). Wheat is the dominant crop in temperate regions or countries and is mainly used for human food and livestock feed (Shewry, 2009).

Most of the wheat grown worldwide is common wheat (*Triticum aestivum* L.), while durum wheat (*T. turgidum* L. spp. *durum*.) is cultivated about 10 % worldwide (Wrigley, 2009). More than 80 % of wheat is consumed within the source of origin, and the remaining 110 million tons enters into international trade, thus making wheat the most traded grain in the world (Wrigley, 2009).

#### 2.1.2. Wheat in the United States

The majority (80 %) of traded wheat (produced in the developed countries) comes from the United States, Canada, European Union (EU), Australia, and Argentina (Worden, 2004). However, the U.S. is the world's largest exporter of common wheat having a market share of 28% (1993-2002) (Wrigley, 2009). There are six main wheat classes grown in the U.S.: Hard Red Winter (HRW), Hard Red Spring (HRS), Soft Red Winter (SRW), Soft White (SWH), Hard White (HWH), and Durum. These wheat classes are classified based upon color, kernel hardness, and growth habit. However, about 70% of the crop is fall planted (Carson and Edwards, 2009). Each wheat class or type has unique milling and end-use properties.

## 2.2. Wheat Classification

Three of the most important wheat classification criteria are kernel texture (hard or soft), bran color (red or white), and growth habit (spring or winter) (Carson and Edwards, 2009). Grain color and appearance both affect the market value of wheat, misclassification of color classes result in poor grain quality and a loss of monetary value (Singh et al., 2006). On the other hand, endosperm texture influences the milling performance; and it is also an important criterion for determining end use of various wheat classes (Glenn and Saunders, 1990). Kernel texture is the physical resistance of wheat kernels to crushing or shearing force as they are ground or milled into smaller particles. It is sometimes termed as “hardness.” Therefore, hardness is directly related to the force and energy consumed during grinding process. The structure of the endosperm contents is what determines the hardness of the grain (Turnbull and Rahman, 2002). Endosperm consists of protein and starch granule matrix, which is separated by cell walls. More specifically, presence and functionality of the basic and cysteine-rich proteins puroindoline a (PINA) and b (PINB) are what determines the hardness characteristics of wheat (Pauly et. al., 2013).

Kernel hardness is also related to protein content and the flour water absorption factor (Dexter et al., 1989). Although there have been contrasting conclusions, it has been reported that a vitreous appearance is generally associated with hardness and high protein content within a class, whereas mealiness or opaqueness is often associated with softness and low protein content (Sadowska et al., 1999). The hardness characteristic is not very well understood. There have been theories suggested that the trait is caused by the differing amounts of adhesion between the starch granules and surrounding protein

matrix (Turnbull and Rahman, 2002). However, others have suggested that the differences in hardness could be because of the continuity of the protein matrix and the strength with which it physically entraps starch granules. The degree of hardness is determined by the continuity of the protein matrix, its structure and the strength with which it physically entraps starch granules (Glenn and Saunders, 1990). Furthermore, the protein matrix structure can influence hardness.

Generally, the hard cultivars are more difficult to crush during milling or grinding. This is due to the strong adhesion between the starch granules and its surrounding storage proteins (Simmonds, 1974; Sadowska et al., 1999). On the other hand, the North American soft cultivars are easy to crush because of the weaker adhesion between the starch granules and protein matrix due to more open air spaces. The adhesion between starch and protein could vary in hard and soft wheat endosperm because of their quantitative or qualitative differences in cellular deposited at the starch-protein interface (Glenn and Saunders, 1990).

### 2.3. Hard Red Spring Wheat

Hard Red Spring wheat constitutes about 25% of the crop in the United States and is composed of spring-sown varieties with hard endosperm and red seed coat (Carson and Edwards, 2009). Hard Red Spring wheat is important in the U.S. domestic and export markets, as HRS varieties are characterized by high protein content, and excellent milling and baking performance (Carson and Edwards, 2009). Hard Red Spring wheat is also a valued improver in flour blending (U.S. Wheat Associates, 2013). In addition, HRS wheat produced in United States and Canada is well suited to the production of high-



volume breads made by the traditional sponge-and-dough baking process (Cracknell and Williams, 2004).

Hard Red Spring wheat is subdivided into three classes as part of the Federal Grain Inspection (FGIS) grading standards, and the division into three subclasses is based on dark, hard and vitreous kernel content (Carson and Edwards, 2009). Wheat is assigned to (1) dark northern spring (DNS) if it contains  $\geq 75\%$  DHV kernels, (2) northern spring (NS) if it contains 25-74% DHV kernels, and (3) red spring (RS) if it has  $< 24\%$  DHV kernels. Due to the variation in percentage of DHV kernels present, these subclasses of HRS wheat differ in protein content (Dexter et al., 1989; Dexter and Edwards, 1998), thus resulting in different milling performance and baking quality.

#### 2.4. The Wheat Kernel

Wheat kernels are dry one-seeded fruits (Posner and Hibbs, 2005). Wheat kernels are rounded in the dorsal (the same side as the germ) and have a longitudinal crease over the length of the ventral side (opposite the germ). The wheat kernel consists of three parts: bran, endosperm, and germ (Fig. 2).

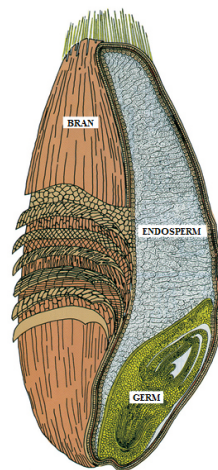


Figure 2. A longitudinal section of wheat kernel (<http://www.ndwheat.com/uploads/resources/376/kernel-wheat-how-flour-milled.pdf>)

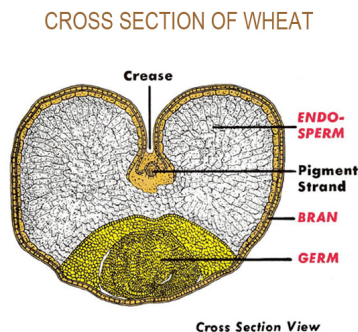


Figure 3. A cross section of wheat kernel  
 (<http://www.namamillers.org/oldsite/images/CrossSectionViewofWheat.gif>)

The pigment strand or pigment in the seed coat is responsible or determines the color of the seed.

#### 2.4.1. Bran

The pericarp and the outermost tissues of the wheat kernel compose what is commercially known as “bran” (Posner and Hibbs, 2005). The bran makes up about 14.5% of the whole-wheat kernel. The pericarp (fruit coat) surrounds the entire seed and is composed of several layers. The outer pericarp is comprised of the epidermis, hypodermis, and remnants of thin-walled cells. The inner pericarp is composed of intermediate cells, cross cells, and tube cells. The seed coat is firmly joined to the tube cells on their outer side and to the nucellar epidermis on its inner side (Delcour and Hosney, 2010). The seed coat consists of three layers: (1) a thick outer cuticle, (2) a layer that contains pigment, and (3) a thin inner cuticle, which surrounds the kernels’ endosperm.

#### 2.4.2. Germ

The germ is structurally a separate entity of the kernel (Posner and Hibbs, 2005). The wheat germ makes up 2.5-3.5% of the kernel (Delcour and Hosenev, 2010). The wheat germ contains the embryo and the scutellum, which are separated from the epithelial layer. The germ is composed of two major parts: the embryonic axis and the scutellum, which functions as a storage organ.

#### 2.4.3. Endosperm

The wheat endosperm contains about 30,000 cells that vary in size, shape, and composition of starch granules and protein depending on the location in the kernel (Posner and Hibbs, 2005). The endosperm consists of the aleurone layer and the starchy endosperm (Delcour and Hosenev, 2010). Aleurone layer, which is a single cell in thickness, surrounds the kernel completely and covers the starchy endosperm and the germ. The starchy endosperm is composed of three types of cells, and these also vary in size, shape, and location within the kernel. The peripheral starchy endosperm cells are the first row of cells inside the aleurone layer, and these cells are usually small and equal in diameter. Prismatic starchy endosperm cells are the next several rows of cells, and they extend inward to about the center of cheeks (Delcour and Hosenev, 2010). Central starchy endosperm cells are more irregular in size and shape compared to the other types of cells. The wheat endosperm cells walls are mainly composed of arabinoxylans, and they contain minor levels of  $\beta$ -glucans and other hemicelluloses. The cell walls are packed with starch granules that are embedded in the protein matrix.

Environmental factors such as temperature impacts grain yield by altering the rate and the duration of grain filling period (Dupont and Altenbach, 2003). When high

temperature and drought are combined together, the effects are far greater. More, specifically, the combination of high temperature and drought reduces the duration of grain filling (Dupont and Altenbach, 2003). Starch is a major determinant for grain yield, in which it accounts for 65-75% of the grain dry weight and up to 80% of the endosperm weight. It has been reported that reductions in starch accumulations at high temperatures account for significant losses in grain yield (Tashiro and Wardlaw, 1989; Bhullar and Jenner, 1985). Although there are series of enzymes involved in synthesizing amylose and amylopectin chains that comprise starch, most of the decline in starch deposition by heat is due to decreased activity soluble starch synthase.

## 2.5. Wheat Kernel Characterization

Grain quality is affected by characteristics such as moisture content, soundness, and vitreousness (Singh et al., 2006). Visual or physical characteristics of a wheat kernel take one of two forms (vitreous and starchy or non-vitreous) depending on the compactness of its components in the endosperm (Carson and Edwards, 2009). Major components in the wheat endosperm are starch granules and proteins that surround the starch granules. Developing endosperm cells have discrete protein bodies, and these protein bodies form a continuous matrix around starch granules during grain maturing. Kernels that are glasslike and translucent in appearance are referred to as vitreous, whereas kernels that lack translucency or are light-colored opaque are called non-vitreous (starchy or piebald). Often times, the cut surface of a hard cultivar can be distinguished from a soft cultivar by the amount of vitreousness it has (Turnbull and Rahman, 2002) (Fig. 4).

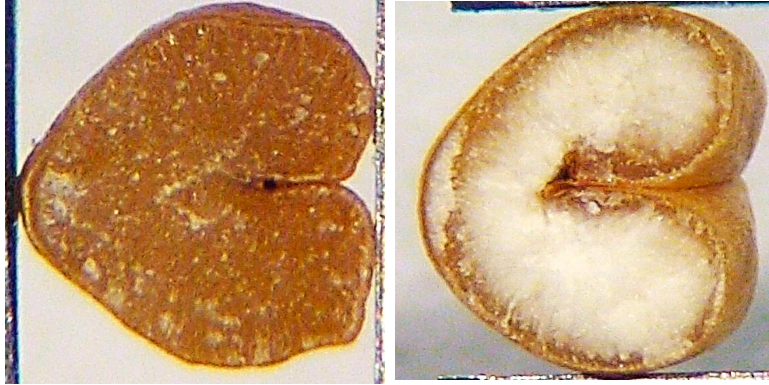


Figure 4. Light microscopy images of cross cut sections of vitreous (left) and starchy kernels (right)

Factors influencing vitreous characteristics of wheat kernels are heredity, weather, tillage, and fertilization (Phillips and Niernberger, 1976). However, vitreousness is mainly controlled by nitrogen availability as well as temperature during grain filling period (Pomeranz and Williams, 1990). Yellow berries or starchy kernels can be distinguished by sorting equipment because lighter-colored or starchy endosperm lacks the vitreous texture characteristics of normal grain (Sharp, 1927).

In vitreous endosperm, the adhesion between the starch granules and storage proteins is much stronger compared to starchy endosperm, thus leading to a more tightly compacted structure (Simmonds, 1974; Sadowska et al., 1999). In other words, starch granules are much more closely associated with the storage proteins in vitreous endosperm of hard wheat. This adhesion between starch granules and the surrounding proteins is important in milling because the fracture differs between hard and soft wheat (Posner and Hibbs, 2005).

Generally, factors that determine the differences in milling yield fall into two classes: (1) factors affecting the proportion of endosperm in the wheat kernel (2) factors affecting the ease and degree to which the endosperm can be separated from non-

endosperm components (Marshall et al., 1986). Kernel size and shape, embryo size and the thickness, and the density of the seed coat are examples of factors that determine the proportion of the endosperm. However, other factors such as grain hardness, bulk density, fiber content, crease depth and width, and cell wall thickness in the sub-aleurone endosperm determine the ease and the degree endosperm can be separated from non-endosperm components.

Therefore, endosperm texture is very important as texture affects the tempering requirements; flour particle size, flour density, starch damage, water absorption, and milling yield to the miller (Turnbull and Rahman, 2002). However, to the processor, endosperm texture is a good indicator of the suitability of flour for a particular product, while endosperm texture is important to the grower as higher premiums are paid for harder wheat.

Cell walls and the cell contents of hard wheat form a coherent whole during milling, and cell walls remain attached to the smaller granular particles produced in the milling process (Simmonds, 1974). Compared to hard wheat, the cell contents of soft wheat are readily crushed and released through the rupture of the cell walls due to weaker adhesion or more air spaces between starch and storage proteins.

Therefore, the nature of the starch-protein interface is an important consideration to the miller, and the kernel vitreousness is a key factor of milling performance (Simmonds, 1974; Samson et al., 2005). In durum wheat milling, starchy kernels yield less coarse semolina and more flour, thus reducing the milling potential (Carson and Edwards, 2009). In contrast, starchiness has little impact on the milling performance of hard wheat when straight-grade types of flour are produced. However, starchiness

reduces the yield of granular hard-wheat farina from the break rolls but with more fine flour produced during the reduction roll passes (Carson and Edwards, 2009). With more fine flour produced in the reduction rolls, it could lower the potential for the production of low-ash patent flours.

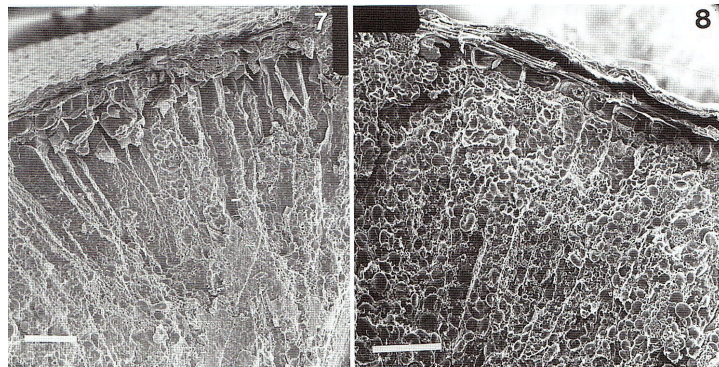


Figure 5. Scanning electron micrographs of cross sections of hard wheat breakage at the cell wall (left) and soft wheat breakage through the cells (right) (Reprinted from Delcour, J.A. and Hoseney, R.C., 2010)

## 2.6. Wheat Flour Proteins and Their Role on Dough Characteristics

Wheat storage proteins are known as prolamins due to their high content of the amino acids, proline and glutamine (Malik, 2009). Wheat flour proteins are classified into four types depending on their solubility (Delcour and Hoseney, 2010) (Fig. 6). Albumins are soluble in water whereas globulins are insoluble in water but soluble in dilute solutions of salt and insoluble at high salt concentration. Gliadin is the wheat prolamins and these proteins are soluble in 70% ethanol. The wheat glutelin is named glutenin, and is soluble in dilute acids or bases (Delcour and Hoseney, 2010). Another classification system divides prolamins into three groups: sulfur-rich, sulfur-poor, and high molecular weight glutenin subunits (HMW-GS) (Malik, 2009).

