

HARD RED SPRING WHEAT QUALITY EVALUATION WITH VARIOUS  
ROLLER MILL TYPES AND BREADMAKING METHODS

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**Title**

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## ABSTRACT

Roller mill type and breadmaking methods might be a source of variation in the evaluation of the end-use quality of Hard Red Spring (HRS) wheat. In this study, various roller mill types and baking methods have been used to investigate whether they affect end-use quality evaluation of HRS wheat cultivars. In addition, a quality scoring system has been developed to determine if ranking of the HRS wheat cultivars would change when different roller mills and breadmaking methods were used. Both the roller mill type and breadmaking method had an effect on the end-use quality of HRS wheat cultivars. When using different roller mills for quality evaluation, HRS wheat samples of MN Bolles and ND Glenn from Gulf/Great Lakes (G/GL) region and ND Glenn from Casselton location had overall quality scores of 6.5 or above when averaged across mill types. When using various baking methods and conditions for quality evaluation, ND 817, MN Bolles, ND Glenn cultivars from Pacific Northwest region, and MN Bolles and ND Glenn from G/GL region received overall baking quality scores of 6.5 or above hence these cultivars were considered to have “excellent” baking quality characteristics under different baking conditions. The results in the current research study indicate that although there are differences in the mill type and breadmaking methods on the end-use quality evaluation, the ranking of HRS wheat flours is not affected by the mill type or baking methods and conditions. In other words, cultivars considered to have “fair” quality tend to have low end-use quality, while “excellent” cultivars will have superior end-use quality regardless of the roller mills and/or baking method and processing conditions used. The proposed overall wheat scoring system could assist farmers and breeders in selection of wheat cultivars considering the wheat end-use quality. Development of a comprehensive scoring system will also enable a more detailed scoring system for screening new lines for suitable end-use.

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## **DEDICATION**

I dedicate this dissertation to my dear father Baasandorj Bech-Ochir, who has sacrificed so much for his children's education. His love, support, encouragement, and daily phone calls are greatly appreciated throughout this journey. I cannot thank enough for all that you have done for us.

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## GENERAL INTRODUCTION

Hard Red Spring (HRS) wheat grown in the United States is important in the U.S. domestic and exports markets in terms of end-use quality. HRS wheat cultivars are characterized by high protein content, excellent milling and baking performance (Carson and Edwards, 2009), which make it ideal for blending with other wheat types for a “valued improved” for flour blending (U.S. Wheat Associates, 2016). Blending of HRS wheat improves dough handling and mixing characteristics as well as water absorption of low protein wheat. In addition, HRS wheat produced in the United States is well suited for the production of high-volume breads made by the traditional sponge-and-dough baking process (Cracknell and Williams, 2004).

HRS wheat is known as a “blending wheat” to increase the gluten strength (U.S. HRS Wheat Regional Crop Quality Report, 2015). As a result, Hard Red Spring wheat grown in the Northern Plains states of North Dakota, Minnesota, Montana, and South Dakota is transported from the farmers to the export facilities by truck, rail and water. On average, close to 80% of the wheat grown in the region get to the markets by rail (U.S. HRS Regional Quality Report, 2015).

Variation in the end-use quality of wheat samples is commonly explained by differences in genotype and/or growing environment (Machet, 2005). Both factors can affect the concentration of composition of important constituents of wheat. In addition to these genotype and environmental factors, the processing conditions can also be a source of variation on the end-use quality. Wheat milling is a key source of variation in flour quality for breadmaking (Machet, 2005), as wheat kernel is heterogeneous in physical and chemical composition. Different laboratory roller mills can have an impact on the end-use quality of HRS wheat (Baasandorj et al., 2015). On the other hand, a commercial hard wheat mill can produce 30 or more flour mill streams (Machet, 2005). Because of the physical and chemical composition of a wheat kernel is

very heterogeneous, different flour millstreams can vary in composition and quality ultimately impacting the end-use quality. Therefore, it is very important to understand that different types of roller mills can have a significant effect on the end-use quality variation.

Breadmaking is the ultimate test for HRS wheat quality evaluation. Therefore, breadmaking method is another source of variation on the end-use quality of wheat, as there is various breadmaking methods developed by the American Association of Cereal Chemists International (AACC-I) include: optimized straight dough method, long fermentation method, sponge and dough method, and no time method. Various breadmaking methods were evaluated and compared (Maeda et al., 2004), and the authors have concluded that straight-dough method with long fermentation was considered suitable for improving the poor dough and baking properties of polished flours (Maeda et al., 2004). Therefore, breadmaking method can have an impact on the end-use quality of wheat, too.

An overall scoring system for quality evaluation for HRS wheat is helpful to objectively rank various HRS wheat cultivars when considering wheat, flour and dough, and breadmaking quality parameters. An overall scoring system can help the wheat farmers to select HRS wheat cultivars based on the end-use quality thus they can alternate high yield cultivars with high quality cultivars. A comparison and ranking of wheat cultivars for their end-use quality characteristics on a score-system will provide a better and accurate evaluation of HRS wheat cultivars. In addition, development of a comprehensive scoring system will enable a more detailed and new potential scoring system for screening new lines for suitable wheat end-use.

## Overall Objectives

The current research was carried out with four specific objectives in mind.

- i. To determine if the ranking of Hard Red Spring wheat cultivars for quality evaluation is affected by mill type
- ii. To determine whether breadmaking methods and loaf size affect the overall ranking of Hard Red Spring wheat cultivars
- iii. To evaluate and compare Hard Red Spring wheat flours for Solvent Retention Capacity and Pasting Properties
- iv. To compare the flour millstreams for their physicochemical characteristics, mixing and breadmaking characteristics, and protein molecular weight distribution

The hypothesis of current study was that the ranking of different HRS wheat cultivars would be consistent when using various roller mills for flour milling. In other words, the ranking and comparison of HRS wheat cultivars stays the same no matter what type of roller mill is used. Similarly, the ranking of HRS wheat cultivars would be consistent when using different breadmaking methods. A proposed overall scoring system for ranking HRS wheat end-use quality would be helpful. In addition, different roller mill types would have different solvent retention capacity (SRC) and pasting properties. Lastly, various millstream flours obtained from MIAG-Multomat would have different mixing and breadmaking characteristics as well as protein molecular weight distribution (MWD).

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## CHAPTER 1. LITERATURE REVIEW

### 1.1. Wheat

Wheat is among the oldest and most extensively grown of all grain crops worldwide. Wheat and bread are integral to human life as well as human food (Wrigley, 2009). Wheat is a member of the grass family (Gramineae), which includes the cereal grains (Delcour and Hosenev, 2010). Wheat is the primary cereal for temperate regions and it is most widely adapted and cultivated crop in the world. There are number of species and subspecies in the genus *Triticum*. However, the most important are the common wheat (*T. aestivum*), which accounts for more than 90% of the world wheat production, durum wheat (*T. turgidum*), which accounts for about 5% (Gooding, 2009).

The wheat plant is quite hardy and can be grown under a wide cultivar of environmental and soil conditions (Delcour and Hosenev, 2010). Wheat can be grown as either a winter or a spring crop (Wrigley, 2009). Therefore, 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).

Therefore, wheat-based food products are considered staples in many countries throughout the world. Various types of products are made from wheat flour depending on the desired end-use (Figure 1.1).

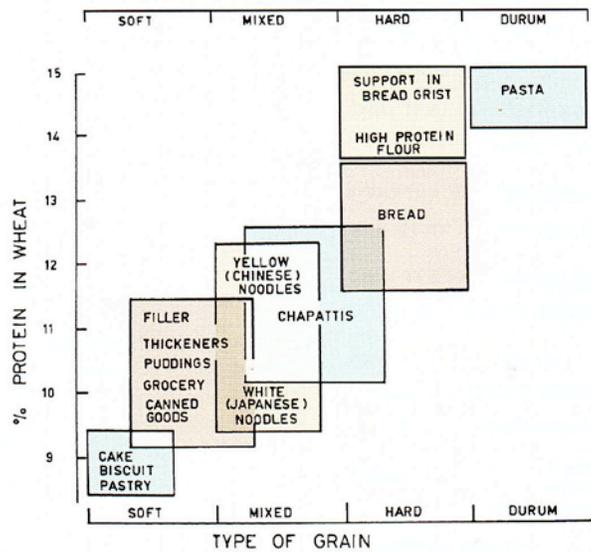


Figure 1.1. Wheat Types and Types of Products Varying in Protein Content (Reprinted from Delcour, J.A. and Hosney, R.C. 2010)

### 1.2. Wheat in the United States

Wheat is an important crop in many countries, including the United States and Canada. Countries such as the United States, Canada, Australia, the European Union, Russia, Ukraine, Kazakhstan, and Argentina account for about 90% of the world wheat exports (Figure 1.2). The United States is the world's leading wheat exporter.

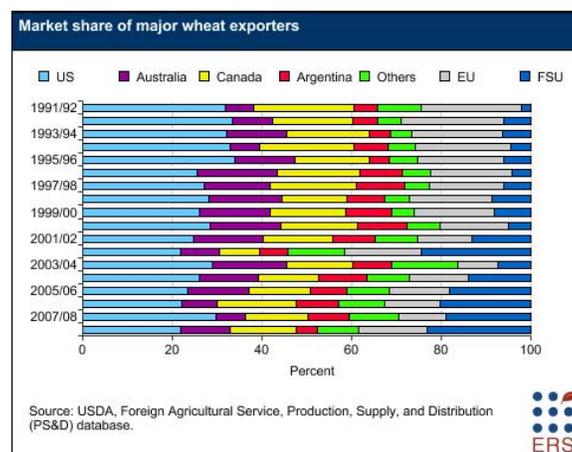


Figure 1.2. The Market Share of Major Exporting Countries ([http://www.ers.usda.gov/media/600559/exportshare\\_1\\_.jpg](http://www.ers.usda.gov/media/600559/exportshare_1_.jpg))

There are six classes of wheat grown in the United States: Hard Red Winter (HRW), Hard Red Spring (HRS), Soft Red Winter (SRW), Soft White (SWH), Hard White (HWH), and Durum. These are designated by color, hardness, and their growing season (U.S. Wheat Associates, 2013). 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.

### 1.3. 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 opacity 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).

#### 1.4. Hard Red Spring Wheat

Hard Red Spring (HRS) wheat constitutes about 25% of the wheat crop in the United States and is composed of spring-sown cultivars with hard endosperm and red seed coat (Carson and Edwards, 2009). HRS wheat is almost exclusively grown in the Northern Great Plains states of Minnesota, Montana, North Dakota, and South Dakota. Furthermore, small portion of HRS wheat acres are grown in the Pacific Northwest (PNW), states of Idaho, Oregon, and Washington.

Hard Red Spring wheat is known as a “blending wheat” to increase the gluten strength as the high protein content and superior gluten quality of hard red spring wheat make it ideal for use in products such as yeast breads, hard rolls and specialty breads such as hearth breads, whole grain breads, bagels and pizza crusts (U.S. HRS Wheat Regional Crop Quality Report, 2015). In addition, using HRS wheat flours in frozen dough products are better because they can be stored long than those made with low protein wheats.

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.

Although there are three dozen HRS wheat cultivars are grown in these 4-state growing regions, only 10 cultivars make up 55% of acreage (Carson and Edwards, 2009). In 2015, top 4 HRS wheat cultivars accounted for 32% of the planted acres in growing regions of MN, MT, ND and SD (Figure 1.3).