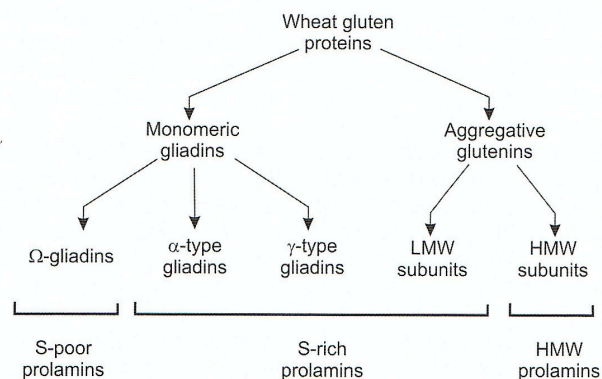


Figure 6. Wheat gluten-forming proteins  
(Reprinted from Khan, K. and Shewry, P.R., 2009)

Flour produced from wheat is unique (compared to other cereals) because it has the ability to form viscoelastic dough when mixed with the appropriate amount of water (Delcour and Hosney, 2010). The viscoelastic property of wheat flour dough is important for the bread making process, as it provides for the formation of strong and cohesive dough. Also, the degree of dough expansion during bread baking depends on the viscoelastic properties (Aamodt et al., 2004). Although wheat flour contains all of the four types of proteins (classified based on solubility), the storage or gluten forming proteins constitute up to 80% of the total flour proteins (Dupont and Altenbach, 2003). These gluten-forming proteins are present in the wheat endosperm, in which they form a continuous matrix around the starch granules (Malik, 2009).

Gluten forming proteins consist of monomeric gliadins and polymeric glutenins. Gliadin has little or no resistance to extension and is responsible for viscous characteristic of the dough (Delcour and Hosney, 2010). In contrast, glutenin is responsible for resistance to extension or elastic characteristics of the dough. And together they form the viscoelastic characteristics of wheat dough (Fig. 7).



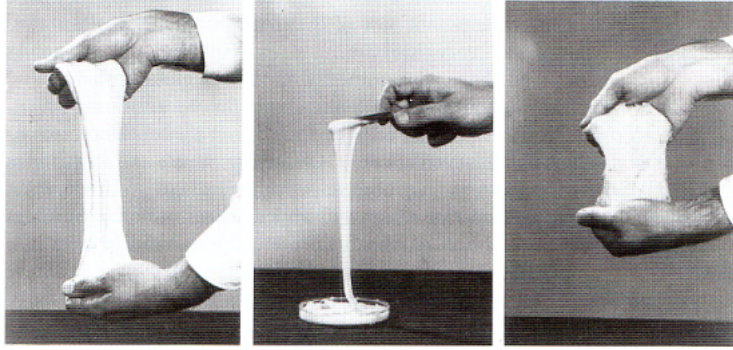


Figure 7. Physical dough properties of wheat gluten (left) and its components: gliadin (center) and glutenin (right)  
(Reprinted from Delcour, J.A. and Hoseney, R.C., 2010)

There have been number of studies that have evaluated the effects of fertilizer and temperature on the amount and composition and/or polymerization of gluten forming proteins (Dupont and Altenbach, 2003). Increases in grain protein, gliadin to glutenin, and HMW-GS to LMW-GS ratios were observed with nitrogen fertilizer. Effects of temperature on storage proteins are unclear; however, night temperature during grain filling has a bigger effect on the enzymes involved in glutenin biosynthesis than the day temperature. However, high night temperatures do not inactivate enzymes involved in gliadin biosynthesis; therefore, gliadin deposition continues.

A greater proportion of low molecular weight glutenin (LMW-G) and gliadin was found in vitreous endosperm, whereas the levels of  $\omega$ -gliadin and high molecular weight glutenin (HMW-G) were found to be similar in both vitreous and starchy kernels (Samson et al., 2005). Moreover, the greater proportion of gliadin in vitreous kernels is associated with a harder texture (Gianibelli et al., 1991), and this would also account for the higher vitreousness of durum wheat (Dexter et al., 1988).

In vitreous endosperm, high gliadin content will allow for better adhesion of the protein matrix on starch granules during kernel desiccation, which leads to a compact

endosperm structure (Dexter et al., 1989; Dexter and Edwards, 2001). In contrast, lower gliadin content will lead to a discontinuous protein matrix and a more friable structure with air vacuoles in the wheat endosperm. This results in lower density endosperm. Thus, there are more air spaces in mealy or starchy endosperm, which give the endosperm a starchy or opaque appearance (Dexter et al., 1989; Glenn and Saunders, 1990), while vitreous endosperm is more compact. Air spaces in non-vitreous or starchy kernels are result of pre-harvest rains. Once water enters into the endosperm, it causes swelling with resultant air spaces and fissures on drying. Therefore, it results in loss of vitreousness.

Dobraszczyk (1994) also reported that vitreous endosperm is tougher than mealy endosperm for a single hard wheat cultivar. An increase in protein content would also account for this compact endosperm, because it lowers the volume of entrapped air (Samson et al., 2005).

## 2.7. Protein Quality and Its Effect on Bread Quality

The quality of the gluten forming proteins in wheat flour confers good or poor baking properties at a given protein content (Carson and Edwards, 2009). Environmental conditions, more specifically, fertilizer and temperature, affect the amount, composition and/or polymerization of the gluten proteins (Dupont and Altenbach, 2003). Gluten, which forms in the presence of water and shear during mixing, is composed primarily of gliadin and glutenin. The presence of HMW-G subunits and the proper balance between gliadin and glutenin has been identified as corresponding with superior baking quality (Carson and Edwards, 2009). Gluten-forming or storage proteins must exhibit sufficient overall strength as well as good balance between elasticity and extensibility when properly developed. In order to retain gas during fermentation, strong dough is desired so

that a loaf can expand sufficiently during proofing and baking to produce high quality bread.

The proportions of polymeric and monomeric gluten-forming proteins and their size distribution both contribute to protein quality (Wrigley et al., 2006). Thus, the proportion defines the relationship between protein content and loaf volume. The proportions of polymeric and monomeric components, and the proportions of large polymers can be determined by size-exclusion high performance liquid chromatography (SE-HPLC). Currently, this method is the most important tool used to quantitatively characterize the overall protein composition of wheat proteins. The unextractable polymeric protein (UPP) can be determined using a two-step extraction procedure, followed by SE-HPLC separation of the polymeric and monomeric proteins (Gupta et al., 1993). The amounts of the polymeric and monomeric components in the two fractions are used to calculate the amount of UPP as the percentage of polymeric protein content (%UPP).

## 2.8. Flour Quality Characteristics and End-Use Quality

In general, the overall baking quality of flour is a combination of starch damage, protein content, and protein quality (Carson and Edwards, 2009). Hard Red Spring wheat flour usually has higher protein content and quality, higher water absorption, and greater bread loaf volume compared to HRW or soft wheat. Vitreous kernels of HRS wheat are higher in protein content compared to non-vitreous kernels (Carson and Edwards, 2009). Thus, it is desirable for production of bread and pasta to have high percentages of vitreous kernels (Carson and Edwards, 2009; Dexter and Edwards, 1998). A study done by Pomeranz et al. (1976) stated that a separated dark, hard, and vitreous (DHV) kernels

contained more protein and the flour produced from them produced larger loaves. They also found that flours from the DHV and yellow or starchy kernels were comparable in bread making quality when expressed on an equal protein basis. Also, the percentages of DHV kernels correlated highly with protein content, baking absorption, and loaf volume. Therefore, they concluded that the protein content rather than percentage of DHV is a more consistent and satisfactory index of bread making quality (Pomeranz and Williams, 1990). Protein content of wheat or flour was much better criterion of bread making quality than was DHV kernel content (Pomeranz et al., 1976).

Hard wheat requires more grinding energy during the milling process to reduce to flour due to the tightly embedded starch granules, thus these starch granules are physically damaged during milling. This results in more damaged starch in flours produced from hard wheat. Due to much weaker association, soft wheat produces flour with low starch damage (Carson and Edwards, 2009). However, a certain amount of starch damage is desirable in bread making, and this is to optimize hydration and also to provide a source of fermentable sugars in the production of fermented bread products.

Damaged starch granules exhibit a higher degree of water absorption than the undamaged granules (Carson and Edwards, 2009). As a result, hard wheat flours exhibit high fermentation rates and dough water absorption, both of which are desirable traits for bread making. Water absorption is a primary quality determinant for bread baking (Morgan et al., 2000). Generally, HRS wheat has high water-absorption capacity and greater loaf volume potential (Carson and Edwards, 2009). Therefore, high water-absorption capacity is desirable in bread baking because it is economically advantageous to add more water than any other ingredient.

Flour protein content is also an important predictor of loaf volume potential in bread baking (Dowell et al., 2008). When all other factors being equal, it is reported that higher protein wheat has higher water absorbing capacity and greater loaf volume potential (Carson and Edwards, 2009). In addition, the wet gluten content of wheat is often used to predict the wheat flour's ability to form a gluten matrix; however, protein content is not necessarily a good measure for the overall quality of that gluten as it relates to the end-use functionality. Therefore, wet gluten provides an indication of protein content, which, in turn, affects loaf volume potential and water absorbing capacity. The amount of wet gluten in a flour sample can be determined using a Glutomatic 2200 S apparatus. During this process, flour is mixed with a salt solution to form dough. Once the dough is formed, the water-soluble proteins and starch are washed out. The amount of wet gluten can then be quantitatively measured.

## 2.9. Hard Red Spring Wheat Subclass Differentials on Wheat Pricing

Not only is the DHV kernel content of HRS wheat an important grading factor that is associated with kernel hardness, milling properties, and baking quality (Wang et al., 2002), but it also has an important role in wheat marketing as well as pricing decisions. Dark or hard kernel content is used as a basis for hard wheats because it is assumed that the percentage of DHV is an indication of the protein content of the wheat (Mangels, 1927). And protein level is a very important component of the marketing system, as it is one of the characters which premiums and discounts are applied (Dahl and Wilson, 1997). The percentage of dark, hard and vitreous kernel content shows greater variation in physical characteristics as well as composition according to season and

location. Therefore, the effect of DHV kernel content of HRS wheat on pricing decisions should also be studied.

Color, protein content and strength, and hardness are intrinsic characteristics of wheat (Wilson, 1989). Both protein quality and quantity are important characteristics that affect the value of wheat across different places and through time. Protein quality affects the gluten strength of dough whereas high protein quantity is desirable for bread baking. Protein quality of wheat is hard to measure; therefore, the quantity of protein is used for trading of wheat (Wilson, 1989). Since the protein content of wheat varies across locations as well as within and among countries with respect to time, premiums for protein are reflected in prices. Explicit premiums for protein can be determined at selected U.S. grain exchanges. Differences in the percentage of DHV kernels present and related protein content within a specific class of HRS wheat would result in different prices and premiums for high protein and higher DHV subclass when wheat is traded (Wilson, 1989).

### 3. OBJECTIVES AND NEED STATEMENT

#### 3.1. Need Statement

Classifying and sorting wheat based on color class is important because milling and baking properties of wheat can vary (Pasikatan and Dowell, 2002). Therefore, color class can determine wheat market price as both domestic and foreign buyers pay premium for wheat of a preferred class. The grade reflects the variation in end-use quality, in turn end-use quality is affected by environment (McCaig et al., 2006). Kernel vitreousness is an important grading factor for HRS wheat.

As HRS wheat is subdivided into three classes based on the percentage of vitreous dark, hard, vitreous kernels present, differences in price can be seen for these subclasses; thus, protein premiums are reflected. This is due to the fact that higher percentage of vitreous kernels generally results in higher protein content, and high percentage HRS flour has higher water absorbing or holding capacity, and it also results in greater loaf volume potential. There have been relatively few studies with regards to DHV content, and its effect on milling performance and end-use quality. However, there have not been recent studies showing effect of DHV kernel content and protein content on the end-use quality characteristics of HRS wheat. To the best of our knowledge, both DNS and NS wheat have been priced together when trading wheat and the historical price difference between the two subclasses is hard to find. However, separating them based on DHV kernel content and determining whether there is both statistical and economical difference could be beneficial for domestic and foreign buyers.

Therefore, segregating based on DHV kernel content would allow them to choose and decide whether choosing for protein premium is worth the risk. This could affect their pricing decision.

### 3.2. Research Objectives

The main objective of this research was to determine the effect of dark, hard and vitreous kernel content of Hard Red Spring wheat on flour and baking characteristics qualitatively; both, by analyzing wheat samples from different regions and different protein levels and also, by looking at one specific variety, Glenn, from two different locations.

The secondary objective was to determine and quantify the effect of DHV kernel content on flour water absorption and potential difference on number of bread loaves that can be produced.



#### 4. EXPERIMENTAL APPROACH

The U.S. HRS wheat regional crop quality survey samples used in this study were obtained from six different states (Minnesota, Montana, North Dakota, South Dakota, Oregon, and Washington). Six samples were obtained from regional composites, and six samples were obtained from protein composites in each growing season.

Regional composites were collected from West Central Minnesota (MN-B), North East Montana (MT-B), North West North Dakota (ND-A), South West North Dakota (ND-D), South Central North Dakota (ND-E), and North Central South Dakota (SD-B). Each composite was then segregated into three sub-samples based on the percentage of vitreous kernels present using a custom-built color sorter machine (Pearson et al., 2012): high, medium, and low. These samples were categorized as high (>85%), medium (between 25 and 85%), and low (<26%). Color sorting was used in this study to segregate wheat samples based on DHV kernel content. The current standard method of evaluating the percentage of vitreous kernels in the United States was used. This was done where manually inspecting a 15-g sample, which was free of shrunken and broken kernels (USDA, 1997). Some minor defects such as bleached, cracked, or checked hard vitreous kernels were considered vitreous. A total of 18 samples were obtained from regional composites each year.

Protein composites were collected from the Eastern and Western sections of six states (Minnesota, Montana, North Dakota, South Dakota, Oregon, and Washington) with protein contents of low (less than or equal to 13.4%), medium (13.5%-14.5%), and high (more than or equal to 14.6%). Each sample was then further segregated into three sub-samples based on the percentage of vitreous kernels present in the sample. Samples were

categorized as high (>85%), medium (between 25 and 85%), and low (<26%). A total of 18 samples were obtained from protein composites each year. Therefore, a total of 36 samples were obtained for each of three consecutive growing years (2010, 2011, and 2012).

In addition to regional crop quality survey samples, a sample of Glenn was obtained from two locations to determine the effect of DHV content of HRS wheat on milling performance and baking quality. Glenn samples were obtained from Minot and Casselton locations. Each wheat sample was then further segregated into three subsamples based on the percentage of vitreous kernels present in the sample using a color sorter. Each sample was categorized as high, medium, and low percentage of DHV kernels present. Therefore, a total of 6 samples were obtained from two locations.

#### 4.1. Sample Preparation and Analysis

Each HRS wheat sample was cleaned on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN) and analyzed for moisture content and test weight using a Dickey John GAC 2100 instrument (DICKEY-John Corporation, Auburn, IL). Thousand-kernel weight (KWT) of the samples was determined using a Totalizer Model 77 apparatus (Seedburo Equipment Co., Chicago, IL). The whole-wheat protein content was measured by near-infra red (NIR) using an Infratec 1226 Grain Analyzer (Perstorp Analytic, Hoganas, Sweden). A Bühler MLU-202 Mill (Bühler Industries Inc., Uzwil, Switzerland) was used to mill the wheat samples according to AACC approved method 26-21.02 (AACC International, 1999a), and the flour extraction was determined as the percentage of straight grade flour produced on a product basis. In addition, break flour yield was also determined.

Flour samples were then analyzed for moisture content (14% m. b.) by NIR using an Inframatic 9140 (Perten Instruments, Springfield, IL) and flour protein content according to AACC approved method 46-30.01 using a LECO FP 528 apparatus (AACC International, 1999b). Starch damage in the flour was determined using the Megazyme starch damage assay procedure according to AACC Approved Method 76-31.01 (AACC International, 1999c). The water absorption and dough strength were measured using a Farinograph (C.W. Brabender Instruments Inc., Hackensack, NJ) according to AACC approved method 54-21.02 applying the constant flour weight method (AACC International, 2011). The wet gluten content and gluten index (GI) were determined using a Glutomatic 2200 S apparatus (Perten Instruments, Springfield, IL) according to AACC approved method 38-12.02 (AACC International, 2000).

Samples were baked according to AACC approved method 10-09.01 (AACC International, 1999d) with the following modifications; fungal  $\alpha$ -amylase (15 SKB) instead of malt dry powder, instant yeast (1.0%) instead of compressed yeast and the addition of 10ppm ammonium phosphate. After baking, bread loaf volume was measured according to AACC approved method 10-05.01 (AACC International, 2001). A three-hour fermentation schedule with two punches was used and the bread was baked in “Shogren-type” pans. The bread was then evaluated on a scale of 1-10, with ten being the best and one being the worst, for crust color, crumb color, crumb grain and texture and symmetry. The results were evaluated to determine the relationship between the percentage/content of DHV kernels of HRS survey wheat samples on milling and baking quality.

Flour protein composition, the proportions of polymeric and monomeric components and the proportions of large polymers were determined by SE-HPLC. Flour proteins were extracted as described by Gupta et al. (1993) with minor modification (Ohm et al., 2009). Two replicates of each flour sample were used for the investigation of size distribution of polymeric proteins. SDS-extractable and unextractable polymeric proteins were obtained according to the procedure of Gupta et al (1993). Flour (10 mg) was suspended in 1 mL of 1% SDS and 0.1M sodium phosphate buffer (pH 6.9) and stirred for 5 min at 2,000 rpm using a pulsing vortex mixer (Fisher Scientific). The mixture was centrifuged for 15 min at 17,000 x g (Centrifuge 5424, Eppendorf) and the extractable protein was dissolved in supernatant and filtered through a membrane filter (0.45  $\mu$ m PVDF membrane, Sun Sri, Rockwood, TN). Immediately after filtering, the sample was heated for 2 min at 80°C (Larroque et al., 2000). The unextractable protein was obtained from the residue. The residues were sonicated for 30 sec at the power setting of 10W output (Sonic Dismembrator 100, Fischer Scientific) with 1 mL of extraction buffer. Then the mixture was centrifuged for 15 min at 17,000 x g (Centrifuge 5424, Eppendorf) and the supernatant was filtered and heated before SE-HPLC analysis as described for extractable proteins.

SE-HPLC was performed using Agilent 1100 series chromatograph (Agilent Technologies, Santa Clara, CA) (Batey et al., 1991). SDS-extractable and unextractable protein fractions were separated by a narrow bore column (300 x 4.5 mm, BIOSEP SEC S4000, Phenomenex, Torrance, CA) with a guard cartridge (Ohm et al., 2009). Injection volume was 10  $\mu$ L. Eluting solution was 50% acetonitrile in water with 0.1%

trifluoroacetic acid at a flow rate of 0.5 mL/min. Solutes were detected at 214 nm using an Agilent 1200 photodiode array detector (Agilent Technologies, Santa Clara, CA).

#### 4.2. Statistical Analysis

Statistical analysis was performed using the SAS statistical methods (Version 9.3, SAS Institute; Cary, NC). An analysis of variance (ANOVA) was performed to assess the effect of treatment on quality characteristics for individual locations. A least significant difference (LSD) with a 5% significance level will be used to declare differences between treatments.

1. The experimental design for regional composites was three factorial layout with year, location, and vitreous content as main factors. Year, location, and vitreous content interaction term was used as error term.
2. The experimental design for protein composites was split-plot arrangement with whole plot in randomized complete block design. Main plot is growing year, and subplot is protein content, vitreous kernel content, and their interaction. Growing location was treated as a block nested in a year.
3. The experimental design for 'Glenn' variety samples was randomized complete block design considering location as a block.

## 5. RESULTS AND DISCUSSION

### 5.1. Regional Composites

Regional composite samples were chosen and selected from four states: MN, MT, ND, and SD. Table 1 shows the kernel quality characteristics of regional composites that vary in the percentage of vitreous kernel present. Percentage of vitreous kernel had significant ( $P<0.0001$ ) effect on the test weight. When averaged across growing years, the test weight increased as the percentage of vitreous kernel increased. However, percentage of vitreous kernel showed no significant ( $P<0.05$ ) difference between growing locations. This suggested that DHV kernel content did not have locational difference among these regions of the four growing states.

Table 1. Kernel quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel

Location	Treatment	Vitreous Kernel (%)	Test Weight (kg/hl)	1000 KWT (g)
MN-B	Low	18.9	78.8	30.8
	Medium	44.0	80.6	31.1
	High	86.9	82.0	32.6
MT-B	Low	15.3	78.9	29.6
	Medium	57.2	80.4	29.0
	High	93.0	81.8	27.8
ND-A	Low	17.2	78.4	28.9
	Medium	54.6	81.3	28.2
	High	88.5	83.2	28.4
ND-D	Low	18.3	78.8	27.4
	Medium	56.1	79.5	27.0
	High	88.9	81.7	26.5
ND-E	Low	20.6	77.8	27.7
	Medium	61.0	79.8	26.9
	High	91.0	81.5	27.4
SD-B	Low	14.9	78.3	27.3
	Medium	50.2	79.4	27.1
	High	88.6	81.3	27.4
LSD ( $P<0.05$ )		9.9	2.9	2.1

LSD = Least significant difference

However, vitreous kernel content had significant ( $P<0.05$ ) effect on the growing years. Table 2 shows the kernel quality characteristics of regional composites across growing years.

Table 2. Kernel quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel across growing years

Year	Treatment	Vitreous Kernel (%)	Test Weight (kg/hl)	1000 KWT (g)
2010	Low	19.3	79.5	30.7
	Medium	59.0	81.0	30.1
	High	89.7	83.1	30.5
2011	Low	22.6	76.7	26.6
	Medium	50.0	79.2	26.7
	High	91.0	81.7	26.6
2012	Low	10.8	78.7	28.6
	Medium	52.6	80.6	27.9
	High	87.7	81.1	28.0
LSD ( $P<0.05$ )		7.0	2.5	1.5

LSD=Least significant difference

Growing condition of individual year was a major factor affecting the DHV kernel content. According to the U.S. HRS wheat Crop Quality report, year 2010 was reported as “excellent growing conditions,” in which adequate moisture and limited disease pressures during growing season allowed for good kernel development in these four major states: Minnesota, Montana, North Dakota, and South Dakota (North Dakota Wheat Commission, 2012).

Average kernel weight was reported to be much higher in 2010 while it was much lower in the following year. The average kernel weight in these 6 growing locations was over 30 g in 2010. This was much higher and comparable to 2011, where the average kernel weight was reported nearly 27 g. In 2011, there was a sharp reduction in planted area in the four state regions. This was due to a significantly delayed planting season and excessive spring rains, which led to smaller crop. There was nearly a 4-gram difference in

the 1000-KWT, which confirmed that crops were much smaller compared to the previous year (Table 2). In 2012, there was a marginal increase in area planted and a “significant” rebound in yields across central and eastern areas of the four-state region (North Dakota Wheat Commission, 2012). When averaged across growing locations, there was a significant ( $P<0.001$ ) difference in the 1000-KWT among growing years; however, there was no difference between vitreous kernel treatments (Table 2).

When averaged across locations, test weight was significantly ( $P<0.001$ ) different between growing years. Although test weight increased with increasing vitreous kernel content, there was no significant ( $P<0.05$ ) difference in the flour yield among vitreous kernel treatments. Milling and flour quality characteristics of the regional composites are presented in table 3.

When averaged across growing years, there was a significant ( $P<0.05$ ) difference in the flour yield (product basis) among locations. However, there was no significant ( $P<0.05$ ) difference in the vitreous kernel treatments. Therefore, the vitreous kernel treatment and the total flour yield showed no significant ( $P<0.05$ ) correlation. This is in agreement with Phillips and Niernberger (1976), who observed that all quality factors were significantly ( $P<0.05$ ) related to DHV content except flour extraction and mixing tolerance. This insignificant difference in flour yield could be due to the growing environment such as weather and planting conditions that were observed in these growing years.



Table 3. Milling and flour quality characteristics of regional composites varying percentages of dark, hard and vitreous kernel

Location	Treatment	Flour Yield (%)	Break Flour Yield (%)	Flour Protein (%)	Starch Damage	Wet Gluten (%)
MN-B	Low	74.8	11.9	11.9	7.2	30.2
	Medium	74.2	10.9	13.1	7.6	34.1
	High	74.8	10.8	13.9	7.9	38.2
MT-B	Low	73.5	11.1	12.4	6.9	33.9
	Medium	73.3	10.8	12.5	7.0	33.7
	High	73.6	10.7	13.0	7.4	36.0
ND-A	Low	73.7	10.8	13.4	6.5	36.0
	Medium	73.1	10.7	14.0	7.3	37.8
	High	73.3	11.1	14.1	7.3	39.0
ND-D	Low	73.1	11.2	13.7	6.5	37.5
	Medium	72.4	10.5	13.8	6.8	37.2
	High	73.8	10.8	14.3	7.1	39.5
ND-E	Low	73.3	10.9	13.2	6.8	35.2
	Medium	73.5	10.7	13.8	7.2	37.1
	High	73.7	10.5	14.2	7.5	38.6
SD-B	Low	73.6	11.4	12.8	6.6	34.1
	Medium	73.2	10.7	13.5	6.9	36.3
	High	73.3	10.7	14.0	7.0	37.4
LSD (P<0.05)		1.6	1.3	0.4	0.4	1.6

LSD=Least significant difference

Environment plays a large part in determining quality and consistency (Dahl and Wilson, 1998). There are several factors that influence the degree to which wheat becomes non-vitreous, including weather conditions, soil fertility and heredity (Phillips and Niernberger, 1976). However, milling quality varies by variety and location. Flour yield was higher in MN-B location, whereas flour yield was lower in other locations.

Table 4 shows the milling and flour quality characteristics of the regional composites during three consecutive growing years when averaged across locations.

Table 4. Milling and flour quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel across growing years

Year	Treatment	Flour Yield (%)	Break Flour Yield (%)	Flour Protein (%)	Starch Damage	Wet Gluten (%)
2010	Low	74.5	11.3	12.1	7.0	33.9
	Medium	75.1	11.2	12.6	7.4	34.7
	High	75.0	11.5	13.2	7.6	37.1
2011	Low	71.7	10.3	13.7	6.6	36.5
	Medium	70.9	9.9	13.9	6.8	37.4
	High	72.0	10.2	14.5	7.3	40.0
2012	Low	74.9	12.1	12.9	6.6	33.1
	Medium	73.9	10.9	13.8	7.2	35.9
	High	74.2	10.6	14.1	7.2	37.3
LSD ( $P<0.05$ )		1.2	0.9	0.3	0.3	1.1

LSD=Least significant difference

Flour yield was higher in 2010, while it was lower in the following year. Smaller kernels could account for lower flour yield that was observed in 2011. However, vitreous kernel treatment showed no significant ( $P<0.05$ ) difference on both total flour and break flour yield in these growing years. Posner and Hibbs (2005) stated that although there are many descriptors used for wheat classification such as kernel vitreousness, none of these characteristics are directly related to milling quality.

Generally, range in protein content is influenced by environment and genetics (Dowell et al., 2006). Although hardness and vitreousness are separate traits that describe different characteristics of wheat grain (Turnbull and Rahman, 2002), the environment affects the expression of both (Osborne et al., 2006). However, the expression of vitreousness is highly influenced by the environmental conditions (Turnbull and Rahman, 2002). Dexter and Edwards (1998) stated that the DHV kernel percentage is related to protein content and hardness. However, others have reported that the relationship between vitreousness and hardness is not straightforward (Nielsen et al., 2003). Haddad

et al. (1999) found that the rheological properties of the grain endosperm were influenced by both variety and grain vitreousness.

Hard wheats are grown under high fertility and low moisture during the growing season, both of which favor the production of high protein levels in the mature grain (Simmonds, 1974). Flour protein content was significantly different across both growing locations ( $P < 0.0001$ ) and growing season ( $P < 0.0001$ ). However, flour protein varied more between growing years than between locations within a year (Tables 3 and 4). These results are in agreement with Waldron et al. (1942), who concluded that weather was the larger factor affecting wheat protein levels rather than soils. And the location x treatment interaction was significant at  $P < 0.05$ , which confirmed that the flour protein content increased as the percentage of vitreous kernel increased within a location.

In addition, the percentage of vitreous kernel was positively associated with flour protein content ( $r = 0.44^{***}$ ). Li and Posner (1987) also concluded in their study that the percentages of DHV kernels were correlated highly with protein content, as well as baking absorption and loaf volume. Mangels (1927) also found positive correlation between protein content and dark, hard and vitreous kernels in their study; however, the coefficient of correlation showed seasonal variation.

Hard wheat has starch granules that are tightly embedded in the protein matrix (Carson and Edwards, 2009). This requires more grinding energy during the milling process to reduce to flour, thus these starch granules are physically damaged. This results in more damaged starch in flours produced from hard wheat. Therefore, starch damage is an important quality parameter because damaged starch granules exhibit a higher degree of water absorption than the undamaged granules (Carson and Edwards, 2009). As a

result, hard wheat flours exhibit high fermentation rates and dough water absorption, both of which are desirable traits for bread making.

Flour starch damage was determined for regional composites (Tables 3 and 4). Starch damage increased as the percentage of the vitreous kernel increased. In addition, there was a positive correlation between vitreous kernel content and starch damage ( $r=0.50^{***}$ ). This is due to a greater gliadin composition in vitreous endosperm as it allows for better adhesion of the protein matrix on starch granules during kernel desiccation, which leads to a compact endosperm structure (Samson et al., 2005). This compact endosperm structure therefore results in greater starch damage during milling process. Although both location and treatment showed significant ( $P<0.001$ ) differences for starch damage, the location x treatment interaction was not significant ( $P<0.05$ ) when considering growing years as replications. This could be due to the fact that starch damage was also significantly ( $P<0.001$ ) different among growing years.

As mentioned, damaged starch granules of vitreous kernels exhibit a higher degree of water absorption than the undamaged granules. High water absorption was observed in both farinograph and baking water absorption (Table 5).

Both farinograph and baking water absorption increased as the DHV content increased. High water absorption was evident and consistent in all regional composites. In addition, farinograph and baking water absorption had very high and positive association ( $r = 0.77^{***}$ ). Both farinograph and baking water absorption were significantly ( $P<0.001$ ) different across growing locations and vitreous kernel treatment; however, the location x treatment interaction was not significant ( $P<0.05$ ).