### MN / MT / ND / SD combined

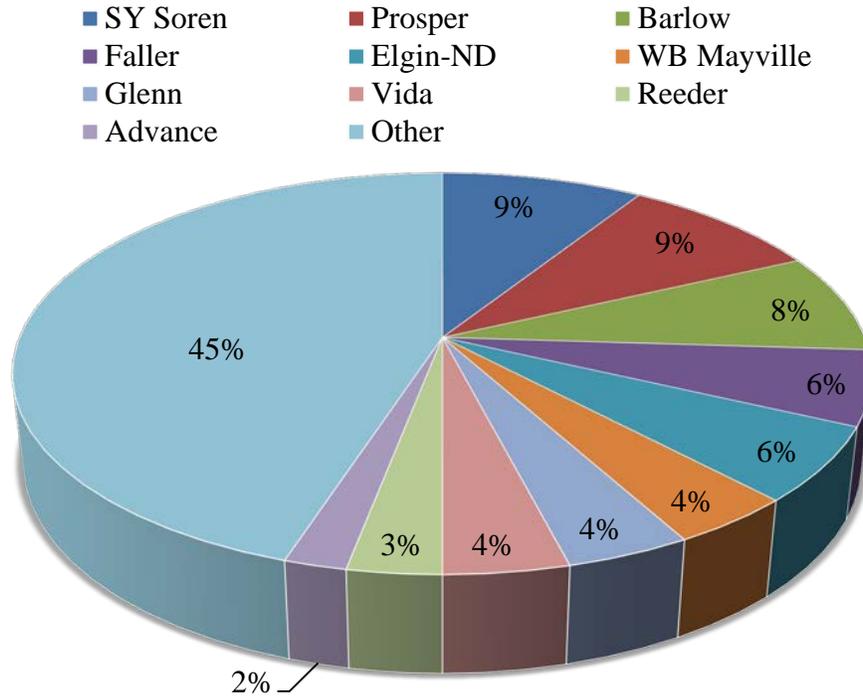


Figure 1.3. Popular Hard Red Spring Wheat Cultivars Based on Percentage of Planted Acres in 4-State Growing Regions (Information is adapted from the 2015 U.S. Hard Red Spring Wheat Regional Crop Quality Report)

Hard Red Spring wheat is important in the U.S. domestic and export markets, as HRS cultivars are characterized by high protein content, and excellent milling and baking performance (Carson and Edwards, 2009). HRS wheat is also a valued improver for flour blending (U.S. Wheat Associates, 2016). 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). In addition, blending of HRS wheat to lower protein wheat improves dough handling and mixing characteristics as well as water absorption.

Hard Red Spring wheat grown in the Northern Plains is transported from the farm to the export facilities by truck, rail and water. On average, close to 80% of the wheat grown in the region get to the markets by rail (U.S. HRS Regional Quality Report, 2015). Figure 1.4 illustrates

the average share of U.S. HRS exports, and the domestic use and wheat exports for last 4 years from these growing regions.

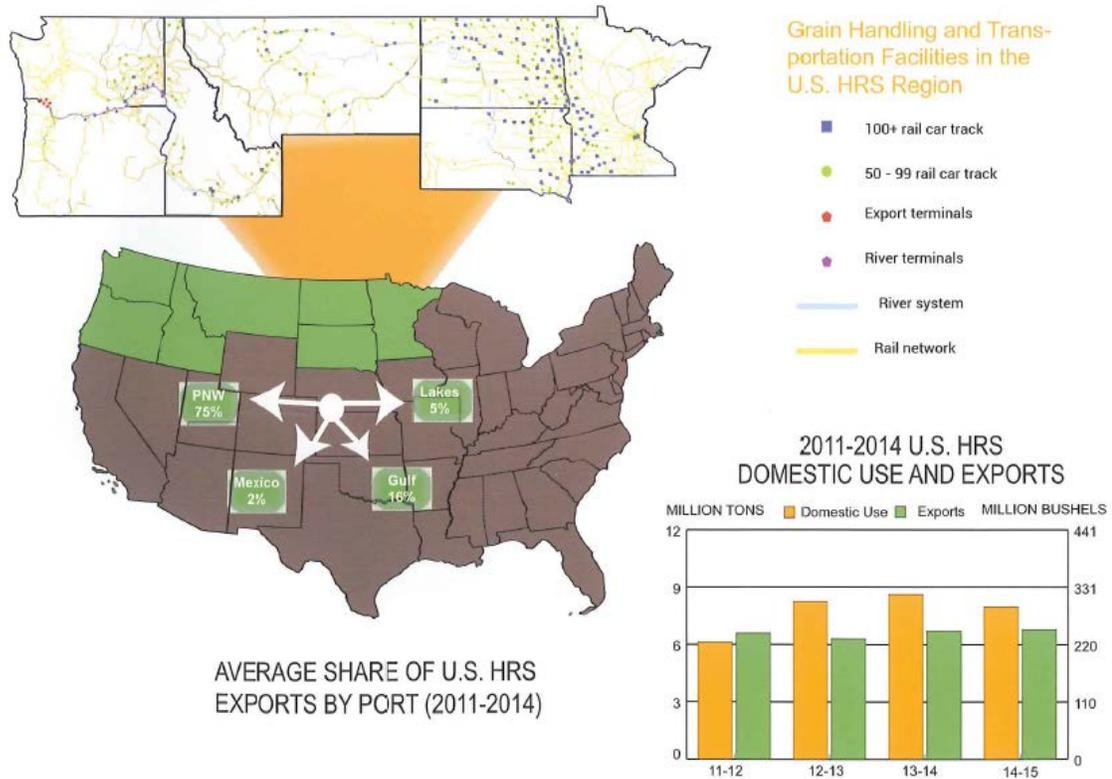


Figure 1.4. Domestic Use and Export Regions for Hard Red Spring Wheat from Growing Regions  
(Reprinted from the 2015 U.S. Hard Red Spring Wheat Regional Crop Quality Report)

### 1.5. 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 (Figure 1.5).

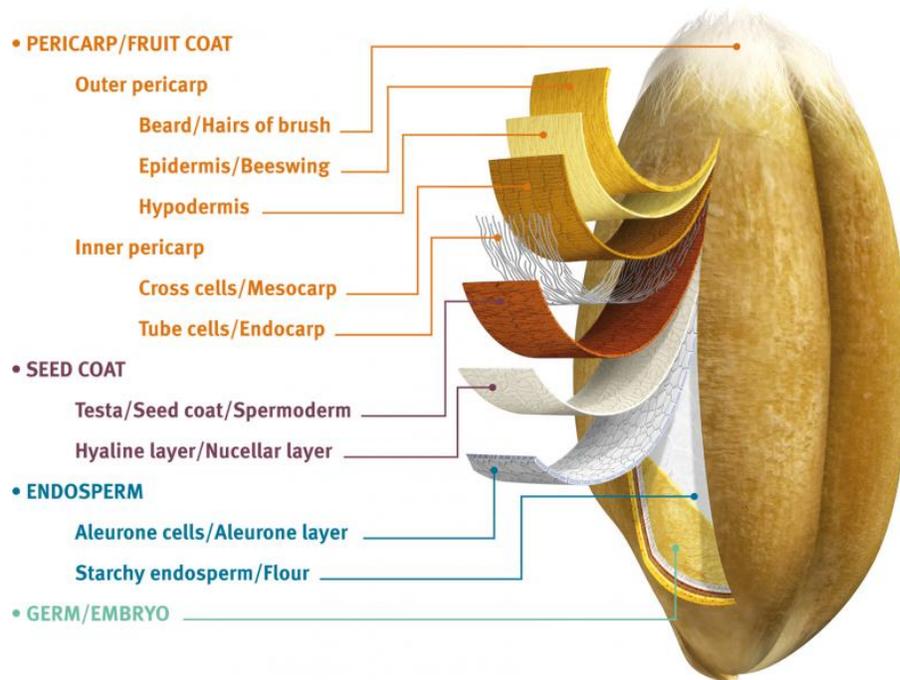


Figure 1.5. A Longitudinal Section of Wheat Kernel  
 (<http://grain-gallery.com/en/wheat/images>)

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

### 1.5.1. 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 Hosenehy, 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.

### 1.5.2. 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 Hoseney, 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.

### 1.5.3. 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 Hoseney, 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 Hoseney, 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 impact 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.

### 1.6. Wheat Kernel Characterization

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 (Baasandorj et al., 2015) (Figure 1.6).



Figure 1.6. Light Microscopy Images of Cross Cut Sections of Vitreous (left) and starchy (right) Kernels

Factors influencing vitreous characteristics of wheat kernels are heredity, weather, and soil fertility (Phillips and Niernberger, 1976). However, vitreousness is mainly controlled by nitrogen availability as well as temperature during grain filling period (Pomeranz and Williams, 1990).

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.

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, starchy kernel has little impact on the milling performance of hard wheat when straight-grade types of flour are produced. However, starchy kernel 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.

### 1.7. Hard Red Spring Wheat Quality Evaluation

When wheat is bought in the cash market or in an export transaction, wheat is evaluated according to the official grades. In the United States the official grade of wheat is determined by the procedures guidelines set by the U.S. Grain Inspection, Packers, and Stockyards Administration (GIPSA). To objectively evaluate a representative wheat sample of minimum of 2000 g from the entire lot is required (Posner and Hibbs, 2005).

#### 1.7.1. Kernel Quality Evaluation

In a wheat quality lab, kernel quality evaluation is the very first step upon receiving a wheat sample. There are various kernel quality tests that are routinely tested for kernel quality evaluation (Table 1.1).

Table 1.1. Common Kernel Quality Parameters and Current Methods

Kernel Quality Parameter	Official Method	Method of Reference
Test Weight	Test Weight per Bushel	AACCI Method 55-10.01
Dockage	Carter Dockage Tester	Official USDA Procedure
Moisture	Dickey-John Moisture Meter	Official USDA Procedure
Ash	Incineration Method	AACCI Method 08-01.01
Protein	Crude Protein-Combustion (LECO)	AACCI Method 6-30.01
Vitreous Kernel	Manual and Visual Inspection	Official USDA Procedure
Thousand Kernel Weight	Count by Electronic Counter	-
Kernel Size Distribution	Kernel Sizer	-
Kernel Hardness	Single Kernel Characterization System	AACCI Method 55-31.01
Falling Number	Enzyme Activity Measurement	AACCI Method 56-81.03

Dockage is the non-wheat material and it is separated using the Carter-Day dockage machine. All U.S. grade and non-grade factors are determined only when dockage is removed. Test weight is a weight of a specific volume of grain. In the United States, test weight is expressed as pounds per Winchester bushel determined on a dockage-free wheat sample. Moisture is very important for grain storability, as low moisture is generally more stable during storage in the bin (U.S. HRS Regional Crop Quality Report, 2015). Wheat ash is another quality factor used in the kernel quality evaluation, and ash content indicates the mineral content in the kernel. Flour millers seek for wheats that will produce-low ash flours (Posner and Hibbs, 2005). Wheat protein is probably the most important factor determining the value of HRS wheat because wheat protein correlates to many processing factors such as high flour water absorption and bread loaf volume.