Table 5. Dough properties and bread baking quality characteristics of regional composites varying different percentages of dark, hard and vitreous kernel

Location	Treatment	Farinograph Water Absorption (%)	Baking Water Absorption (%)	Loaf Volume (cc)
MN-B	Low	61.1	63.0	915.8
	Medium	62.6	64.4	945.0
	High	65.1	66.9	989.2
MT-B	Low	61.7	64.0	906.7
	Medium	62.5	64.6	904.2
	High	64.1	66.2	928.3
ND-A	Low	62.5	64.8	1013.3
	Medium	64.5	66.2	993.3
	High	65.8	67.6	1027.5
ND-D	Low	62.7	64.6	975.0
	Medium	63.4	65.4	1007.5
	High	65.1	66.9	1043.3
ND-E	Low	61.9	64.1	973.3
	Medium	63.3	65.4	983.3
	High	64.9	66.9	1017.5
SD-B	Low	61.3	63.3	917.5
	Medium	62.7	64.7	910.8
	High	64.3	66.2	942.5
LSD (P<0.05)		0.8	0.7	56.9

LSD=Least significant difference

Water absorption is a primary quality determinant for bread baking (Morgan et al., 2000). Generally, high protein wheat, such as HRS wheat has high water-absorption capacity and greater loaf volume potential (Carson and Edwards, 2009). Therefore, high water-absorption capacity is desirable in bread baking because it is economically advantageous to add more water than any other ingredient.

Bread loaf volume was significantly ( $P<0.001$ ) different for growing years and locations, and vitreous kernel treatments ( $P<0.01$ ). Bread loaf volume increased with increasing percentage of vitreous kernel content; however, the location x treatment interaction showed no significant ( $P<0.05$ ) difference. In addition, bread loaf volume had

very high and significant ( $P < 0.001$ ) associations with flour protein, wet gluten, farinograph and baking water absorption with the correlation coefficients  $r = 0.80, 0.69, 0.59,$  and  $0.58,$  respectively. However, the flour protein content and bread loaf volume showed the strongest correlation. This was expected, because protein content is an important factor in determining bread making potential as higher protein content generally yields larger loaf volumes (Bushuk et al., 1969). Park et al. (2006) also found similar results in their study and concluded that protein content was highly correlated with bread loaf volume ( $r = 0.80$ ).

Flour protein composition, the proportions of polymeric and monomeric components and the proportions of large polymers were determined by SE-HPLC. Flour protein content had significant effect on both SDS-extractable and -unextractable protein fractions (Table 6). More specifically, SDS-extractable protein fractions that were eluted at F2, F3, and F4 sections of the chromatogram had very high and significant ( $P < 0.0001$ ) correlation with flour protein content.

Table 6. Correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-extractable and -unextractable proteins and flour protein content and vitreous kernel for regional composites

HPLC Protein Fractions (% Flour)	Protein Content	Vitreous Content
<b>SDS extractable</b>		
F1	0.45 **	-0.06
F2	0.86 ***	0.29 *
F3	0.94 ***	0.50 ***
F4	0.74 ***	0.21
<b>SDS unextractable</b>		
F1	0.65 ***	0.30 *
F2	0.54 ***	0.29 *
<b>(% Protein)</b>		
<b>SDS extractable</b>		
F1	-0.13	-0.35 *
F2	-0.24	-0.28 *
F3	0.33 *	0.38 *
F4	-0.69 ***	-0.43 ***
<b>SDS unextractable</b>		
F1	0.29 *	0.15
F2	-0.21	-0.04

\*\*\*  $P < 0.0001$ ; \*\*  $P < 0.001$ ; \*  $P < 0.05$

This means the low molecular weight (LMW) polymeric proteins, gliadin, and LMW soluble proteins were highly correlated with flour protein content. Although the LSD mean separation showed no significant ( $P < 0.05$ ) difference (except for MN-B location), gliadin content composition (E3) increased as the percentage of the vitreous kernel increased. Table 7 shows SDS-extractable and -unextractable protein fractions of regional composite samples.

Table 7. SDS-extractable and -unextractable protein fractions of regional composites varying different percentages of dark, hard and vitreous kernel

Location	Treatment	SDS Extractable (% Flour)				SDS Unextractable (% Flour)			
		E1	E2	E3	E4	U1	U2	U3	U4
MN-B	Low	0.53	1.45	5.32	1.76	1.00	1.11	0.53	0.22
	Medium	0.52	1.55	5.98	1.84	1.19	1.22	0.55	0.21
	High	0.54	1.63	6.50	1.90	1.29	1.28	0.55	0.20
MT-B	Low	0.49	1.48	5.59	1.72	1.08	1.30	0.56	0.19
	Medium	0.48	1.49	5.74	1.71	1.23	1.19	0.51	0.18
	High	0.50	1.56	6.07	1.77	1.31	1.20	0.48	0.17
ND-A	Low	0.52	1.60	6.03	1.86	1.33	1.25	0.57	0.21
	Medium	0.48	1.54	6.05	1.80	1.71	1.32	0.82	0.24
	High	0.50	1.61	6.31	1.86	1.53	1.37	0.72	0.23
ND-D	Low	0.48	1.55	6.02	1.84	1.63	1.20	0.71	0.23
	Medium	0.50	1.59	6.25	1.87	1.54	1.25	0.60	0.21
	High	0.51	1.64	6.59	1.91	1.62	1.29	0.56	0.20
ND-E	Low	0.67	1.63	5.97	1.77	1.13	1.17	0.59	0.23
	Medium	0.59	1.66	6.36	1.81	1.32	1.27	0.57	0.21
	High	0.57	1.68	6.59	1.83	1.41	1.35	0.60	0.21
SD-B	Low	0.53	1.60	5.62	1.81	1.34	1.22	0.53	0.19
	Medium	0.55	1.68	6.01	1.86	1.37	1.30	0.55	0.19
	High	0.55	1.71	6.30	1.88	1.40	1.32	0.60	0.20
LSD ( $P<0.05$ )		0.04	0.08	0.42	0.09	0.22	0.21	0.24	0.04

LSD=Least significant difference

When averaged across growing years, the LSD mean separation showed no difference in gliadin with the exception of MN-B location. This could be because there was a significant ( $P<0.0001$ ) difference in the growing years, in which would contribute to the insignificant difference when growing years were treated as replications. Therefore, the location x treatment interaction was not significant ( $P<0.05$ ) for gliadin composition. This significant difference in the gliadin composition can be seen in Figure 8. This figure illustrates the correlation between size exclusion HPLC-absorbance areas of SDS-extractable proteins with vitreous kernel content and bread loaf volume.



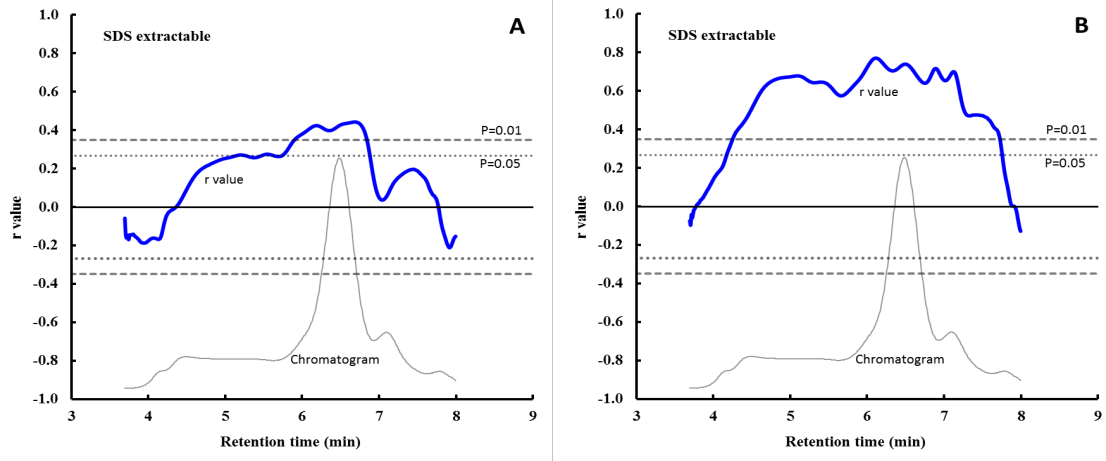


Figure 8. Spectrum of correlation coefficients ( $r$ ) between size-exclusion HPLC absorbance areas of SDS-extractable proteins and vitreous kernel (A) and bread loaf volume (B) over retention time for regional composites

As shown in Figure 8, F3 of the SDS-extractable protein was significantly ( $P < 0.0001$ ) different. This F3 fraction (retention time between 6.0-6.9 min) refers to the gliadin composition. Gliadin composition was significant ( $P < 0.001$ ) and highly correlated with vitreous kernel content ( $r = 0.50^{***}$ ). This finding is in agreement with Samson et al. (2005) and Dexter et al. (1989), who found that greater gliadin composition was found in vitreous endosperm.

SDS-extractable proteins were highly correlated with bread loaf volume (Figure 8). Primary components of each fraction were high molecular weight (HMW) protein for F1, low molecular weight (LMW) for F2, gliadins for F3, and albumin and globulins for F4. These SDS-extractable protein fractions were significantly ( $P < 0.001$ ) and highly correlated with bread loaf volume. As mentioned, vitreous kernel content showed very high and significant relationship with gliadin composition; however, vitreous kernel showed very weak association but significant ( $P < 0.05$ ) with bread loaf volume ( $r = 0.27^*$ ). This means that protein content could be a better indication of bread loaf volume than the percentage of DHV kernel content. Pomeranz and Williams (1990) also

concluded that protein content rather than the percentage of DHV was more consistent and satisfactory index of bread making quality in their study.

In addition to the SDS-extractable proteins, the unextractable protein fractions were also determined (Table 7). Protein fractions U1 and U2 increased with the increasing percentage of vitreous kernel. However, the LSD mean separation showed no significant ( $P < 0.05$ ) difference between vitreous kernel treatments. These fractions refer to the high molecular weight (HMW) and low molecular weight (LMW) polymeric proteins. These polymeric proteins had significant ( $P < 0.05$ ) correlation with the vitreous kernel content with correlation coefficients of  $r = 0.30^*$  and  $r = 0.29^*$ , respectively (Figure 9). This figure also illustrates the relationship between the SDS-unextractable proteins with farinograph peak time and bread loaf volume. Although the vitreous kernel had high correlations with HMW and LMW SDS-unextractable polymeric proteins, these fractions of the chromatogram had stronger and positive correlation with flour protein content ( $r = 0.65^{***}$  and  $r = 0.54^{***}$ ) and bread loaf volume ( $r = 0.50^{***}$  and  $r = 0.44^{***}$ ), respectively. Therefore, the flour protein content could be a better indication of bread baking quality rather than the percentage of vitreous kernel content.

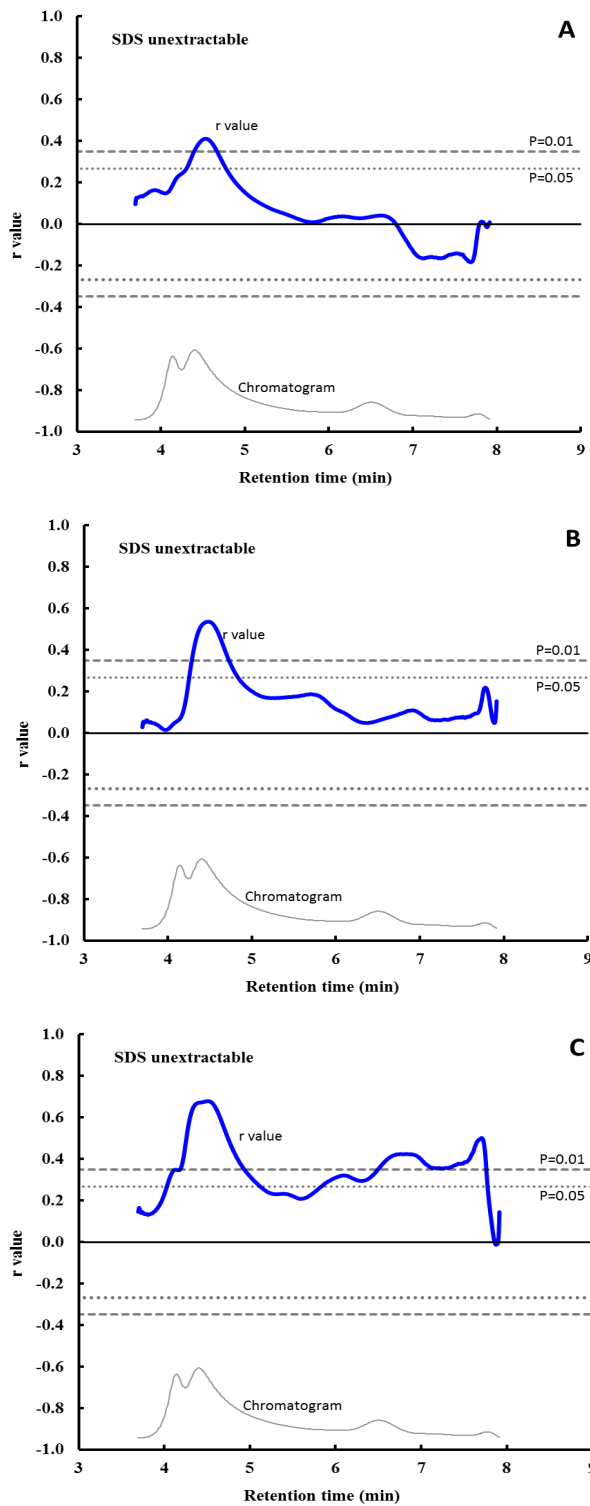


Figure 9. Spectrum of correlation coefficients ( $r$ ) between size-exclusion HPLC absorbance areas of SDS-unextractable proteins and vitreous kernel (A), farinograph peak time (B), and bread loaf volume (C) over retention time for regional composites

## 5.2. Protein Composites

Protein composites were collected from the Eastern and Western sections of six states (Minnesota, Montana, North Dakota, South Dakota, Oregon, and Washington) with protein levels of low (less than or equal to 13.4%), medium (13.5%-14.5%), and high (more than or equal to 14.6%). Each sample was then further segregated into three sub-samples based on the percentage of vitreous kernels present. These samples differed in the percentage of DHV kernels within each sample and were categorized as high (>85%), medium (between 25 and 85%), and low (<26%). As composite samples were segregated based on the protein level and as well as vitreous kernel content, these two factors and the interaction of protein and vitreous kernel were treated as a subplot for the statistical analysis. Table 8 summarizes the Analysis of Variance (ANOVA) for kernel quality and milling characteristics of protein composites collected from three growing years.

Table 8. Analysis of variance of vitreous kernel content, test weight, 1000-kernel weight, flour extraction, and break flour yield for protein composites

Source	<i>F</i> -value									
	Vitreous Content		Test Weight		1000-Kernel Weight		Flour Yield		Break Flour Yield	
Year (Y)	1.45	ns	17.18	*	2.05	ns	8.62	ns	6.7	ns
Location (Y)	7.85	**	1.11	ns	16.51	***	4.49	*	1.77	ns
Protein (PT)	1.05	ns	32.24	***	102.85	***	21.8	***	1.63	ns
Vitreous (VT)	1428.8	***	143.74	***	0.53	ns	0.17	ns	12.15	**
PT*VT	1.76	ns	5.47	*	0.52	ns	0.57	ns	0.61	ns
Y*PT	0.91	ns	12.39	***	1.43	ns	1.44	ns	0.67	ns
Y*VT	2.42	ns	7.91	**	1.4	ns	0.3	ns	1.54	ns
Y*PT*VT	1.44	ns	1.82	ns	1.47	ns	0.71	ns	1.4	ns

\*\*\* *P*-value<0.0001; \*\* *P*-value<0.001; \* *P*-value<0.05; ns non significant

Both protein level and vitreous kernel content had significant ( $P<0.0001$ ) effect on the test weight, and the interaction of these two factors was also significant ( $P<0.05$ ).

Although test weight is a good indicator of milling yield, it is not always an indication of

the amount of flour that should be extracted from a certain quality of wheat (Posner and Hibbs, 2005). In addition to test weight, thousand kernel weight can also give the miller important information about the wheat's milling potential. However, in this study, only protein level showed significant ( $P < 0.0001$ ) effect on the 1000-KWT. Kernel quality and milling quality characteristics of protein composites are presented in Table 9.

When averaged across eastern and western locations, the test weight increased as both protein level and the percentage of the vitreous kernel content increased. This was observed and consistent in all protein composites obtained from three consecutive growing years. The increase in test weight could be due to a more compact structure in vitreous endosperm, while lower test weight in starchy kernels could be due to a more open or air spaces that resulted in lower density in non-vitreous kernels. Sharp (1927) also observed that within a sample vitreous kernels were higher in protein and in density compared to starchy kernels.

Table 9. Kernel and milling quality characteristics of protein composites varying different percentages of dark, hard and vitreous kernel across growing years

Year	Protein Level	Vitreous Level	Vitreous Kernel	Test Weight	1000-Kernel Weight	Flour Yield	Break Flour Yield	
			(%)	(kg/hl)	(g)	(%)	(%)	
2010	13.4%≤	Low	20.5	79.5	31.4	74.9	11.7	
		Medium	64.1	81.8	32.1	74.3	10.2	
		High	86.6	84.1	32.5	75.3	10.2	
	13.5-14.5%	Low	17.3	80.4	31.6	74.2	11.2	
		Medium	56.5	81.1	31.9	74.7	10.7	
		High	93.2	84.0	32.2	75.4	11.4	
	14.6%≥	Low	14.0	79.2	29.4	73.2	11.5	
		Medium	64.5	81.3	28.8	73.5	11.6	
		High	89.9	83.6	29.4	71.8	10.7	
	2011	13.4%≤	Low	16.0	79.5	31.5	73.2	10.5
			Medium	62.2	82.4	30.2	73.4	10.8
			High	92.2	83.8	30.5	73.7	9.8
13.5-14.5%		Low	20.2	79.1	29.8	72.8	10.2	
		Medium	62.0	81.1	29.2	73.3	10.2	
		High	90.2	83.6	29.4	72.8	9.6	
14.6%≥		Low	21.5	73.1	26.4	70.5	11.0	
		Medium	58.5	78.7	27.8	71.1	9.7	
		High	92.5	81.5	27.6	70.3	10.5	
2012		13.4%≤	Low	9.0	80.4	32.2	75.7	12.2
			Medium	62.0	81.0	31.3	75.8	10.9
			High	91.7	82.0	30.7	75.6	10.2
	13.5-14.5%	Low	10.0	79.8	31.7	75.9	12.0	
		Medium	48.7	81.4	31.1	75.2	10.8	
		High	90.3	81.9	31.3	74.6	10.3	
	14.6%≥	Low	6.4	78.0	28.6	74.6	12.3	
		Medium	53.3	81.0	28.2	74.4	10.9	
		High	87.5	81.8	28.3	74.5	11.1	
	LSD <sup>1</sup> (P<0.05)			8.78	2.89	1.44	1.99	1.29
	LSD <sup>2</sup> (P<0.05)			11.6	2.90	2.38	2.35	1.34

<sup>1</sup> Least significant difference between treatment means within same year

<sup>2</sup> Least significant difference between treatment means in different year

The physical discontinuity of protein matrix in the non-vitreous wheat kernel is observed, and there is a difference in the distribution of densities between vitreous and the non-vitreous wheat kernels (Neethirajan et al., 2006). Therefore, the ability to transmit or reflect light is different for the vitreous and non-vitreous kernels. Samson et

al. (2005) also noted that starchy kernels exhibit a white and opaque endosperm, which is related to the existence of air pockets that diffract and diffuse light. Visual differences between the cut surface of vitreous and non-vitreous wheat kernel can be detected using a Scanning Electron Microscope (SEM) (Fig. 10 and 11).

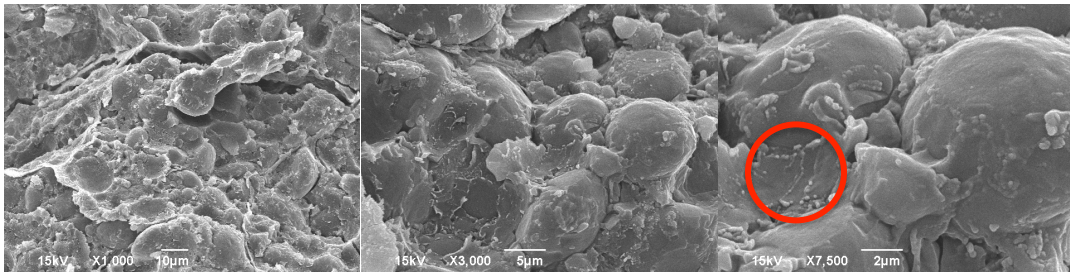


Figure 10. Scanning electron microscopy (SEM) images of cross cut sections of vitreous kernel at different magnifications

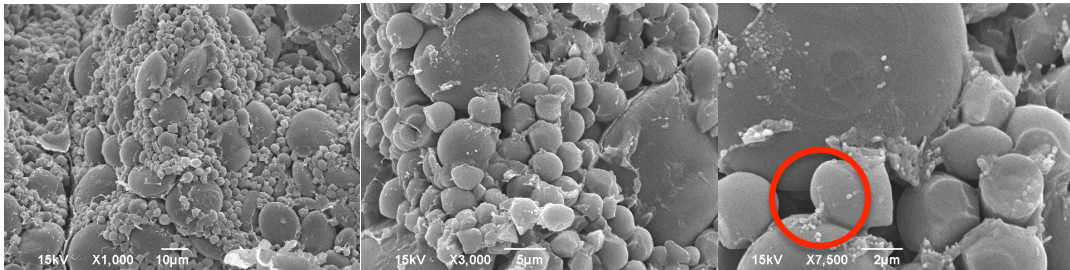


Figure 11. Scanning electron microscopy (SEM) images of cross cut sections of starchy kernel at different magnifications

It appears that small and large starch granules are loosely packed in the protein matrix, and there are more air spaces in non-vitreous or starchy kernel (Fig. 11). In contrast, much of the starch granule is covered with protein material; thus, the starch granules and protein matrix are tightly packed (Fig. 10). Turnbull and Rahman (2002) also found that vitreous endosperms were tightly packed with essentially no air spaces, whereas starchy kernels appeared white and mealy, and also had a discontinuous endosperm with numerous air spaces. These findings are also in agreement with Sadowska et al. (1999).

Turnbull and Rahman (2002) also found that the cut surface of mature hard wheat contained a compact uniform endosperm structure with starch granules that were firmly embedded in the surrounding protein matrix. Dobraszczyk et al. (2002) studied both hard and soft wheat cultivars and concluded that the differences in the rheological properties of vitreous and mealy endosperm were due to the variations in porosity. In addition, high percentage of small starch granules is typical of hard wheat compared to soft wheat (Edwards et al., 2007). Therefore, a more compact structure or tight adhesion between starch granules and surrounding protein matrix in vitreous endosperm results in more dense wheat kernel.

Vitreous kernel content had very high and positive association with test weight ( $r = 0.77^{***}$ ). Although protein level had very high and significant ( $P < 0.0001$ ) relationship to both 1000-KWT and total flour yield, vitreous kernel content showed no significant ( $P < 0.05$ ) difference on these quality parameters. Pomeranz et al. (1976) also concluded that flour yield was not affected by the percentage of DHV kernels or protein content. Results in our study are also in agreement with Phillips and Niernberger (1976), who found that the flour yield did not correlate with the DHV kernel content. Therefore, the degree of vitreousness had no effect on the milling yield. However, they concluded that other quality parameters were correlated to DHV kernel content. In our study, flour protein content and vitreous kernel showed no significant ( $P < 0.05$ ) relationship. Thus, when protein level is held constant, there is no relationship between flour protein content and vitreous kernel treatment.

In contrast, vitreous kernel had significant ( $P < 0.05$ ) effect on test the weight and break flour yield. The percentage of vitreous kernel content had negative association with



break flour yield ( $r = -0.53^{***}$ ). In other words, the break flour yield decreased with a greater percentage of vitreous kernels. The goal of the break system in a roller mill is to produce the most middlings, which are the pieces of endosperm without bran, and also to produce the least amount of flour. Therefore, the less amount of flour being produced in the break system with greater percentage of vitreous kernels is desired, as vitreousness promotes fracturing of the endosperm into large pieces and minimizes flour release. Too much flour in the break system might indicate smaller bran fragments that may result in more specks or ash in flour.

The results were in agreement with Haddad et al. (1999), who found that high vitreousness in a hard variety resulted in a distinct decrease in the break flour yield. This is expected, as the adhesion between starch granules and the storage proteins is much stronger in vitreous endosperm. This tight adhesion between starch granules and protein matrix is due to a greater gliadin composition, which allows better adhesion during kernel desiccation, which leads to a compact endosperm (Dexter et al., 1989; Dexter and Edwards, 2001). Moreover, a greater proportion of gliadin in vitreous kernels is associated with harder texture. Turnbull and Rahman (2002) also reported that an “extra protein” present in hard wheat is responsible for the tight adhesion between the starch granules and the protein matrix.

The adhesion between starch granules and the surrounding proteins is important in milling because the fracture differs between hard and soft wheat (Posner and Hibbs, 2005). Cell walls and the cell contents of hard wheat form a coherent whole during milling, and cell walls remain attached to the smaller granular particles produced in the milling process (Simmonds, 1974). Compared to hard wheat, the cell contents of soft

wheat are readily crushed and released through the rupture of the cell walls due to a weaker adhesion or more air spaces between starch granules and the storage proteins. However, for common wheat where the desired end product is flour, starchiness has little or no impact on milling performance when straight-grade flour is produced (Pomeranz et al., 1976; Dexter et al., 1988).

Table 10 shows the ANOVA for flour quality and bread baking characteristics of protein composites. Both protein level and vitreous kernel had a significant ( $P<0.001$ ) effect on the flour protein content (Table 10).

Table 10. Analysis of variance of flour protein content, starch damage, wet gluten, farinograph water absorption, baking water absorption, and bread loaf volume for protein composites

Source	<i>F</i> -value											
	Flour Protein		Starch Damage		Wet Gluten		Farinograph Water Absorption		Baking Water Absorption		Bread Loaf Volume	
Year (Y)	241.7	**	0.3	ns	4.16	ns	28.8	*	58.1	*	0.23	ns
Location (Y)	0.05	ns	1.23	ns	0.87	ns	1.71	ns	2.6	ns	8.72	**
Protein (PT)	288.5	***	25.3	***	155.5	***	148.9	***	118.1	***	81.9	***
Vitreous (VT)	27.62	***	27.6	***	17.5	***	70.1	***	61.1	***	11.9	**
PT*VT	1.66	ns	2.75	ns	0.58	ns	1.98	ns	2.55	ns	1.64	ns
Y*PT	3.45	*	0.64	ns	1.55	ns	29.2	***	21.3	***	2.69	ns
Y*VT	1.76	ns	0.23	ns	0.25	ns	3.04	*	3.36	*	0.18	ns
Y*PT*VT	0.44	ns	0.45	ns	0.55	ns	0.39	ns	0.43	ns	1.06	ns

\*\*\*  $P$ -value $<0.0001$ ; \*\*  $P$ -value $<0.001$ ; \*  $P$ -value $<0.05$ ; ns non significant

Flour protein content increased with greater percentage of vitreous kernel content in composite samples; however, there was no significant ( $P<0.05$ ) correlation between these two factors ( $r = 0.26^{ns}$ ). This indicated that when protein level was held constant, the effect of DHV content was not significant ( $P<0.05$ ) on the flour protein content.

Pasikatan and Dowell (2004) also reported that shifts in the protein content were small and likely due to differences in color or vitreousness.

Both protein level and vitreous kernel content had significant ( $P < 0.001$ ) effect on starch damage. There was a significant and positive association between the DHV kernel and starch damage with correlation coefficient of  $r = 0.61$ . This could be explained by a greater hardness found in vitreous endosperm; thus resulting in greater starch damage in vitreous kernels (Glenn and Saunders, 1990). This is very important because a certain amount of starch damage is desirable in bread baking as to optimize hydration and also to provide a source of fermentable sugars in the production of bread products. As mentioned, hard wheat flours exhibit high fermentation rates and dough water absorption, both of which are desirable traits for bread baking. However, in this study, it was found that starch damage had no significant ( $P < 0.05$ ) relationship with both farinograph and baking water absorption.

Water absorption is a primary quality determinant for bread baking (Morgan et al., 2000). Generally, high protein wheat, such as HRS wheat has higher water-absorption capacity and greater loaf volume potential compared to other HRW or soft wheat (Carson and Edwards, 2009). Both flour protein and vitreous kernel content had positive correlation with farinograph and baking water absorption. These results were supported by Phillips and Niernberger (1976), who also found that farinograph water absorption was positively and highly correlated with the percentage of DHV kernels. However, in our study, the flour protein showed stronger correlation with both farinograph and baking water absorption ( $r = 0.72^{***}$  and  $r = 0.63^{***}$ , respectively). Pomeranz et al. (1976) also found a high correlation between baking water absorption and protein content.

Furthermore, they found that the separated DHV kernels contained more protein and flour produced from them produced larger loaves. This is in agreement with the results obtained from our study, in which flour protein content was highly correlated with bread loaf volume ( $r = 0.86^{***}$ ). Pomeranz et al. (1976) also found that the percentages of DHV kernels correlated with protein content, baking water absorption, and loaf volume. However, they have found that when protein was held constant, partial correlation between DHV content and loaf volume was not significant. Thus, the researchers concluded that protein content rather than the percentage of DHV was more consistent and satisfactory index of bread baking quality (Pomeranz and Williams, 1990; Phillips and Niernberger, 1976). Similarly in our study, the percentage of vitreous kernel showed no significant ( $P < 0.05$ ) correlation with flour protein content and bread loaf volume.

As mentioned, both protein level and vitreous kernel content showed significant effect on all flour quality and bread baking parameters. Although both farinograph and baking water absorption increased with greater percentage of DHV kernel, the LSD mean separation showed no significant ( $P < 0.05$ ) difference for bread loaf volume between protein level and vitreous kernel treatments across growing years. However, an increasing trend was observed in bread loaf volume for protein composites obtained from different growing years. In general, bread loaf volume increased with greater percentage of vitreous kernel present in the sample (Table 11). For example, bread loaf volume increased as the DHV percentage increased in high protein sample ( $14.6\% \geq$ ) obtained in 2011.