Kernel vitreousness is an important factor milling performance of Hard Red Spring wheat, as vitreousness is the ability to fracture during the milling process. Baasandorj et al. (2015) have also reported that high vitreous kernel percentage resulted in high flour water absorption determined by farinograph. Thousand-kernel weight (TKW) measures the mass of 1000 wheat kernel. TKW can provide important information to the miller about the milling

potential of certain wheat (Posner and Hibbs, 2005). Kernel size another important factor in milling of wheat. Kernel size influences grinding performance of roller mill, as wheat kernels of different sizes break up differently. Kernel hardness characteristics are related to important milling properties such as tempering, roll gap settings, and flour starch damage content (Overview of U.S. Wheat Inspection, 2007). Falling number is an indirect measurement of  $\alpha$ -amylase activity, where low falling number (measured in seconds) indicates high  $\alpha$ -amylase activity resulting from pre-harvest sprout damage.

### 1.7.2. Milling Quality Evaluation

Grinding is considered the most important process in the milling system (Posner and Hibbs, 2005). There are four stages in grinding process and each has its own objective. The objective of the break system is to open up the wheat kernel and remove the endosperm and germ from the bran coat with the least amount of bran contamination. In addition, the second break is to scrape off endosperm without cutting up the bran. Sizing system detaches bran pieces attached to the large middlings and also produce clean middlings while minimizing flour production (Posner and Hibbs, 2005). In the reduction system, the objective is now to reduce those middlings produced in the sizing system to flour in the most economical way while retaining the most desirable baking characteristics. Lastly, the tailing system recovers small pieces of endosperm by reducing their size in relation to the bran and germ particles.

There are four principal forces used in grinding machines; however, some use one or combination of two or more forces depending on the type of mill used. These forces are compression, shear, friction or abrasion, and impact.

Roller mill is the principal grinding machine in a commercial wheat flour mill, as it has range of grinding action and economy of operation (Posner and Hibbs, 2005). Grinding action of

the roller mill is achieved by two rolls rotating in an opposite direction (the ratio is known as roll differentials), as it subjects the particles to shear and compressive forces (Figure 1.7).

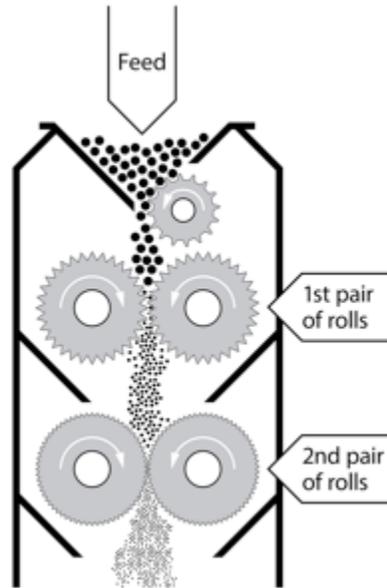


Figure 1.7. A Typical Roller Mill Illustration

(Adapted from

[http://cdn2.hubspot.net/hub/216938/hubfs/images/Typical\\_roller\\_mill\\_illustration\\_used\\_in\\_process\\_equipment\\_marketing.png?t=1458765636392&width=234&height=324](http://cdn2.hubspot.net/hub/216938/hubfs/images/Typical_roller_mill_illustration_used_in_process_equipment_marketing.png?t=1458765636392&width=234&height=324))

This is caused by corrugations on the roll surfaces and as well as the pressure that is exerted by the rolls while pulling particles toward the nip (Haque, 1991). The rate and uniformity of flow of stock to rolls, the roll velocities, the ratio of speed of the fast rolls (roll differential), the gap between the rolls, the type and condition of the roll surfaces, and the properties of particles all affect the magnitude of the stresses imposed on the particles during roller milling (Posner and Hibbs, 2005).

Experimental milling is one of the most significant tests performed in a laboratory (Posner and Hibbs 2005). The objective of experimental milling is to perform flour milling in a practical way with a small wheat sample in order to provide technical information about the raw material and the functionality of the end product. The difference between experimental mill and

laboratory mill is that the experimental mill allows the determination of the wheat milling quality (Posner and Hibbs, 2005). For example, in the experimental milling, the miller is able to change roll characteristics, gap differential, or action to determine the best grinding characteristics for particular wheat. On the other hand, the laboratory mill produces a flour that only adequate for analytical, rheological and baking tests or other end-use evaluation. The flour obtained from various small-scale laboratory mills can differ from the experimental roller mills in the flour quality characteristics. This is because the miller or the operator is able to optimize the milling conditions and settings in experimental milling as to obtain optimal results from the raw material (Posner and Hibbs 2005). Individual lab mills also show differences in the flour extraction rate (Gaines et al., 1997; Baasandorj et al., 2015b).

#### 1.7.2.1. Laboratory and Experimental Roller Mills

Brabender Quadrumat Jr. mill has four grinding rolls and these rolls have a fixed gap between them. This allows the material to pass through three sequential grinding stage (Figure 1.8).

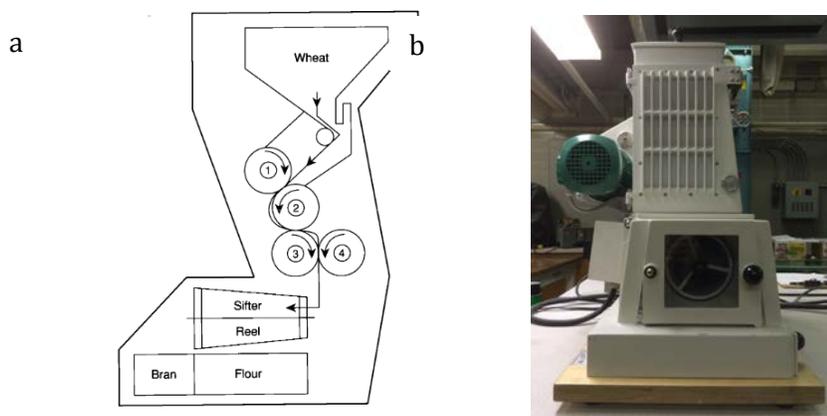


Figure 1.8. A Typical Brabender Quadrumat Jr. Laboratory Flour Mill (a) Mill Flow Diagram (b) Picture Image (Reprinted from AACC International Method 26-50.01)

All mills are corrugated and 70 mm in diameter. As can be seen in Figure 1.8, the grinding is a continuous process thus no sieving is done after each grinding stage. However, after grinding stages are complete, the stocks are dropped into a rotating reel and flour is sifted and the remaining bran is collected separately. Because the roll diameters are smaller, there is small grinding zone, which results in minimal bran disintegration (Posner and Hibbs, 2005). The amount of wheat sample can be milled on this roller mill is 50-500 g.

Brabender Quadrumat Sr. mill is a fully automatic mill based on the four-roll principle. There are two Quadrumat grinding units, the first one is used as the break unit; whereas, the second unit is used for sizing and reduction unit (Figure 1.9).

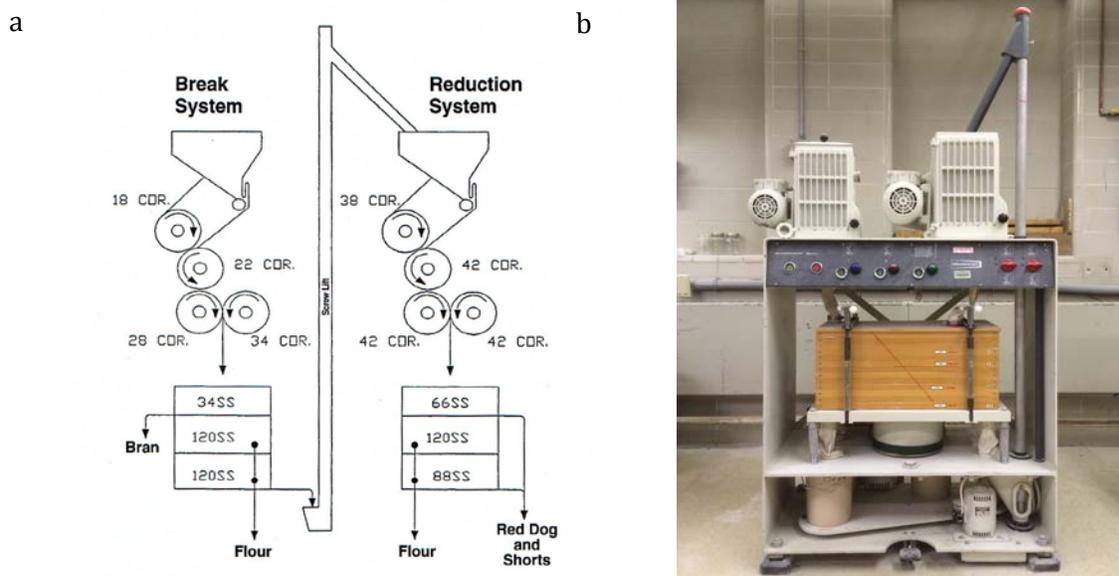


Figure 1.9. A Typical Brabender Quadrumat Sr. Laboratory Flour Mill (a) Mill Flow Diagram (b) Picture Image (Reprinted from Posner, E.S. and Hibbs, A.N. 2005)

The planifier is divided horizontally into two: three sieves for reduction unit and above three sieves are for the break side (Posner and Hibbs, 2005). The material from the break side is sifted and separated. The screw conveyor then elevates the sizing stock from under the sifter to

the reduction unit. As seen in flowsheet of the mill (Figure 1.9a), all rolls are corrugated. The amount of wheat sample that can be milled in this mill is 150-500g.

Buhler mill has six grinding stages with corresponding sifting sections unlike Quadrumat Jr. and Sr. mills. There are three rolls each for break side and reduction side. The break rolls are corrugated whereas reduction rolls are smooth. (Figure 1.10).

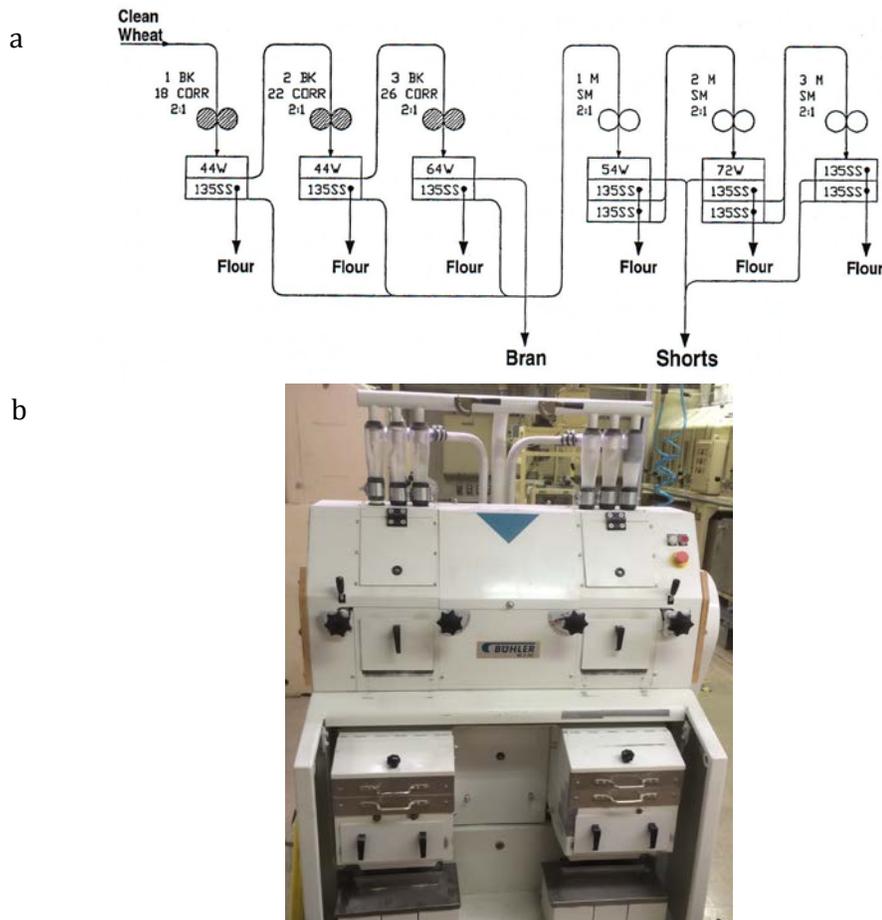


Figure 1.10. A Typical Buhler MLU-202 Laboratory Flour Mill (a) Mill Flow Diagram (b) Picture Image  
(Reprinted from Posner, E.S. and Hibbs, A.N. 2005)