Table 11. Flour and bread quality characteristics of protein composites varying different percentages of dark, hard and vitreous kernel across growing years

Year	Protein	Vitreous Level	Flour Protein	Starch Damage	Wet Gluten	Farinograph Water Absorption	Baking Absorption	Loaf Volume	
			(%)	(%)	(g)	(%)	(cc)		
2010	13.4%≤	Low	11.1	7.0	30.7	60.7	62.0	856.3	
		Medium	11.2	7.6	30.2	62.0	63.4	855.0	
		High	11.7	8.3	32.4	63.8	65.3	906.3	
	13.5-14.5%	Low	11.6	7.1	32.2	62.8	64.0	873.8	
		Medium	12.6	7.2	34.9	62.9	64.1	958.8	
		High	13.1	7.9	36.2	64.6	65.9	950.0	
	14.6%≥	Low	13.3	6.5	37.6	63.0	64.7	966.3	
		Medium	13.6	6.8	39.2	64.2	65.3	958.8	
		High	13.7	7.2	39.0	65.3	66.4	993.8	
	2011	13.4%≤	Low	11.3	6.8	27.7	54.0	56.4	853.8
			Medium	11.2	7.8	29.6	56.3	59.0	836.3
			High	12.0	8.5	30.2	60.0	62.2	876.3
13.5-14.5%		Low	12.4	7.1	32.0	58.9	61.0	936.3	
		Medium	12.8	7.5	33.1	60.6	62.6	962.5	
		High	13.2	7.9	36.5	62.2	64.0	947.5	
14.6%≥		Low	14.6	6.9	38.1	63.5	65.0	976.3	
		Medium	14.5	6.9	39.4	64.9	66.0	1028.8	
		High	14.8	7.0	40.8	67.1	68.0	1070.0	
2012		13.4%≤	Low	10.8	7.4	27.3	59.8	64.3	843.8
			Medium	11.5	7.9	30.2	61.5	66.3	866.3
			High	12.1	8.4	30.7	62.9	67.0	905.0
	13.5-14.5%	Low	12.0	6.8	31.8	61.4	65.8	928.8	
		Medium	12.9	7.3	33.3	62.8	67.2	970.0	
		High	13.2	7.6	34.9	63.6	67.4	987.5	
	14.6%≥	Low	13.7	6.6	36.2	63.2	67.5	981.3	
		Medium	14.4	7.1	37.6	64.6	68.6	956.3	
		High	14.8	7.2	38.7	65.5	69.5	1021.3	
	LSD <sup>1</sup> (P<0.05)			0.70	0.73	3.02	1.61	1.57	62.6
	LSD <sup>2</sup> (P<0.05)			0.67	0.74	3.00	1.67	1.70	85.3

<sup>1</sup> Least significant difference between treatment means within same year

<sup>2</sup> Least significant difference between treatment means in different year

In addition to vitreous kernel treatment, the protein level had a significant (P<0.0001) effect on the bread loaf volume. As the level of the protein content increased in the sample, the flours produced larger bread loaves (Table 11). When comparing samples with different protein levels from 2011, bread loaf volume increased as the

protein level increased. For example, flours produced from low protein (13.4% ≤) composite resulted in smaller bread loaves with 855cc, whereas flours from medium (13.5-14.5%) and high (14.6%≥) protein composites produced larger bread loaves with averages of 949cc and 1025cc, respectively.

Flour protein composition, the proportions of polymeric and monomeric components and the proportions of large polymers were determined by SE-HPLC. Flour protein content had significant effect on the SDS-extractable proteins (Table 12).

Table 12. Correlation coefficients (r) between size-exclusion HPLC absorbance areas of SDS-extractable and -unextractable proteins and flour content and vitreous kernel for protein composites

HPLC Protein Fractions (% Flour)	Protein Content	Vitreous Content
<u>SDS extractable</u>		
F1	0.29 *	-0.14
F2	0.88 ***	0.11
F3	0.94 ***	0.28 *
F4	0.79 ***	0.10
<u>SDS unextractable</u>		
F1	0.50 ***	0.52 ***
F2	0.51 ***	0.07
<u>(% Protein)</u>		
<u>SDS extractable</u>		
F1	-0.11	-0.22
F2	-0.37 **	-0.33 *
F3	0.53 ***	0.23
F4	-0.53 ***	-0.28 *
<u>SDS unextractable</u>		
F1	-0.33 *	0.33 *
F2	-0.35 **	-0.14

\*\*\*  $P < 0.0001$ ; \*\*  $P < 0.001$ ; \*  $P < 0.05$

More specifically, SDS-extractable protein fractions that were eluted at F2, F3, and F4 sections of the chromatogram had very high and positive association with flour protein content with correlation coefficients of 0.88, 0.94, and 0.79, respectively. In other words, LMW-polymeric proteins, gliadin, and LMW-soluble proteins had very high

correlation with flour protein content. In contrast, vitreous kernel showed significant ( $P < 0.05$ ) difference only on the gliadin composition. Figure 12 shows the correlation between size-exclusion HPLC absorbance areas of SDS-extractable proteins and vitreous kernel (left) and bread loaf volume (right).

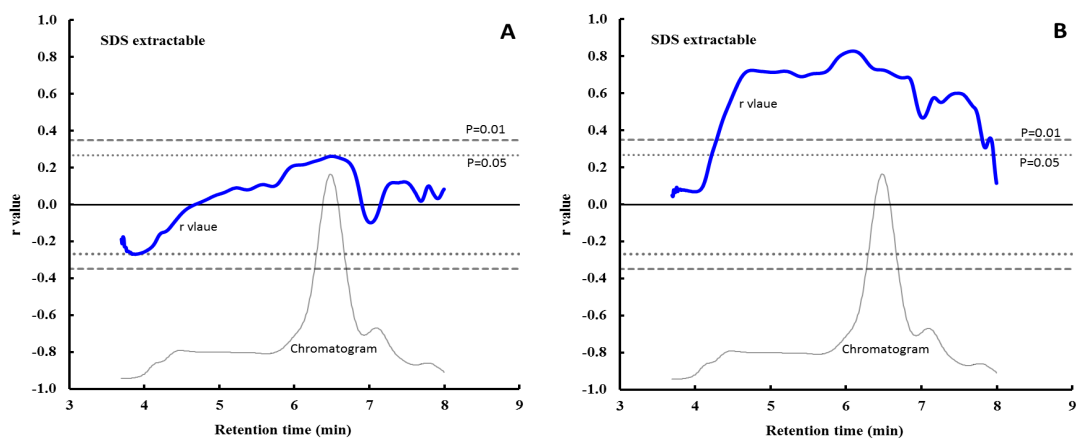


Figure 12. Spectrum of correlation of coefficients ( $r$ ) between size-exclusion HPLC absorbance areas of SDS-extractable proteins and vitreous kernel (A), and bread loaf volume (B) over retention time for protein composites

SDS-extractable protein fraction that was eluted at F3 section (retention time between 6.0-6.9 min) of the chromatogram was significantly different at  $P < 0.01$ . In addition, there was a significant ( $P < 0.05$ ) correlation between gliadin composition and the vitreous kernel content ( $r = 0.28^*$ ). This was expected, as high gliadin composition allows better adhesion of the protein matrix on starch granules in vitreous endosperm, thus leading to a more compact endosperm structure (Samson et al., 2005). Park et al. (2006) also found that the protein composition varied with flour protein content, because of both total soluble protein and gliadin levels increased proportionally to increased protein content. This significant ( $P < 0.05$ ) difference in the gliadin composition in vitreous kernels observed in our study was in agreement with Samson et al. (2005), in which they observed a greater proportion of gliadin and as well as LMW-glutenin in

vitreous endosperm. Results were also in agreement with Park et al. (2006), who observed a higher correlation between the level of gliadin and loaf volume was found when its level was calculated based on flour rather than the protein.

In addition to vitreous kernel content, the correlation between SDS-extractable proteins and bread loaf volume is shown in Figure 12. Although only gliadin composition was significantly ( $P < 0.01$ ) different, the bread loaf volume showed very strong and positive correlation with SDS-extractable protein fractions that were eluted at F2, F3, and F4 sections of the chromatogram. This could mean that flour protein content may be a better indicator of bread loaf volume rather than the percentage vitreous kernel. These findings are in agreement with Phillips and Niernberger (1976), who also concluded that protein content was a better indicator of bread quality, as measured by loaf volume, than the percentage of DHV kernels.

Figure 13 shows the correlation between size-exclusion HPLC absorbance areas of SDS-unextractable proteins with vitreous kernel, farinograph peak time, and bread loaf volume. SDS-unextractable protein fraction that was eluted at the F1 section of the chromatogram showed very strong and positive correlation with quality parameters such as vitreous kernel, farinograph peak time, and bread loaf volume. In other words, although HMW-G was not significantly different at  $P < 0.05$ , HMW-G contributed the most on these quality parameters, in which the correlation with such quality parameters showed significant difference at  $P < 0.01$  (Figure 13). Samson et al. (2005) also found that the levels of  $\omega$ -gliadin and high molecular weight glutenin (HMW-G) were found to be similar in both vitreous and starchy kernels.



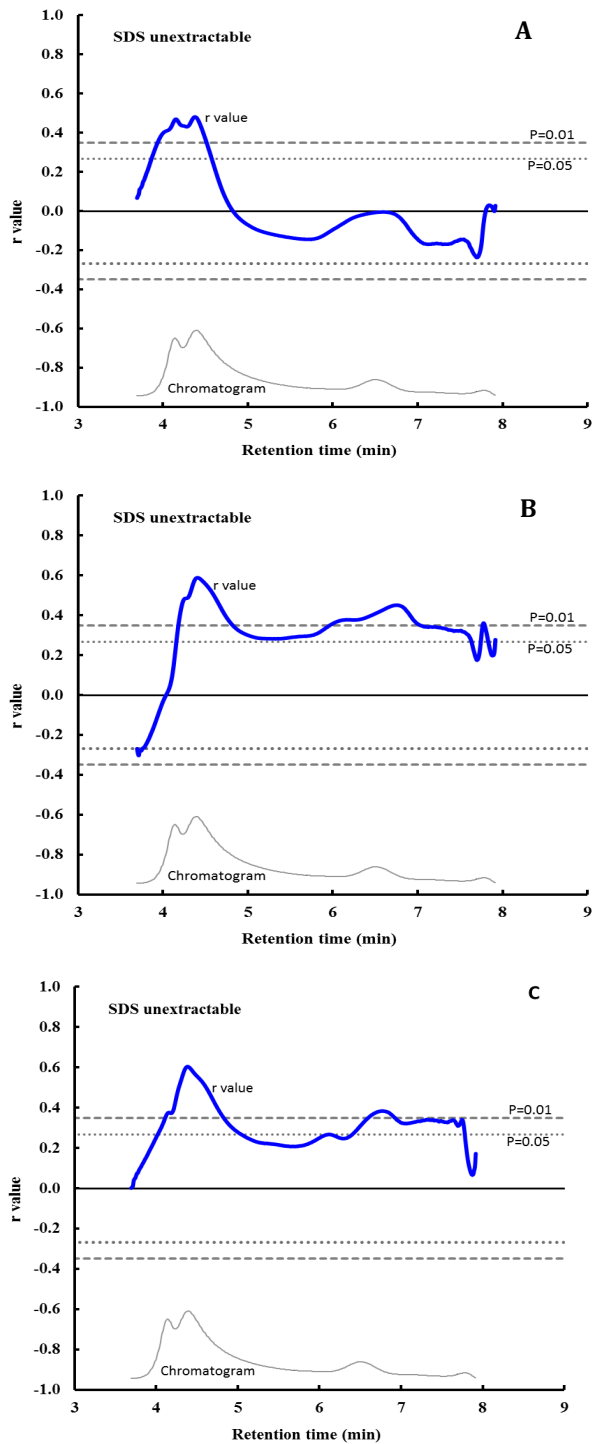


Figure 13. Spectrum of correlation coefficients ( $r$ ) between size-exclusion HPLC absorbance areas of SDS-unextractable proteins and vitreous kernel (A), farinograph peak time (B), and loaf volume (C) over retention time for protein composites

### 5.3. Glenn Variety

Hard Red Spring wheat variety “Glenn” was obtained from two locations to determine the effect of DHV content on HRS wheat flour and baking quality. Glenn samples were obtained from Minot and Casselton locations. Each wheat sample was then further segregated into three sub-samples based on the percentage of vitreous kernels present in the sample using a custom-built color sorter (Pearson et al., 2012). Each sample was categorized as high (>85%), medium (between 25 and 85%), and low (<26%) percentage of DHV kernels present. Glenn variety was selected and obtained to see the differences (if any) in the flour and baking quality characteristics. Furthermore, it was the objective in this section to determine whether the difference is due to the percentage of the dark, hard and vitreous kernels. This is because in the previous sections of our study both regional and protein composites (mixture of varieties) were used for quality characteristics. Thus, the objective was to determine the whether there is a difference in the DHV kernel content when considering a Glenn variety from two different locations. Therefore, a total of 6 samples were obtained from two locations. Kernel quality characteristics and flour yield of Glenn varieties are presented in Table 13.

Table 13. Kernel quality characteristics and milling yield of Glenn variety varying different percentages of dark, hard and vitreous kernel

Location	Treatment	Vitreous kernel (%)	Test Weight (kg/hl)	1000-KWT (g)	Flour Yield (%)	Break Flour Yield (%)
Minot		51.1a	80.9a	26.2b	73.0a	10.9a
Casselton		47.9a	82.7a	30.8a	72.4a	9.2b
	Low	6.7c	80.4a	28.1c	73.0a	10.0a
	Medium	47.2b	82.0a	28.5b	72.8a	9.0a
	High	94.7a	83.1a	29.0a	72.4a	10.2a

Mean values followed by same letter in the column are not significantly different

There was no significant ( $P<0.05$ ) difference in the test weight and the total flour yield between samples from these two locations. Although the test weight increased with greater percentage of vitreous kernels, there was no significant difference ( $P<0.05$ ) among vitreous kernel treatments for both locations.

Test weight is a good indication of flour yield, and high test weight has been correlated with high flour yield (Troccoli and Fonzo, 1999). Average test weight of 82.7 kg/hl from Casselton location was higher than that of Minot. However, no significant ( $P<0.05$ ) difference was shown in the test weight when comparing Glenn samples from these two locations. In addition, no significant ( $P<0.05$ ) difference appeared in the flour yield among vitreous kernel treatments. The flour yield was slightly higher in low vitreous kernel treatments in this study. However, starchiness has little impact on the milling performance of hard wheat when straight-grade types of flour are produced (Carson and Edwards, 2009).

In contrast, there was a significant ( $P<0.001$ ) difference in 1000-KWT between these two locations. Thousand-KWT was much lower in Minot with about 4g differences when comparing to Casselton (Table 13). In other words, Glenn sample from Minot location had smaller kernels compared to Casselton location. Environment and growing conditions in these two locations could have contributed to the differences observed in the 1000-KWT. Kernel weight increased as the percentage of DHV kernel increased, and the difference between vitreous kernel treatments was significant ( $P<0.01$ ). This was expected, as there is more tight and compact structure in vitreous endosperm. In contrast, there is more open or air spaces in starchy or non-vitreous kernels, thus resulting in lower density. A lower density in non-vitreous kernel found in our study is in agreement with

Samson et al. (2005), who found that vitreous endosperm showed a greater physical resistance to compression, a higher density, higher protein content, and a preferential accumulation of gliadin versus glutenin. Sharp (1927) also noted that a lower density in starchy kernels is attributed to the presence of air pockets, which may also cause light refraction, thus resulting in an opaque appearance. In addition, Samson et al. (2005) also noted that starchy kernels exhibit a white and opaque endosperm, which is related to the existence of air pockets that diffract and diffuse light.