The Buhler mill can be designed to produce semolina; in that case, the three reduction rolls are corrugated. All products are pneumatically conveyed; stocks from roll is sifted and

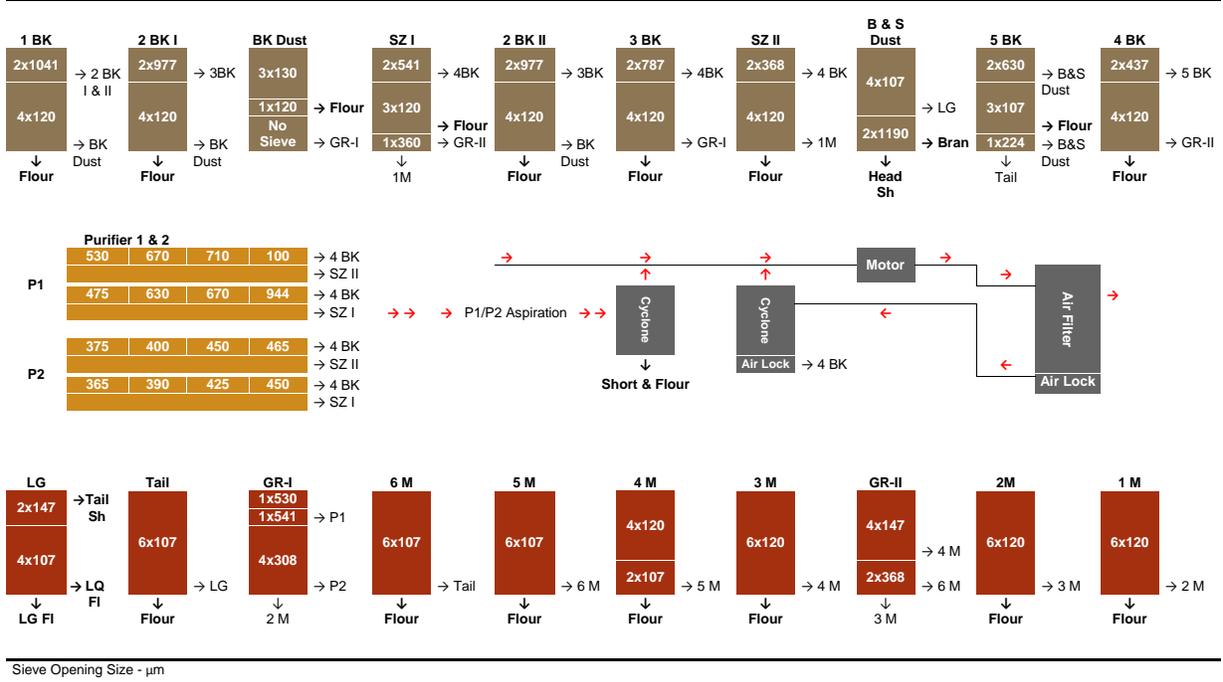
coarse materials gets sent to the subsequent roll. Six flour or semolina streams can be produced on the Buhler mill (Figure 1.10a).

The standard Miag mill has eight roller mill sections each with one pair of diagonally arranged rolls of 250mm diameter and 100mm length (Posner and Hibbs, 2005). The sifter is arranged underneath each roll; five sections on each side thus making total of 10 sections. Flour is collected in drawers from various grinding stages, and the scalps and overs are moved to the subsequent grinding stage by pneumatic conveying system. The feed rate for this mill is 800g/min for soft wheat and 1,500g/min for hard wheat (Posner and Hibbs, 2005). The roll gap adjustments can be made while the mill is operating, unlike the previous laboratory roller mills. The miller can adjust the break releases for the first three breaks in order to reach an optimum flour extraction. However, the break release adjustment varies from one wheat class to another.

a

### Miag Multomat Mill Flow Chart

(Hard Red Spring & Durum Wheat Quality Laboratory, Cereal Crops Research Unit, USDA-ARS-RRVARC, Fargo, ND)



b



Figure 1.11. A MIAG Multomat Flour Mill Located in Harris Hall, NDSU in Fargo, ND (a) Mill Flow Diagram (b) Picture Image (Printed with permission)

### 1.7.2.2. Milling Quality Evaluation Parameters

There are number of milling quality tests that are performed routinely to check the flour being produced from certain mill. Table 1.2 lists the common milling quality parameters routinely checked for milling evaluation.

Table 1.2. Common Milling Quality Parameters and Current Methods

Milling Quality Parameter	Official Method	Method of Reference
Flour Color	Reflectance Colorimeter Method	AACCI Method 14-22.01
Flour Particle Size	Particle Size Distribution	AACCI Method 55-60.01
Starch Damage	Spectrophotometric Method	AACCI Method 76-31.01
Protein Loss	-	-

Flour color is considered as a major quality parameter (Posner and Hibbs, 2005). Flour color is used as a means of milling process control. Operative millers adjust their milling settings as to obtain flour with bright color, as flour with dull color indicates the presence or contamination of bran specks in the final flour. Flour particle size distribution is another milling quality parameter where millers use to adjust and operate the mill. Particle size distribution of flour is determined using mechanical sieving (Posner and Hibbs, 2005). Particle size distribution relates to water absorption capacity, rate of hydration, and mechanical damage during the milling process. Wheat starch granules are mechanically damaged during the milling process, and this is of a great importance to the baker or the other end-user. A certain amount of starch damage is desirable in breadmaking. However, an excess amount of starch damage is inferior to dough handling properties.

### 1.7.3. Flour and Dough Properties of Hard Red Spring Flour

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

breadmaking 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).

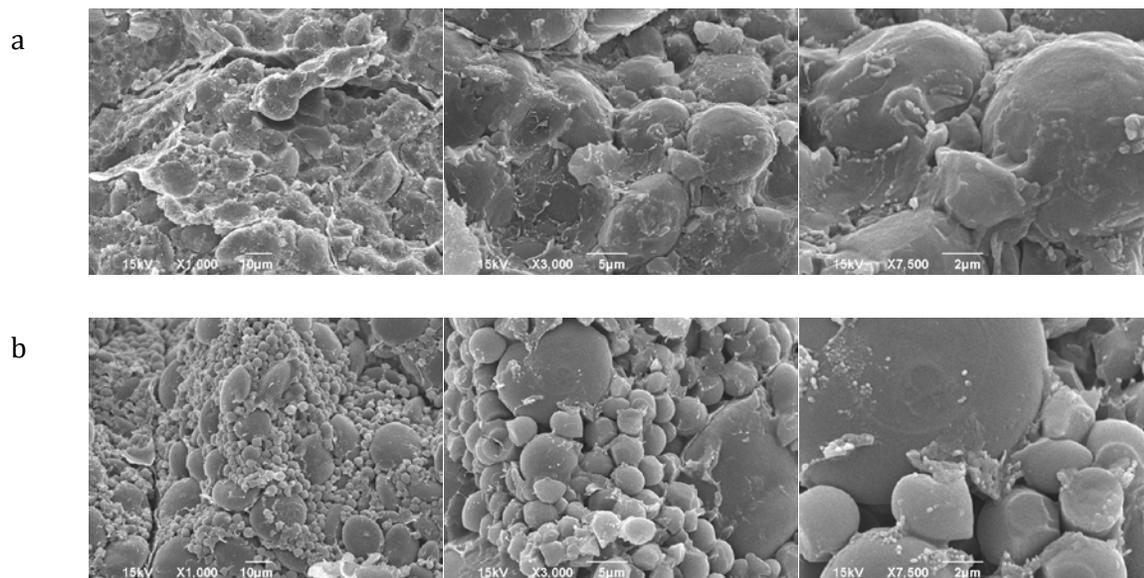


Figure 1.12. Scanning Electron Microscopy (SEM) Images of Cross Cut Sections of Vitreous (a) and Starchy Kernels (b) at Different Magnifications (Adapted from Baasandorj et al., 2016)

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. 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). Baasandorj et al. (2015a) have also found that high gliadin content was associated with vitreous kernel.

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 Hoseneý, 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 Hoseneý, 2010). Another classification system divides prolamins into three groups: sulfur-rich, sulfur-poor, and high molecular weight glutenin subunits (HMW-GS) (Figure 1.13) (Malik, 2009).

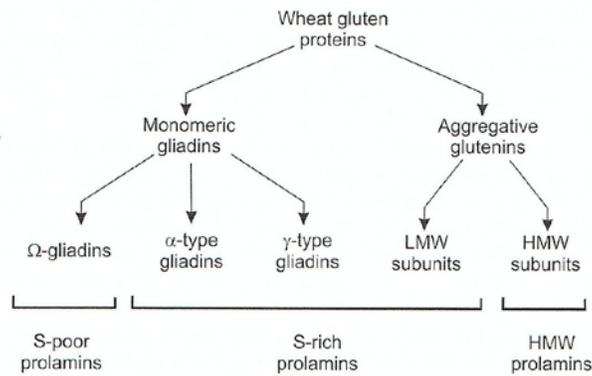


Figure 1.13. Wheat Gluten-Forming Proteins (Reprinted from Khan, K. and Shewry, P.R., 2009)

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 (Figure 1.14).

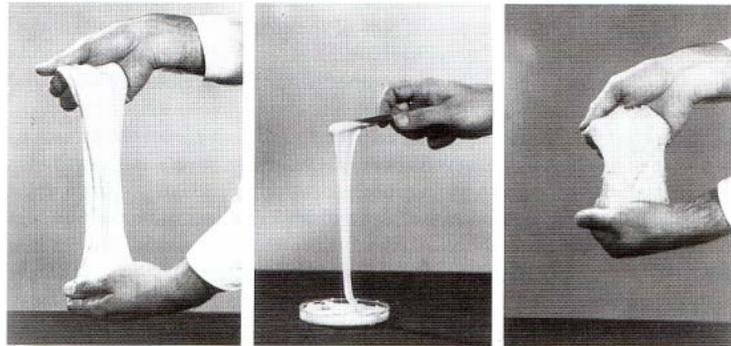


Figure 1.14. Physical Dough Properties of Wheat Gluten (left) and Its Components: Gliadin (center) and Glutenin (right)  
(Reprinted from Delcour, J.A. and Hosney, R.C., 2010)

#### 1.7.3.1. Flour and Dough Quality Evaluation

Flour produced from milling can also be evaluated for quality. Table 1.3 lists the common flour and dough quality parameters and their official methods for quality analysis.

Table 1.3. Common Flour and Dough Quality Parameters and Current Methods

Flour and Dough Quality Parameter	Official Method	Method of Reference
Wet Gluten	The Glutomatic Machine	AACCI Method 38-12.02
Solvent Retention Capacity	Solvent Retention Capacity Profile	AACCI Method 56-11.02
$\alpha$ -Amylase Activity	Rapid Visco Analyzer	AACCI Method 22-08.01
Resistance of Dough to Mixing	Mixograph	AACCI Method 54-40.02
Rheological Behavior of Flour	Farinograph	AACCI Method 54-21.02
Dough Extensibility	Extensigraph	AACCI Method 54-10.01

The wet gluten test provides the amount of gluten in the flour, and it also estimates gluten quality in wheat flour samples (Overview of U.S. Wheat Inspection, 2007). Solvent retention capacity (SRC) test provides the weight of solvent held by flour after centrifuging. There are four solvents used for SRC test: lactic acid SRC is associated with gluten protein characteristics, sodium carbonate SRC is related to the levels of starch damage whereas sucrose SRC is related with pentosan components. Water SRC is influenced by all water absorbing components in flour. The rapid visco analyzer (RVA) test measures flour starch properties, and it can also measure sprout damage, which is indicated by the stirring number. The mixograph test analyzes small amount of samples for dough gluten strength. This test determines the measures flour water absorption and dough mixing characteristics by measuring the resistance of dough against the mixing action of pins (Overview of U.S. Wheat Inspection, 2007). The farinograph is probably the most important and commonly used flour quality test in the world. The farinograph test is similar mixograph test but the flour needed for this test is greater. The farinograph determines the flour water absorption and dough strength by measuring the resistance of dough against the mixing action of blades. The extensigraph test measures the dough extensibility and resistance to extension by measuring the force required to stretch the dough with a hook until it breaks.

#### 1.7.4. Breadmaking Quality Evaluation

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 breadmaking 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 breadmaking quality (Pomeranz and Williams, 1990). Protein content of wheat or flour was much better criterion of breadmaking 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 breadmaking, and this is to optimize hydration and also to provide a source of fermentable sugars in the production of fermented bread products. Baasandorj et al. (2016) have estimated that the optimum flour starch damage for hard wheat flour was found to be 6.6-8.5%. 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 breadmaking.

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. Baasandorj et al. (2015a) have reported that the DHV level was found to have a significant and positive effect on variations in flour protein content and water absorption capacity for breadmaking, which resulted in more dough weight and consequently more bread loaves.

#### 1.7.4.1. Breadmaking Methods

In a wheat quality lab there are various physical and physicochemical testing methods that provide very useful information about how certain flour milled will perform. However, the bake test yields the most reliable index to flour's potential performance in production. Often times, both test formula and mixing time are optimized and balanced if the purpose of bake test is to estimate the loaf volume and crumb grain potential of an unknown flour. Thus, none of added ingredients becomes limiting factor. On the contrary, of the aim of the bake test is to verify uniformity of the flour and to evaluate its suitability for the production requirements of a bakery or to determine the effects of formula changes or efficacy of new ingredient, more standard bake method is required.

The most important quality factors in bake test are bread loaf volume, specific volume, and crumb firmness (Sahli, 2015). The most common method of assessing the product volume is by the rapeseed displacement method (AACCI, 2011). The specific volume is the ratio of bread loaf volume to bread weight, and it is commonly used to assess bread quality (Belz et al., 2012). In addition, bread texture is an important factor for consumer acceptance. Crumb firmness is also an important factor as it is related with the perception of bread freshness (Sahli, 2015).

There are several breadmaking methods used in baking quality evaluation (Table 1.4).

Table 1.4. Various Breadmaking Methods

Breadmaking Methods	Method of Reference
Basic Straight-Dough Method-Long Fermentation Method	AACCI 10-09.01
Optimized Straight Dough Method	AACCI 10-10.03
Sponge-Dough, Pound Loaf Method	AACC 10-11.01
No-Time Method	Baker et al. 1988

Maeda et al. (2004) illustrates different breadmaking methods and their processing steps in Figure 1.15.

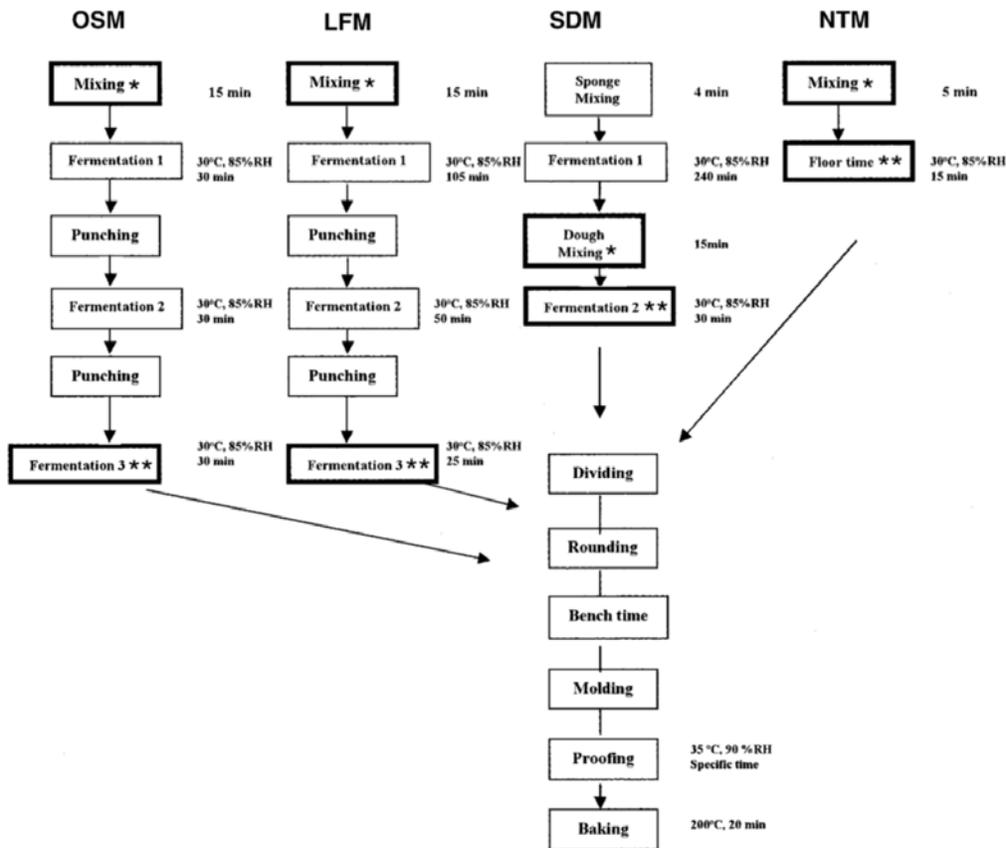


Figure 1.15. Flow Diagram of Various Baking Methods, OSM, Optimized Straight Dough Method; LFM, Long Fermentation Method; SDM, Sponge-Dough Method; NTM, No-time Method (Adapted from Maeda et al., 2004)

Although there are various breadmaking methods for quality evaluation, ‘Basic Straight-Dough’ and ‘Sponge-Dough’ methods are the most important methods for baking quality. In addition to these methods ‘Optimized Straight-Dough’ method is also a common method in terms of bread baking quality. These methods are approved by the American Association of Cereal Chemists International (AACCI) and are commonly used in wheat quality laboratories around the world.

Straight-dough breadmaking method (AACCI Approved Method 10-09.01) provides evaluation of bread wheat flour quality by straight-dough process, which employs long fermentation. ‘Straight-Dough’ method is intended mainly for laboratory assessment of flour quality. In the straight dough method, all the ingredients are mixed in one step. One-hundred grams or 200 g flour is used to make pup loaves; however, larger doughs may be mixed and scaled to desired weight. The total fermentation time is 180 min, with first dough punch after 105 min, second after additional 50 min, molding after additional 25 min.

Sponge-and-dough baking method (AACCI Approved Method 10-11.01) provides a baking test for assessing flour quality by a sponge-dough method. Sponge-dough method is a two-step process. In the sponge dough method, the dough ingredients are mixed in two steps. First, the sponge is made by mixing part of the total flour with water, yeast, and yeast food. Then the sponge is allowed to ferment for 4 hours. In the second step, the sponge is mixed with the rest of the flour, water, and other ingredients to make the dough.

#### 1.8. Protein Quality Evaluation Its Influence on Baking 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).

#### 1.8.1. Size-Exclusion High Performance Liquid Chromatography with Multi Angle Light Scattering

Size-exclusion high performance liquid chromatography (SE-HPLC) have been used extensively to separate wheat flour proteins according to protein molecular weight distribution

(Ohm et al., 2010). Size-exclusion chromatography (SEC) is the separation of mixtures based on the molecular size of the components i.e. protein, carbohydrate etc. This separation is achieved based on the size differential of the components as they pass through the stationary phase with different pore sizes (Size Exclusion Chromatography Principles and Methods, 2014). When the solutes pass through stationary phase mixture components get separation based on their size (Figure 1.16).

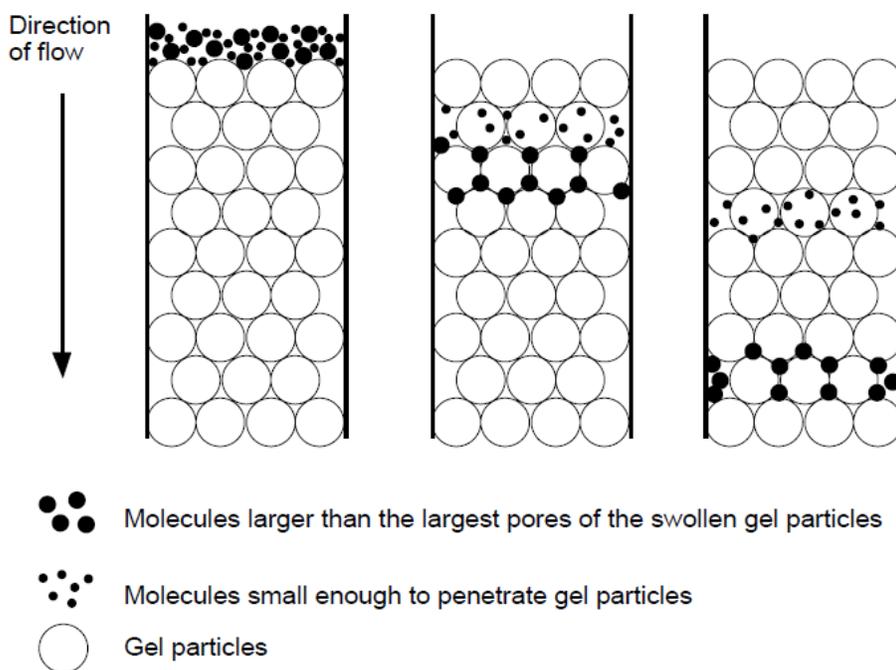


Figure 1.16. A Principle of Size-Exclusion Chromatography  
(Adapted from <https://drgp.institute.wordpress.com/author/dharmendragaur/page/3/>)

In the case of proteins, large molecules are excluded from the pores so they pass through the space in between the gel particles and will elute first. In contrast, smaller proteins can now enter the pores of these beads thus they move slower through the stationary phase and elute later.