Break flour yield was significantly ( $P<0.05$ ) different between Minot and Casselton locations. However, there was no significant difference ( $P<0.05$ ) in the break flour yield among vitreous kernel treatments. There was a very high but negative correlation ( $r = -0.94^{**}$ ) between break flour yield and 1000-KWT. This means that there is less break flour yield with larger kernels. Similar findings were also observed for the regional composite samples.

Table 14. Flour and bread quality characteristics of Glenn variety varying different percentages of dark, hard and vitreous kernel

Location	Treatment	Flour Protein Content (%)	Starch Damage	Wet Gluten (%)	Farinograph Water Absorption (%)	Baking Water Absorption (%)	Bread Loaf Volume (cc)
Minot		18.8a	6.8b	37.2a	64.1a	67.0b	1036a
Casselton		16.1b	8.6a	29.2b	64.1a	68.7b	903b
	Low	17.2a	7.5a	31.8a	63.4b	67.2c	984a
	Medium	17.4a	7.7a	33.7a	64.2a	67.9b	976a
	High	17.8b	7.9a	34.1a	64.7a	68.3a	948a

Mean values followed by same letter in the column are not significantly different

Significant ( $P<0.01$ ) differences were observed for flour protein content, starch damage, wet gluten, and bread loaf volume characteristics between Minot and Casselton locations. Glenn sample from Minot location had higher flour protein content compared to Casselton. This was expected, as small kernels tend to have higher protein content. As

mentioned, there was about 4 g differences in the 1000-KWT between two locations. Smaller kernels observed in Minot location could be due to a high air temperature. This is in agreement with Randall and Moss (1990). They reported that kernel weights were impacted by high growing temperature; however, the effects did not seem to be related to changes in end-use quality. High and negative correlation ( $r = -0.93^{**}$ ) was found between 1000-KWT and flour protein content. Thus, the protein content increased as the 1000-KWT decreased or with smaller kernels. In addition, flour protein content increased with greater percentage of vitreous kernel treatment. This is expected, as vitreous kernels are generally associated with higher protein content. There was a significant ( $P < 0.01$ ) difference in flour protein between medium and high percentages of vitreous kernel treatments. Similarly, wet gluten increased with greater percentage of DHV kernels; however, there was no significant ( $P < 0.01$ ) difference between treatments. The wet gluten was strongly and positively ( $r = 0.99^{***}$ ) correlated with flour protein content.

Starch damage was significantly ( $P < 0.05$ ) different between Minot and Casselton locations; however, there was no significant ( $P < 0.01$ ) difference among vitreous kernel treatments. Starch damage was positively associated with 1000-KWT and negatively associated with both break flour yield and flour protein content, respectively. In other words, there is greater starch damage with large kernels, while starch damage decreases with smaller kernels or with high protein content. Generally, damaged starch absorbs more water than the undamaged starch granules (Carson and Edwards, 2009). Due to the compact endosperm structure, there is a tight adhesion between the starch granules and its surrounding protein matrix. As a result, greater starch damage is found in vitreous kernel

during milling process. Therefore, vitreous kernels of HRS wheat exhibit higher water absorption compared with starchy kernels.

Conversely, vitreous kernel content was highly ( $r = 0.97^{**}$ ) associated with farinograph water absorption. The findings are in agreement with Phillips and Niernberger (1976). There was no difference in the farinograph water absorption between two locations; however, farinograph water absorption increased with high DHV kernel content. This water absorption difference between vitreous kernel treatments was significant ( $P < 0.05$ ). Similarly, there was a significant ( $P < 0.01$ ) difference in the baking water absorption between both locations and vitreous kernel treatments. Baking water absorption also increased with greater percentage of vitreous kernel treatment.

Bread loaf volume was significantly ( $P < 0.01$ ) different between Minot and Casselton locations. Bread loaf volume was 1036 cc for Minot, which was about 130 cc greater than that of Casselton location. This was expected, as high flour protein content observed in Minot location could contribute to a higher bread loaf volume. Sandstedt and Fortman (1944) examined hard red winter (HRW) wheat varieties across locations and indicated that wheat varieties grown at the same locations had loaf volumes and mixing times that were similar. However, they indicated that larger variability resulted among locations for individual varieties. In our study, bread loaf volume was highly correlated with protein content, which could have contributed the most for the difference observed in bread loaf volume between two locations. In addition, the SDS-extractable proteins were significantly ( $P < 0.05$ ) different between two locations, and the protein fractions were positively associated with bread loaf volume. SDS-extractable and unextractable protein fractions of Glenn samples are presented in Table 15.

Table 15. SDS-extractable and -unextractable protein fractions of Glenn variety varying different percentages of dark, hard and vitreous kernel

Location	Treatment	SDS-extractable (%flour)				SDS-unextractable (%flour)			
		E1	E2	E3	E4	U1	U2	U3	U4
Minot		0.84a	2.29a	8.23a	2.41a	2.03a	1.91a	0.83a	0.29a
Casselton		0.50b	1.71b	6.54b	2.08b	1.95a	1.93a	1.06a	0.33a
	Low	0.76a	2.01a	7.21a	2.21a	1.81a	1.94a	0.93a	0.31a
	Medium	0.63a	1.96a	7.26a	2.23a	2.10a	1.87a	1.06a	0.34a
	High	0.62a	2.04a	7.67a	2.29a	2.06a	1.94a	0.84a	0.29a

Mean values followed by same letter in the column are not significantly different

SDS-extractable protein fractions that were eluted at F1, F2, F3, and F4 sections of the chromatogram were significantly ( $P<0.05$ ) higher for samples obtained from Minot location. As mentioned bread loaf volume was higher for samples obtained from Minot location; thus, the SDS-extractable protein fractions could contribute to this significant ( $P<0.01$ ) difference. However, there was no significant ( $P<0.05$ ) difference between vitreous kernel treatments, although the gliadin composition (E3) increased with greater percentage of vitreous kernel treatment.

#### 5.4. Flour Water Absorption and Its Influence on Dough Weight

The objective of this section was to determine and quantify the effect of DHV kernel content on flour water absorption, and also to identify potential difference in the number of bread loaves that can be produced. As found in both the regional and protein composites, there was a significant ( $P<0.05$ ) difference in the flour water absorption among different percentages of vitreous kernel treatments. Flour water absorption increased with greater percentage of vitreous kernels.

In this section of our study, we investigated whether this physical difference in the flour absorption had economical importance. In other words, the objective was to see whether there was an economical difference when evaluating flours that were produced

from HRS wheat assigned to different subclasses when varied in the percentages of DHV kernels.

A local commercial bakery, Pan-O-Gold Baking Company (Fargo, ND), was contacted to determine the potential economic impact of water absorption rates. Below is an example used to quantify the difference in the number of bread loaves when considering two HRS flours that had 1% difference in the flour water absorption:

During one day's shift, Pan-O-Gold Baking Company ran 32,636 kg of dough. One percent of 32,636, of dough were 326.4 kg. If one were to calculate how many bread loaves could be produced from 326.4 kg of dough, we would simply multiply this amount by 1000g and divide by 779.6 g (amount needed to make a 1 ½ loaf of bread):

$$(326.4 \text{ kg} \times 1000 \text{ g/kg}) / 779.6 \text{ g} = 419 \text{ extra bread loaves}$$

It should be noted that 779.6 g is scaled to get 680.4 g loaf of bread, considering the loss of fermentation, and moisture during baking conditions. According to Cliff Sheeley, plant manager at the Pan-O-Gold Baking Company in Fargo, ND, this 419 extra bread loaves produced was “worth watching.”

Average price from a plain white loaf of bread ranges from \$1.74 to a Country Hearth Brand loaf \$3.10. Thus,

$$\text{Plain white: } 419 \text{ extra loaves} \times \$1.74 \text{ per loaf} = \$729.06/\text{day}$$

$$\text{Country hearth: } 419 \text{ extra loaves} \times \$3.10 \text{ per loaf} = \$1,298.9/\text{day}$$

These are the potential change in gross revenue, which can be obtained from flours with high water absorbing capacity during one day's shift.

In this study, we were able to evaluate the difference in flour water absorption produced from HRS assigned to different classes. In addition, this study investigated how



flour water absorption could play an important role in the number of bread loaves that can be produced. Therefore, we were able to show the effects of flour water absorption on potential gross income that can be generated from flours with high water absorption.

## 6. CONCLUSIONS

In this research project, we studied the effects of dark, hard and vitreous (DHV) kernel content of HRS on flour and baking quality characteristics. The U.S. regional crop quality survey samples from three consecutive growing years (2010, 2011, and 2012) were used. Both regional and protein composites were segregated into three different market classes using a custom built color-sorting system. Samples were milled, and flour quality and bread baking characteristics were evaluated for both regional and protein composites in addition to 'Glenn' variety at two locations.

As found in both regional and protein composites, there was a significant ( $P<0.05$ ) difference in the flour water absorption between vitreous kernel treatments. Flour water absorption increased with greater percentages of DHV kernel, which had a high and positive association with flour water absorption. An example was shown to quantify the flour water absorption difference on the total dough weight and also in the potential number of bread loaves. This further showed the importance of flour water absorption on potential economic value that can be gained with having flour produced from HRS wheat with greater DHV content. Therefore, the findings, in this study, show the importance of dark, hard and vitreous kernel characteristics on flour and baking quality of HRS wheat. In addition, it also enables the flour milling and baking industry to choose between different subclasses of HRS wheat with varying DHV content for their intended end-use applications.

## 7. FUTURE RESEARCH DIRECTIONS

This study was undertaken to compare different percentages of DHV kernel on HRS wheat flour and baking quality. However, due to the nature of this study and the experimental approach there were several limitations:

1. A kernel hardness characteristic was overlooked in this study. Thus, we could not make the assumptions and generalization about the relationship between kernel harness and vitreous kernel characteristics. However, a more detailed analysis is necessary to draw more definite conclusion on the relationship of wheat kernel characteristics such as harness and vitreousness.

2. In this study, we were able to show that flour water absorption was of economic importance. The example we provided in the study only showed the potential change in gross revenue that can be obtained from flours with high water absorbing capacity. However, we could not show whether there was a gain in net revenue by producing the extra 419 loaves of bread from flour with higher water absorption, simply because companies that we contacted were not willing to share detailed cost information. To further differentiate the net revenue from gross revenue, below are suggestions can be helpful in future research:

- Additional price information is needed to calculate the level of change in net revenue for producing 419 extra loaves
- Additional cost information is also needed to estimate possible changes in net revenue. There are two approaches that could be used:
  - Changes can be made in the milling process to obtain similar flour quality characteristics such as flour starch damage and water absorption from

different subclasses (DNS and NS) of HRS wheat. The costs of making these milling changes would need to be calculated and include possible adjustments in the milling capacity.

- Changes can be made in buying different subclasses of HRS wheat. Historical price information for DNS and NS would need to be obtained to estimate the cost differences between using flour made from these subclasses. Unfortunately, to the best of our knowledge, there are no known public sources for this price information.

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APPENDIX

Table A 1. Analysis of variance for kernel quality and milling characteristics of regional composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Test Weight	Year	2	10.66	22.4	<.0001
	Location (Loc)	5	1.59	3.3	0.024
	Treatment (Trt)	2	36.05	75.9	<.0001
	Year*Loc	10	1.71	3.6	0.007
	Year*Trt	4	1.80	3.8	0.019
	Loc*Trt	10	0.29	0.6	0.792
	Error	20	0.48		
Vitreous Kernel	Year	2	154.78	4.6	0.023
	Location (Loc)	5	67.70	2.0	0.121
	Treatment (Trt)	2	23271.15	691.0	<.0001
	Year*Loc	10	64.66	1.9	0.103
	Year*Trt	4	106.87	3.2	0.036
	Loc*Trt	10	33.48	1.0	0.480
	Error	20	33.68		
1000-KWT	Year	2	65.79	43.1	<.0001
	Location (Loc)	5	25.44	16.7	<.0001
	Treatment (Trt)	2	0.80	0.5	0.599
	Year*Loc	10	3.62	2.4	0.048
	Year*Trt	4	0.27	0.2	0.946
	Loc*Trt	10	1.22	0.8	0.629
	Error	20	1.53		
Flour Yield	Year	2	57.17	62.1	<.0001
	Location (Loc)	5	2.57	2.8	0.045
	Treatment (Trt)	2	1.17	1.3	0.303
	Year*Loc	10	1.44	1.6	0.190
	Year*Trt	4	1.58	1.7	0.187
	Loc*Trt	10	0.26	0.3	0.979
	Error	20	0.92		
Break Flour Yield	Year	2	8.46	15.2	<.0001
	Location (Loc)	5	0.26	0.5	0.799
	Treatment (Trt)	2	1.46	2.6	0.096
	Year*Loc	10	1.01	1.8	0.121
	Year*Trt	4	1.16	2.1	0.119
	Loc*Trt	10	0.20	0.4	0.947
	Error	20	0.56		

Table A 2. Analysis of variance for flour quality characteristics of regional composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Flour Protein	Year	2	9.10	137.4	<.0001
	Location (Loc)	5	2.35	35.5	<.0001
	Treatment (Trt)	2	4.80	72.5	<.0001
	Year*Loc	10	0.58	8.8	<.0001
	Year*Trt	4	0.16	2.5	0.077
	Loc*Trt	10	0.21	3.2	0.012
	Error	20	0.07		
Starch Damage	Year	2	1.02	19.3	<.0001
	Location (Loc)	5	0.70	13.3	<.0001
	Treatment (Trt)	2	1.77	33.5	<.0001
	Year*Loc	10	0.33	6.3	0.0003
	Year*Trt	4	0.11	2.0	0.1344
	Loc*Trt	10	0.04	0.7	0.7365
	Error	20	0.05		
Farinograph Water Absorption	Year	2	1.49	7.4	0.004
	Location (Loc)	5	3.35	16.7	<.0001
	Treatment (Trt)	2	40.65	201.9	<.0001
	Year*Loc	10	2.41	12.0	<.0001
	Year*Trt	4	1.78	8.8	0.000
	Loc*Trt	10	0.37	1.8	0.119
	Error	20	0.20		
Farinograph Peak Time	Year	2	11.76	18.1	<.0001
	Location (Loc)	5	2.37	3.6	0.017
	Treatment (Trt)	2	2.14	3.3	0.058
	Year*Loc	10	1.14	1.8	0.138
	Year*Trt	4	1.03	1.6	0.218
	Loc*Trt	10	0.97	1.5	0.213
	Error	20	0.65		
Farinograph Stability Time	Year	2	15.42	17.2	<.0001
	Location (Loc)	5	10.68	11.9	<.0001
	Treatment (Trt)	2	1.37	1.5	0.241
	Year*Loc	10	2.17	2.4	0.044
	Year*Trt	4	4.08	4.6	0.009
	Loc*Trt	10	1.28	1.4	0.239
	Error	20	0.90		