Wheat flour proteins contain mixtures of glutenins, gliadins, albumins, and globulins (Mendichi et al., 2008). Glutenins are polymeric proteins in which individual subunits are linked by disulphide bonds, while gliadins are monomeric proteins that consist of single chain

polypeptides that contribute to viscous properties of dough (Field et al., 1983a,b; Eliasson, 1993). Glutenins have been described as “nature’s largest polymers” and they are the main components responsible for differences in end-use quality among different cultivars (Weegels et al., 1996). More specifically, high molecular weight (HMW) glutenin subunits have been widely studied because of the relationship between these proteins and wheat quality characteristics.

Glutenin subunits have been studied due to their relationship with bread baking characteristics; however, more emphasis on the high molecular weight glutenin subunits (HMW-GS) (Mendichi et al, 2008). There is a strong correlation between baking quality and glutenin polymers (Field et al., 1983ab; Gupta et al., 1993). The molecular weight and size of these two glutenin polymers can be determined by SE-HPLC with Multi Angle Light Scattering (MALS) detector, as these glutenin polymers have very broad distribution of molecular and size. The combination of MALS with an SEC system is very powerful and reliable technique for determining the MWD of macromolecules.

The MALS technique has been long used to determine the molecular weight distribution (MWD), size and confirmation of both synthetic and natural polymers (Mendichi and Schieroni, 2001). Bean and Lockhart (2001) investigated the characterization of wheat gluten protein using the MALS in conjunction with SE-HPLC. Mendichi et al., (2008) also concluded in their study that size exclusion chromatography with MALS technique was shown to be a useful distinguishing glutenin polymers coming from different wheat cultivars. However, the authors have added that it was important to choose the appropriate experimental conditions.

In the MALS technique, the amount of light scatter is directly proportional to the product of the molar mass and the molecular concentration (Wyatt, 2012). The variation of scattered light with scattering angle is proportional to the average size of the scattering molecules.

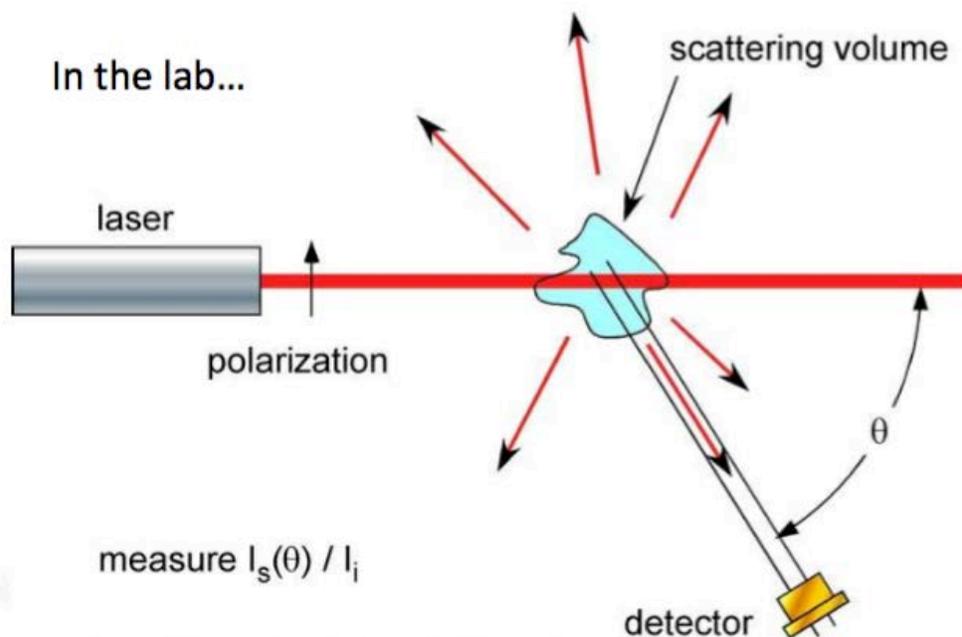


Figure 1.17. The Illustration of Basic Principles of Multi-Angle Light Scattering (MALS) Technique

MALS coupled to SEC can provide absolute molar mass information at every point of the eluting sample (Wyatt Technology, 2012). This allows identification of the protein and its association state and to also detect traces of higher order aggregates. In addition, MALS combined with UV and RI detection is a powerful tool to characterize protein conjugates.

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## **CHAPTER 2. EVALUATION OF HARD RED SPRING WHEAT QUALITY USING FOUR DIFFERENT ROLLER MILL TYPES BASED ON A SCORING SYSTEM**

### **2.1. Abstract**

Hard red spring (HRS) wheat constitutes about 25% of the wheat crop in the United States and is exclusively grown in the Northern Plains states of MN, MT, ND and SD. HRS wheat is known to have high protein content and excellent milling and baking performance. Domestic and overseas buyers pay premium price for HRS wheat because of its high quality and unique characteristics. The objective of this research was to determine if the ranking of HRS wheat cultivars for quality evaluation was affected by mill type. A cultivar scoring system was developed that considered their milling, flour, dough, and bread-baking qualities. This scoring system was designed to rank wheat cultivars for scores between 1 and 10, 1 being “average” and 10 being “most desirable”. A total of twelve HRS wheat samples from 10 cultivars (Forefront, Elgin, Bolles, 817, Ingmar, Glenn, Dapps, Faller, Focus, and Prosper) were milled on Quad. Jr, Quad. Sr, Buhler MLU-202, and MIAG-Multomat roller mills. Mill type and wheat cultivar had significant ( $P < 0.001$ ) effect on the milling, dough, and baking quality scores. Cultivar by mill type interaction did not appear to be so strong as to cause discrepancy in quality evaluation of wheat cultivars since ranking of twelve HRS cultivars was consistent for the overall quality score across different mill types. Based on the overall quality score MN Bolles ND Glenn from G/GL region and ND Glenn from Casselton location had overall quality scores of 6.5 or above when averaged across mill types. This indicated that overall quality for these HRS wheat cultivars would be consistently high when used for different roller mills for quality evaluation. Thus, these cultivars would be considered “good” overall quality wheat cultivars. In contrast, ND Prosper and SD Focus from Casselton location, and SD Forefront from G/GL region were considered

“fair” overall quality wheat cultivars receiving overall quality scores of 6.0 or less. The proposed overall wheat scoring system could assist farmers and breeders in selection of wheat cultivars considering the wheat end-use quality. Development of a comprehensive scoring system will also enable a more detailed scoring system for screening new lines for suitable end-use.

## 2.2. Introduction

Hard Red Spring (HRS) wheat constitutes about 25% of the crop in the United States. HRS wheat is exclusively grown in the Northern Plains, 4-state growing regions (MN, MT, ND and SD) (Carson and Edwards, 2009). However, in recent years HRS wheat also being grown in the Pacific Northwest, specifically in WA, OR, and ID. HRS wheat cultivars are known to have high protein content and excellent milling and baking performance (Carson and Edwards, 2009), hence it is used a “blending wheat” to increase gluten strength and protein content for bread production and Asian noodles in both U.S. domestic and export markets. Flour produced from HRS wheat is generally used in yeast breads, hard rolls and specialty breads, bagels, and pizza crust because of the high protein content. In addition, using hard red spring wheat flours in frozen dough products are better because they can be stored long than those made with low protein wheats.

About 3 dozen of HRS wheat cultivars are grown in the 4-state growing regions; however, less than 10 cultivars make up more than the 50% of acreage (Carson and Edwards, 2009). In 2015, it was reported that top 4 HRS wheat cultivars accounted for 32% of the planted acres in MN, MT, and ND (U.S. HRS Wheat Regional Crop Quality Report, 2015). This means that the farmers had chosen to grow HRS cultivars that would give them higher yield. This does not necessarily mean that the quality of these wheat cultivars was superior. In fact, there is an inverse relationship where wheat quality is often sacrificed by yield during the growing period.

As mentioned HRS wheat is blended to increase gluten strength and protein content for bread production and Asian noodles in both U.S. domestic and export markets. Therefore, the domestic and overseas buyers pay premium price for HRS because of its high quality and unique characteristics. Of the HRS wheat grown in the U.S., 52% was used domestically and 48% was exported to international markets, based on the 5-year averages 2010-2014 (Campbell, 2016). Therefore, the emphasis should be put more on the quality of the HRS wheat. This is because the farmers select HRS wheat cultivars with high growing yield, not necessarily with high wheat quality.

A better overall scoring system is needed for evaluating HRS wheat cultivars; thus, the farmers can alternate high yield cultivars with high quality cultivars. A comparison and ranking of wheat cultivars for their end-use quality characteristics on a score-system will provide a better and accurate evaluation of HRS wheat cultivars. In addition, development of a comprehensive scoring system will enable a more detailed and new potential scoring system for screening new lines for suitable wheat end-use.

The objective of this study was to determine if the ranking of Hard Red Spring wheat cultivars for quality evaluation was affected by mill type. In addition, second objective was to develop an overall scoring system/method for assisting in comparing and ranking of HRS wheat cultivars.

## 2.3. Materials and Methods

### 2.3.1. Wheat Sample

Five bushels of 6 Hard Red Spring wheat cultivar composites (SD Forefront, ND Elgin, MN Bolles, ND 817, SY Ingmar, and ND Glenn) were obtained from Gulf/Great Lake Export Region as part of the 2014 Overseas Varietal Analysis (OVA). Additional five bushels of 6 HRS

wheat cultivars of ND Dapps (2014), ND Elgin (2013), ND Faller (2014), SD Focus (2014), ND Glenn (2012), and ND Prosper (2014) from Casselton location were obtained from the North Dakota State Seed Department, thus making a total of 12 HRS wheat cultivars (Table 2.1).

Table 2.1. Hard Red Spring Wheat Cultivar Composite Ratios (%) from Different Locations in North Dakota

Cultivar	Sample Type	Year	Casselton	Crookston	Watertown
			Blending Ratio (%)		
G-SD Forefront	OVA	2014	33.3	33.3	33.4
G-ND Elgin	OVA	2014	33.3	33.3	33.4
G-MN Bolles	OVA	2014	33.3	33.3	33.4
G-ND 817	OVA	2014	33.3	33.3	33.4
G-SY Ingmar	OVA	2014	50.0	50.0	-
G-ND Glenn	OVA	2014	33.3	33.3	33.4
C-ND Dapps	Experiment Station	2014	100.0	-	-
C-ND Elgin	Experiment Station	2013	100.0	-	-
C-ND Faller	Experiment Station	2014	100.0	-	-
C-SD Focus	Experiment Station	2014	100.0	-	-
C-ND Glenn	Experiment Station	2012	100.0	-	-
C-ND Prosper	Experiment Station	2014	100.0	-	-

### 2.3.2. Kernel Quality Analysis

The wheat was cleaned on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN) to remove shrunken and broken kernels. Test weight and moisture contents (dockage-free portion) were determined with a GAC 2100 tester (Dickey-John, Auburn, IL, USA). Whole wheat ash and protein content were measured by near-infrared spectroscopy with an Infractec 1241 grain analyzer (Perstorp Analytic, Hoganas, Sweden).

The current standard method of evaluating the percentage of vitreous kernels in the United States was used for determination of DHV kernel content. This was done by manually inspecting a 15-g sample, which was free of shrunken and broken kernels (USDA, 1997).

Wheat kernel samples (10g) were weighed and prepared after removal of all dockage, shrunken and broken kernels, and other foreign materials. The number of each sample was

counted with a model 77 totalizer (Seedburo Equipment, Chicago, IL, USA). Number of counted kernels was converted to 1,000 kernel weight and recorded:

$$1,000 \text{ kernel weight (g)} = (1,000/\text{number of kernels}) \times 10 \text{ g}$$

Wheat kernels were sorted for sizing with a shaker in which a set of Tyler standard sieves (number 7 and 9 [2.92 and 2.24 mm]) was used (Arrow testing sieve shaker, Seedburo Equipment, Chicago, IL, USA). Wheat (100g) was sized on the shaker for 200 s.

Approximately 300 kernels of wheat were prepared for kernel hardness. Samples were poured into the access hopper of the SKCE 4100 device (Perten, Huddinge, Sweden) and analyzed according to AACC International Approved Method 55-31.01. Parameters such as kernel weight (mg), kernel diameter (mm), moisture content (%), and kernel hardness index value were determined. Two hundred grams of wheat samples was sent to the North Dakota Grain Inspection for full-grade grain characteristics.

The ground wheat flour falling number was determined using a Falling Number (Perten Instruments, Springfield, IL, USA) according to AACCI Approved Method 56-81.03.

### 2.3.3. Flour Milling

Wheat samples were tempered to 16% moisture for 18 h before milling. All 12 wheat samples were milled in four different laboratory mills: Brabender Quadrumat Jr. and Quadrumat Sr. (Brabender Instruments, Hackensack, NJ, USA), Buhler MLU-202 (Buhler Industries, Uzwil, Switzerland), and MIAG-Multomat (Miag, Braunschweig, Germany).

A total of 4 kg of wheat samples were milled on Brabender Quadrumat Jr. according to AACCI Approved Method 26-50.01 and Quadrumat Sr., and Buhler MLU-202 according to AACCI Approved Method 26-21.02. Two hundred gram lots at a time were milled for Quadrumat Jr. and Sr. mills due to the sieving capacity. Approximately 50 kg of wheat samples

were milled on MIAG Multomat; the feed rate of wheat to the mill was set at 1360 g/min. The break releases for the first, second, and third breaks were set at 30%, 53%, and 65%, respectively. Flour extractions were determined as the percentage of straight-grade flour produced. Flours obtained from MIAG mill were then rebolted through an 84 SS sieve on an Allis-Chalmers rebolter Ser. No. 204 (Allis-Chalmers MFG., Milwaukee, WI, USA) to remove any foreign material. Flour was then blended on a Cross-Flow Blender Serial No. L6-0280 (Patterson-Kelly Co., East Stroudsburg, PA, USA) for 30 minutes.

#### 2.3.4. Flour and Dough Quality Analysis

Moisture content of each sample was determined with air-oven drying at 135°C according to AACCI Approved Method 44-19.01. Ash content of each flour sample was determined according to AACCI Approved Method 08-01.01. Flour (3g) was weighed and placed in an ash crucible. Flour ash contents of each sample were expressed as a percentage of the initial sample weight. Starch damage in the flour was determined with a Megazyme starch damage assay procedure according to AACCI Approved Method 76-31.01. Flour protein content was determined according to AACCI Approved Method 46-30.01 with a LECO FP 528 nitrogen/protein analyzer (LECO, St. Joseph, MI, USA). Protein loss was determined by subtracting flour protein from whole-wheat protein and this was done for all four roller mill types.

Flour particle size was determined using a RoTap (Seedburo Equipment Co., Chicago, IL, USA) shaker according to AACCI Method 55-60.01. Flour (100g) was weighed and sifted on the sieves with screen openings of 250µm, 180µm, 150µm, 125µm, 75µm, and 45µm for 5 minutes. Flour fractions retained on each sieve was weighed and expressed as percentage of flour in each particle size range.

Flour color scores were determined by light reflectance according to AACCI Approved Method 14-22.01 with a Minolta color difference meter (CR 310, Minolta Camera, Osaka, Japan). The flour falling number was determined using a Falling Number (Perten Instruments, Springfield, IL, USA) according to AACCI Approved Method 56-81.03. The wet gluten content and gluten index were determined with a Glutomatic 2200 S system (Perten Instruments, Springfield, IL, USA) according to AACCI Approved Method 38-12.02.

The water absorption and dough strength were measured with a farinograph (C. W. Brabender Instruments, Hackensack, NJ, USA) according to AACCI Approved Method 54-21.02, applying the constant flour weight method. The dough extensibility was measured using an Extensigraph (C.W. Brabender Instruments Inc., Hackensack, NJ) according to AACCI Approved Method 54-10.01.

#### 2.3.5. Breadmaking

Flour samples (100g) were baked according to AACCI Approved Method 10-09.01 with the following modifications; fungal  $\alpha$ -amylase (15 SKB) instead of dry malt powder, instant yeast (1.0%) instead of compressed yeast and the addition of 10 ppm ammonium phosphate. After baking, bread loaf volume was measured according to AACCI Approved Method 10-05.01. 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 symmetry.

#### 2.3.6. Bread Firmness

The texture analysis of bread loaves was done one day after baking. Breads were sliced crosswise using an electric bread slice. A texture analyzer (Texture Technologies Corp.,

Scarsdale, NY) was used to determine the bread firmness according to AACCI Approved Method 74-09.01.

### 2.3.7. Quality Scoring for HRS Wheat Cultivars on Their End-Use Quality Characteristics

The overall quality score for ranking these 12 HRS cultivars (that were milled on four laboratory mills) consisted of (1) wheat quality, (2) milling quality, (3) flour and dough quality, and (4) baking quality scores in which the weights/percentages were given to each of these quality characteristics. Points were awarded for each trait by subdividing these categories into various quality tests for evaluating these traits.

Each of these 4 quality scores further consisted of various quality tests in which weights were again given to calculate individual quality score. Within each quality test, scores between 1 and 10 were assigned for each quality test to calculate the overall score, with ten being the best and one being the worst (Table 2.2-2.5).

Table 2.2. Wheat Quality Score Consisting of Various Quality Tests with Weights Assigned

Score	Test Weight lbs./bu.	Vitreous Kernel %	1000-KWT g	Whole - Wheat Protein % (12% m.b.)	Whole-Wheat Ash % (12% m.b.)	Falling Number sec.
1	<52	<10	<22	<11	>2.8	<109
2	52-53.9	20-29	22-23.9	11-11.9	2.6-2.79	110-159
3	54-55.9	20-29	24-25.9	12-12.9	2.4-2.59	160-209
4	56-57.9	30-39	26-27.9	13-13.9	2.2-2.39	210-259
5	58-59.9	40-49	28-29.9	14-14.9	2.0-2.19	260-309
6	60-61.9	50-59	30-31.9	15-15.9	1.8-1.99	310-359
7	62-63.9	60-69	32-33.9	16-16.9	1.6-1.79	360-409
8	64-65.9	70-79	34-35.9	17-17.9	1.4-1.59	410-459
9	66-67.9	80-89	36-37.9	18-18.9	1.2-1.39	460-509
10	>68	90-100	>38	>19	<1.2	>510
Weight (%)	30	10	10	20	10	20

Table 2.3. Milling Quality Score Consisting of Various Tests with Weights Assigned

Score	Flour	Flour Ash	Starch	Protein Loss
	Extraction		Damage	
	(%)	(%)	% (12% m.b.)	% (12% m.b.)
1	<44	>0.76	3.9-4.5	>1.5
2	44-47.9	0.72-0.75	4.6-5.2	1.40-1.49
3	48-51.9	0.68-0.71	5.3-5.9	1.30-1.39
4	52-55.9	0.64-0.67	6.0-6.6	1.20-1.29
5	56-59.9	0.60-0.63	6.7-7.3	1.10-1.19
6	60-63.9	0.56-0.59	7.4-8.0	1.00-1.09
7	64-67.9	0.52-0.55	8.1-8.7	0.90-0.99
8	68-71.9	0.48-0.51	8.8-9.4	0.80-0.89
9	72-75.9	0.44-0.47	9.5-10.1	0.70-0.79
10	>76	<0.44	10.2-10.8	0.70<
Weight (%)	30	30	20	20

Table 2.4. Flour and Dough Quality Score Consisting of Various Quality Tests with Weights Assigned

Score			Farinograph			Extensograph		
	Wet Gluten	Falling Number	Water Abs.	Peak Time	Stability	Ext. (135 min)	Res. (135 min)	Area (135 min)
	(%)	(sec)	(%)	(min)	(min)	SQ Cm	B.U.	SQ Cm
1	<27	<120	<55	<1.9	<4.9	>23	>580	<95
2	27-28.9	120-179	55-56.9	2-3.9	5-9.9	21-22.9	580-659	95-109
3	29-30.9	180-239	57-58.9	4-5.9	10-14.9	19-20.9	660-739	110-124
4	31-32.9	240-299	59-60.9	6-7.9	15-19.9	17-18.9	740-819	125-139
5	33-34.9	300-359	61-62.9	8-9.9	20-24.9	15-16.9	820-899	140-154
6	35-35.9	360-419	63-64.9	10-11.9	25-29.9	13-14.9	900-979	155-169
7	37-38.9	420-479	65-66.9	12-13.9	30-34.9	11-12.9	980-1059	170-184
8	39-40.9	480-539	67-68.9	14-15.9	35-39.9	9-10.9	1060-1139	185-199
9	41-42.9	540-599	69-70.9	16-17.9	40-44.9	7-8.9	1140-1219	200-214
10	>43	>600	>71	>18.0	>45	<7.0	>1220	>215
Weight (%)	10	15	20	10	30	5	5	5

Table 2.5. Baking Quality Score Consisting of Various Quality Tests with Weights Assigned

Score	Baking Absorption (%)	Dough Handling <sup>1</sup>	Loaf Volume <sup>2</sup> (cc)	Grain Texture	Crumb Color	Crust Color	Symmetry
1	<60	1	<600	1	1	1	1
2	60-61.9	2	670-740	2	2	2	2
3	62-63.9	3	740-810	3	3	3	3
4	64-65.9	4	810-890	4	4	4	4
5	66-67.9	5	880-950	5	5	5	5
6	68-69.9	6	950-1020	6	6	6	6
7	70-71.9	7	1020-1090	7	7	7	7
8	72-73.9	8	1090-1160	8	8	8	8
9	74-75.9	9	1160-1230	9	9	9	9
10	>76	10	>1230	10	10	10	10
Weight (%)	20	10	30	10	10	10	10

<sup>1</sup>Dough handling, grain texture, crumb color, crust color, and symmetry scoring ranges were based on the Guidelines for Scoring Experimental Bread, AACCI Approved Method 10-12.01.

<sup>2</sup>Bread loaf volume scores were based on 100g pup loaves.

Upon getting an overall score from these four quality traits (wheat, milling, dough, and baking quality), a final score was calculated by giving weights on these four quality scores. The weights were assigned for these quality traits, and emphasis was placed on dough and baking quality, as these are the most influential basis used to determine the overall quality (Figure 2.1).



Figure 2.1. Overall Quality Scoring System Consisting of Wheat, Milling, Flour and Dough, and Baking Quality Scores

Thus, 12 HRS wheat cultivars were compared and ranked for their end-use quality characteristics based on the final overall quality score.

## 2.4. Statistical Analysis

The experimental design 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 was used to declare differences between treatments. The experimental design was two-factorial layout with mill type and wheat cultivars as main factors. Mill type and wheat cultivars interaction term was used as error term.