Table A 3. Analysis of variance for bread baking characteristics of regional composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Wet Gluten	Year	2	41.91	49.5	<.0001
	Location (Loc)	5	23.73	28.0	<.0001
	Treatment (Trt)	2	59.64	70.4	<.0001
	Year*Loc	10	6.62	7.8	<.0001
	Year*Trt	4	1.89	2.2	0.1019
	Loc*Trt	10	4.39	5.2	0.0009
	Error	20	0.85		
Baking Water Absorption	Year	2	40.18	211.6	<.0001
	Location (Loc)	5	3.01	15.9	<.0001
	Treatment (Trt)	2	36.40	191.7	<.0001
	Year*Loc	10	2.27	12.0	<.0001
	Year*Trt	4	0.90	4.8	0.007
	Loc*Trt	10	0.32	1.7	0.147
	Error	20	0.19		
Baking Mix Time	Year	2	1.05	38.2	<.0001
	Location (Loc)	5	0.18	6.6	0.001
	Treatment (Trt)	2	0.09	3.4	0.055
	Year*Loc	10	0.26	9.3	<.0001
	Year*Trt	4	0.10	3.6	0.023
	Loc*Trt	10	0.02	0.9	0.582
	Error	20	0.03		
Bread Loaf Volume	Year	2	34550.12	31.0	<.0001
	Location (Loc)	5	16876.23	15.1	<.0001
	Treatment (Trt)	2	8694.56	7.8	0.003
	Year*Loc	10	2380.25	2.1	0.071
	Year*Trt	4	555.67	0.5	0.737
	Loc*Trt	10	551.78	0.5	0.874
	Error	20	1114.77		

Table A 4. Analysis of variance for kernel quality characteristics for protein composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Test Weight	Year (Y)	2	5.83	17.18	0.0228
	Location (Y) (Error I)	3	0.34	1.11	0.3661
	Protein (P)	2	9.89	32.24	<.0001
	Vitreousness (V)	2	44.09	143.74	<.0001
	P*V	4	1.68	5.47	0.0028
	Y*P	4	3.80	12.39	<.0001
	Y*V	4	2.43	7.91	0.0003
	Y*P*V	8	0.56	1.82	0.1219
	Residual (Error II)	24	0.31		
Vitreous Kernel	Year (Y)	2	204.89	1.45	0.3633
	Location (Y) (Error I)	3	141.70	7.83	0.0008
	Protein (P)	2	19.08	1.05	0.3641
	Vitreousness (V)	2	25869.00	1428.77	<.0001
	P*V	4	31.81	1.76	0.1705
	Y*P	4	16.41	0.91	0.476
	Y*V	4	43.80	2.42	0.0764
	Y*P*V	8	26.02	1.44	0.2322
	Residual (Error II)	24	18.11		
1000-KWT	Year (Y)	2	16.52	2.05	0.2745
	Location (Y) (Error I)	3	8.05	16.51	<.0001
	Protein (P)	2	50.17	102.85	<.0001
	Vitreousness (V)	2	0.26	0.53	0.5973
	P*V	4	0.25	0.52	0.722
	Y*P	4	0.70	1.43	0.2539
	Y*V	4	0.68	1.4	0.2654
	Y*P*V	8	0.72	1.47	0.2202
	Residual (Error II)	24	0.49		

Table A 5. Analysis of variance for milling characteristics of protein composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Flour Yield	Year (Y)	2	36.26	8.62	0.0571
	Location (Y) (Error I)	3	4.21	4.49	0.0122
	Protein (P)	2	20.41	21.8	<.0001
	Vitreousness (V)	2	0.16	0.17	0.845
	P*V	4	0.53	0.57	0.6864
	Y*P	4	1.35	1.44	0.2521
	Y*V	4	0.28	0.3	0.8777
	Y*P*V	8	0.66	0.71	0.6806
	Residual (Error II)	24	0.94		
Break Flour Yield	Year (Y)	2	4.64	6.7	0.0782
	Location (Y) (Error I)	3	0.69	1.77	0.1795
	Protein (P)	2	0.64	1.63	0.2176
	Vitreousness (V)	2	4.75	12.15	0.0002
	P*V	4	0.24	0.61	0.6579
	Y*P	4	0.26	0.67	0.622
	Y*V	4	0.60	1.54	0.2238
	Y*P*V	8	0.55	1.4	0.2473
	Residual (Error II)	24	0.39		



Table A 6. Analysis of variance for flour quality characteristics for protein composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Flour Protein	Year (Y)	2	1.33	241.7	0.0005
	Location (Y) (Error I)	3	0.01	0.05	0.9862
	Protein (P)	2	33.83	288.49	<.0001
	Vitreousness (V)	2	3.24	27.62	<.0001
	P*V	4	0.19	1.66	0.1928
	Y*P	4	0.40	3.45	0.0231
	Y*V	4	0.21	1.76	0.1699
	Y*P*V	8	0.05	0.44	0.886
	Residual (Error II)	24	0.12		
Starch Damage	Year (Y)	2	0.05	0.3	0.7628
	Location (Y) (Error I)	3	0.15	1.23	0.3221
	Protein (P)	2	3.17	25.29	<.0001
	Vitreousness (V)	2	3.46	27.58	<.0001
	P*V	4	0.35	2.75	0.0513
	Y*P	4	0.08	0.64	0.6377
	Y*V	4	0.03	0.23	0.9193
	Y*P*V	8	0.06	0.45	0.8812
	Residual (Error II)	24	0.13		
Wet Gluten	Year (Y)	2	7.71	4.16	0.1363
	Location (Y) (Error I)	3	1.85	0.87	0.4726
	Protein (P)	2	332.79	155.5	<.0001
	Vitreousness (V)	2	37.40	17.48	<.0001
	P*V	4	1.25	0.58	0.6787
	Y*P	4	3.31	1.55	0.2206
	Y*V	4	0.53	0.25	0.9087
	Y*P*V	8	1.17	0.55	0.8102
	Residual (Error II)	24	2.14		

Table A 7. Analysis of variance for farinograph quality parameters of protein composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Farinograph Water Absorption	Year (Y)	2	30.02	28.82	0.011
	Location (Y) (Error I)	3	1.04	1.71	0.1921
	Protein (P)	2	90.83	148.9	<.0001
	Vitreousness (V)	2	42.75	70.09	<.0001
	P*V	4	1.21	1.98	0.1299
	Y*P	4	17.82	29.21	<.0001
	Y*V	4	1.85	3.04	0.0368
	Y*P*V	8	0.24	0.39	0.9175
	Residual (Error II)	24	0.61		
	Farinograph Peak Time	Year (Y)	2	3.47	4.27
Location (Y) (Error I)		3	0.81	2.1	0.1264
Protein (P)		2	8.84	22.87	<.0001
Vitreousness (V)		2	1.47	3.81	0.0366
P*V		4	0.24	0.61	0.6606
Y*P		4	0.94	2.44	0.0746
Y*V		4	0.26	0.67	0.6191
Y*P*V		8	0.52	1.35	0.2655
Residual (Error II)		24	0.39		
Farinograph Stability Time		Year (Y)	2	34.99	11.76
	Location (Y) (Error I)	3	2.98	1.95	0.1483
	Protein (P)	2	7.69	5.04	0.0149
	Vitreousness (V)	2	5.20	3.41	0.0497
	P*V	4	3.14	2.06	0.1176
	Y*P	4	3.24	2.13	0.1086
	Y*V	4	0.90	0.59	0.6735
	Y*P*V	8	1.96	1.29	0.2957
	Residual (Error II)	24	1.52		

Table A 8. Analysis of variance for bread baking characteristics of protein composites

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Baking Water Absorption	Year (Y)	2	87.00	58.09	0.004
	Location (Y) (Error I)	3	1.50	2.6	0.0754
	Protein (P)	2	68.00	118.11	<.0001
	Vitreousness (V)	2	35.20	61.14	<.0001
	P*V	4	1.47	2.55	0.0655
	Y*P	4	12.27	21.31	<.0001
	Y*V	4	1.93	3.36	0.0257
	Y*P*V	8	0.25	0.43	0.8919
	Residual (Error II)	24	0.58		
	Baking Mix Time	Year (Y)	2	0.49	15.74
Location (Y) (Error I)		3	0.03	0.76	0.5274
Protein (P)		2	0.79	19.49	<.0001
Vitreousness (V)		2	0.04	1.05	0.365
P*V		4	0.02	0.43	0.7831
Y*P		4	0.04	0.99	0.4331
Y*V		4	0.10	2.56	0.0642
Y*P*V		8	0.03	0.67	0.7143
Residual (Error II)		24	0.04		
Bread Loaf Volume		Year (Y)	2	1821.64	0.23
	Location (Y) (Error I)	3	8028.36	8.72	0.0004
	Protein (P)	2	75391.00	81.92	<.0001
	Vitreousness (V)	2	10963.00	11.91	0.0003
	P*V	4	1509.84	1.64	0.1967
	Y*P	4	2476.16	2.69	0.0553
	Y*V	4	166.44	0.18	0.9461
	Y*P*V	8	975.38	1.06	0.4221
	Residual (Error II)	24	920.28		

Table A 9. Analysis of variance for kernel quality and milling characteristics of Glenn samples

Source	Source	DF	Mean Square	<i>F</i> value	Pr > F
Test Weight	Location	1	3.08	3.73	0.1933
	Treatment	2	2.25	2.72	0.269
	Error	2	0.85		
Vitreous Kernel	Location	1	15.36	0.48	0.5599
	Treatment	2	3875.89	121.25	0.0082
	Error	2	31.97		
1000-KWT	Location	1	31.74	6348	0.0002
	Treatment	2	0.46	91	0.0109
	Error	2	0.01		
Flour Yield	Location	1	0.53	1.13	0.4
	Treatment	2	0.23	0.48	0.6769
	Error	2	0.47		
Break Flour Yield	Location	1	4.06	39.8	0.0242
	Treatment	2	0.04	0.39	0.7184
	Error	2	0.10		

Table A 10. Analysis of variance for flour quality and farinograph parameters of Glenn samples

Dependent Variable	Source	DF	Mean Square	<i>F</i> value	Pr > F
Flour Protein	Location	1	11.20	126.01	0.0078
	Treatment	2	0.17	1.91	0.3437
	Error	2	0.09		
Starch Damage	Location	1	4.70	83.12	0.0118
	Treatment	2	0.10	1.74	0.3644
	Error	2	0.06		
Wet Gluten	Location	1	96.10	252.68	0.0039
	Treatment	2	2.98	7.84	0.1131
	Error	2	0.3803105		
Farinograph Water Absorption	Location	1	0	0	1
	Treatment	2	0.86	43	0.02
	Error	2	0.02		
Farinograph Peak Time	Location	1	28.17	174.23	0.0057
	Treatment	2	0.36	2.24	0.3089
	Error	2	0.17		
Farinograph Stability Time	Location	1	0.24	0.05	0.845
	Treatment	2	3.91	0.8	0.5548
	Error	2	4.88		

Table A 11. Analysis of variance for bread baking characteristics of Glenn samples

Dependent Variable	Source	DF	Mean Square	<i>F</i> value	Pr > F
Baking Water Absorption	Location	1	4.19	772.24	0.0013
	Treatment	2	0.63	115.55	0.0086
	Error	2	0.01		
Baking Mix Time	Location	1	2.04	784	0.0013
	Treatment	2	0.10	39	0.025
	Error	2	0.01		
Bread Loaf Volume	Location	1	26666.67	128.64	0.0077
	Treatment	2	732.30	3.53	0.2206
	Error	2	207.29		

Table A 12. Kernel and flour quality, and bread baking characteristics of composites from 2010

Sample	Test Weight (kg/hl)	Vitreous Kernels (%)	1000 KWT (g)	Flour Extraction (%)	Flour Protein (%)	Wet Gluten (%)	Farinograph Absorption (%)	Baking Absorption (%)	Loaf Volume (cc)
MN -B	80.1	56	33.6	71.2	12.6	33.1	64.7	63.2	945
MT-B	80.5	76	31.9	69.2	12.1	34.0	64.2	62.7	915
ND-A	81.4	70	31.2	70.0	13.0	36.1	65.0	63.5	978
ND-D	80.4	82	31.8	68.8	13.2	35.2	64.7	63.2	960
ND-E	80.9	73	31.2	70.0	13.0	35.6	63.8	62.3	928
SD-B	79.8	76	30.5	69.6	13.0	36.4	64.8	63.3	915
East Low	81.3	49	32.6	71.9	11.6	30.2	63.8	62.3	893
East Mid	81.8	69	31.6	70.1	12.8	34.8	65.8	64.3	950
East High	81.0	70	33.2	69.7	14.0	37.6	66.7	65.2	1050
West Low	81.0	65	33.4	69.5	11.3	29.2	63.3	61.8	840
West Mid	80.7	74	32.3	69.3	12.9	35.6	65.2	63.7	968
West High	79.8	84	31.2	68.1	14.0	38.2	65.7	64.2	1035

Table A 13. Kernel and flour quality, and bread baking characteristics for composites from 2011

Sample	Test Weight (kg/hl)	Vitreous Kernels (%)	1000 KWT (g)	Flour Extraction (%)	Flour Protein (%)	Wet Gluten (%)	Farinograph Absorption (%)	Baking Absorption (%)	Loaf Volume (cc)
MN -B	78.3	78	26.0	69.0	13.6	36.5	64.4	62.9	953
MT-B	80.4	84	30.7	68.0	12.6	35.3	64.4	62.9	910
ND-A	80.1	81	28.6	68.0	14.3	40.0	65.8	64.3	1055
ND-D	77.9	80	23.7	66.7	14.5	39.9	65.8	64.3	1070
ND-E	77.6	75	23.8	65.0	13.4	36.2	65.6	64.1	1023
SD-B	77.6	82	24.0	67.5	14.2	38.8	63.5	62.0	1005
East Low	81.5	64	31.0	70.6	11.7	30.1	63.9	62.4	928
East Mid	80.0	73	28.7	70.4	12.8	34.4	64.9	63.4	970
East High	77.9	84	23.9	69.2	14.3	39.0	65.9	64.4	1013
West Low	81.4	86	30.5	68.0	11.4	30.1	63.1	61.6	860
West Mid	80.9	92	28.5	68.1	12.7	34.1	63.8	63.0	928
West High	79.3	80	25.4	66.1	14.3	40.0	65.1	64.5	998

Table A 14. Kernel and flour quality, and bread baking characteristics of composites from 2012

Sample	Test Weight (kg/hl)	Vitreous Kernels (%)	1000 KWT (g)	Flour Extraction (%)	Flour Protein (%)	Wet Gluten (%)	Farinograph Absorption (%)	Baking Absorption (%)	Loaf Volume (cc)
MN -B	80.5	41	31.6	69.7	13.1	33.8	61.7	61.2	948
MT-B	78.4	82	26.0	69.0	13.8	37.0	62.8	62.3	943
ND-A	79.8	83	27.2	68.3	14.4	39.0	63.8	63.3	1123
ND-D	79.2	85	26.1	68.8	14.6	38.4	64.8	64.3	968
ND-E	77.0	79	26.4	68.6	14.4	38.1	64.1	63.6	1043
SD-B	80.0	72	27.0	69.1	13.5	33.8	61.9	61.4	988
East Low	81.4	55	31.7	70.6	12.1	31.1	60.8	60.8	950
East Mid	80.7	64	30.3	70.0	13.2	34.4	64.2	62.2	978
East High	80.7	71	28.7	68.8	14.4	38.2	64.1	63.3	1020
West Low	81.1	94	29.7	68.7	11.6	28.8	62.1	61.5	865
West Mid	81.4	78	29.6	69.1	13.1	35.5	63.8	62.9	930
West High	79.7	88	26.7	67.8	14.7	38.9	64.6	64.2	1080