## 2.5. Results and Discussion

### 2.5.1. Roller Mills in Quality Evaluation of HRS Wheat Flours

Four roller mills were used in the quality evaluation of HRS wheat cultivars. There were differences observed in the milling quality for these roller mills. Table 2.6 shows the milling quality evaluation of these roller mills. Flour yield increased as the size of the mill increased. The difference in the flour yield could be due to the milling process associated with each roller mill type. The number of grinding stages and sieving process associated with each mill may explain the difference in the flour yield. Quad. Jr. mill had the lowest flour yield owing to the only four grinding rolls with no sieving stage. In contrast, Buhler mill had significant and highest flour yield, as there are 6 grinding rolls with sieving process followed after each grinding stage. In other words, more grinding and sieving stage results in better separation of bran and germ from the endosperm, thus resulting in greater flour yield. Baasandorj et al. (2015) have also reported very low flour yield for Quad. Jr. mill but higher values for Quad. Sr. and Buhler mills.

Table 2. 6. Milling Quality Evaluation of Roller Mills

Mill Type	Flour Yield	Flour Ash	Flour Protein	Protein Loss	Starch Damage	Color		
	(%)	(%)	(%)	% (12% m.b)	(%)	L	a	b
Quad. Jr	53.3d	0.47c	12.6a	0.96b	6.5b	90.1c	-0.91a	9.0a
Quad. Sr	65.5c	0.40d	12.3b	1.27a	5.4c	90.7a	-1.04c	9.7a
Buhler	76.2a	0.58a	12.6a	0.98b	8.7a	90.4b	-0.88a	9.1b
MIAG	74.1b	0.52b	12.5a	1.04b	8.0a	90.4b	-0.97b	8.6c

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types.

When averaged across for 12 HRS wheat cultivars, Quad. Sr. mill had significantly ( $P<0.05$ ) lower flour ash content compared with the other mills. Ash content indicates the purity of wheat flour, and lower ash content often indicates a good separation of bran and germ from the endosperm. Comparing the two Quad. mills it was observed that Quad. Sr. mill had had lower ash content, which could be due to a number of sieving processes employed in the milling process. Thus, separating more bran and aluerone layers from the endosperm. As mentioned the Buhler mill had higher flour ash content compared with other mills and this could be due to a high flour extraction. Generally, ash content increases with flour extraction, which indicates that the more bran and germ is present in the flour. The flour millers often face this situation where the flour ash content could be compromised by the higher flour yield.

Protein loss is simply the difference between wheat protein and the straight-grade flour, and it is typically 1% (Posner, 2009). Protein loss of 1% was observed for all mills except Quad. Sr. It was observed that Quad. Sr. mill had significantly higher protein loss compared with other mills. High protein loss in Quad. Sr. mill could explain the low ash content. As more protein-rich bran and aleurone layer was removed during the milling process, thus resulting in greater protein loss. Flour starch damage was significantly ( $P<0.05$ ) different between mill types; however, there was no difference between Buhler and MIAG mills (Table 2.6).

When averaged across wheat cultivars, Quad. Sr. had the lowest starch damage of 5.4 followed by Quad. Jr mill. Both Buhler and MIAG mills had significantly higher starch damage compared to the Quad. mills. Baasandorj et al. (2015) also reported similar flour starch damage values for Quad. Jr., Sr., and Buhler mills. Factors such as high roll differentials, high roll temperature, and finer apertures on sieves increase starch damage (Posner, 2009). In the Buhler and MIAG mills, there are more grinding and reductions rolls at high roll differential, which could damage more starch in the flour. In contrast, in the smaller mills Quad. Jr. and Sr., rolls are corrugated and are rotating at relatively low differential, thus could account for lower starch damage. Therefore, these roller mills show differences in the milling quality evaluation for HRS wheat flours.

Flour water absorption is one of the most important parameters because it affects the rheological quality of the dough and final product (Matsuki et al., 2015). Hydration properties of wheat flour play an important role in the formation of homogenous flour dough (Guitierrez et al., 2003). Therefore, an optimum level of water is needed to hydrate flour components and to develop the gluten during the mixing stage. Insufficient level of water results in flour particles to not stick together while too much water causes handling problems during mixing and proofing stages (Hatcher et al., 1999). In order to obtain uniform hydration of flour dough at the optimum level water absorption, the flour particle size plays an important role.

Flour and dough quality parameters were evaluated for roller mills (Table 2.7).

Table 2.7. Flour and Dough Quality Evaluation of Roller Mills\*

Mill Type	Wet Gluten (%)	Falling Number (sec.)	Farinograph				Extensigraph	
			Water Abs. (%)	Peak Time (min.)	Stability (min.)	Ext. (135min) (cm.)	Res. (135 min) (EU)	Area (135min) (cm <sup>2</sup> )
			Quad. Jr	33.2b	441a	62.8b	13.9a	20.8a
Quad. Sr	32.4c	427b	61.2c	11.1b	19.1a	14.3ab	1083a	200.7a
Buhler	33.1b	422c	63.4ab	6.8c	10.1b	14.8a	834b	160.0c
MIAG	33.7a	398b	63.8a	7.3c	13.1b	14.7a	858b	162.1c

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types.

When averaged across wheat cultivars, it was observed that the mill type influenced farinograph parameters. Flour water absorption was found to be high for both Buhler and MIAG mills, while it was lower in Quad. Jr. and Sr. mills. In contrast, farinograph peak time and stability were found to be higher for Quad. Jr. and Sr. mills when compared to Buhler and MIAG mills. This difference in the farinograph parameters could be due to the flour particle size, which is a result of the milling process in each roller mill. One of the key differences in wheat flours produced by different milling techniques is the different particle size obtained (Maldonado and Rose, 2013). In addition to particle size, protein molecular weight distribution (MWD) could also affect the differences in the farinograph parameters, especially peak time and stability. Higher protein MWD in flours from Quad. Jr. and Sr. mills could explain the higher farinograph peak time and longer stability. Baasandorj et al. (2015) also reported that both SDS-extractable and – unextractable high molecular weight (HMW) polymeric proteins were found to be significantly higher for Quad. Jr mill compared with Buhler mill.

Figure 2.2 illustrates the flour particle size distribution for different roller mills.

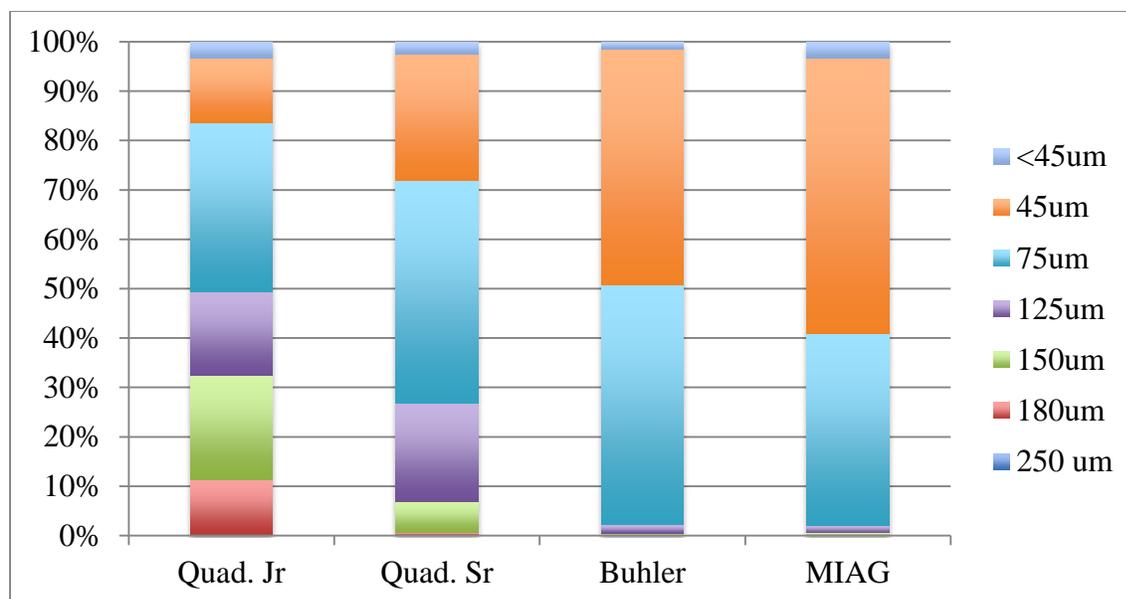


Figure 2.2. Flour Particle Size Distribution for Roller Mills at Various Sieve Openings

As expected, the flour particle size distribution varied among mill types owing to the milling process associated in each mill. It was observed that flours produced from Quad. Jr. and Sr. mill had non-uniform and much coarser particle size, while Buhler and MIAG mills produced very uniform and fine flours (Figure 2.2). This indicates that coarser flour produced from Quad. Jr. and Sr. mills resulted in slow rate of water hydration and also took longer time to develop the dough, which is indicated by the faringograph peak time. In contrast, flours produced from Buhler and MIAG mills resulted in significantly higher ( $P < 0.05$ ) water absorption, indicating that the fine flour particles resulted in faster water uptake and ultimately took shorter time to develop the dough. Flour starch damage could also be responsible for high water absorption, as damaged starch granules exhibit a higher degree of water absorption than the undamaged granules (Carson and Edwards, 2009). Therefore, a combination of high starch damage and fine flour particle size produced in Buhler and MIAG mills explain the higher water absorption.

The viscoelastic property of wheat flour dough is important for the breadmaking 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). The balance between viscous and elastic characteristic of dough is very important, as excess in one results in sticky or “bucky” dough that is difficult to handle during mixing. Dough rheology properties were also determined for four roller mills by the extensigraph (Table 2.7). Flour produced from Quad. Jr. and Sr. mills resulted in dough having significantly ( $P<0.05$ ) higher resistance to extension parameters of 1010 and 1083, respectively. In contrast, resistance to extension was lower for Buhler and MIAG mills. These results indicated that, in terms of dough quality parameters, flours produced from Quad. Jr. and Sr. mills have better dough rheological properties when compared to Buhler and MIAG mills.

Breadmaking quality was evaluated for roller mills (Table 2.8). Flours obtained from Quad Jr. and Sr. mills had significantly ( $P<0.05$ ) lower baking absorption values while Buhler and MIAG mills had higher baking absorption. Baking absorption showed very high and significant ( $P<0.01$ ) correlations with farinograph water absorption for all mill types (data not shown). Flour absorption during baking is related to the flour particle size as well as damaged starch from mechanical means (Posner and Hibbs, 2005). This difference in the baking absorption among mill types could be due to the flour particle size distribution and starch damage, as fine particle size flour and high starch damage resulting in higher baking absorption for Buhler and MIAG mills.

Table 2.8. Breadmaking Quality Evaluation for Roller Mills\*

Mill Type	Bake		Dough Handling	Loaf Volume (cc)	Symmetry	Crust Color	Crumb Grain	Crumb Color	Firmness (g cm)
	Baking Abs. (%)	Mix Time (min.)							
Quad. Jr	62.0c	4.4ab	9.6a	969b	8.2a	9.3a	7.5a	7.3b	117a
Quad. Sr	63.9b	4.5a	9.3ab	968b	8.0a	9.3a	7.5a	7.4b	106b
Buhler	72.0a	4.2b	8.9b	990ab	8.5a	9.2a	7.6a	7.7a	83c
MIAG	71.6a	4.4ab	9.2ab	1000a	8.5a	9.4a	7.8a	7.8a	91c

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types.

Flours from Buhler and MIAG mills produced significantly ( $P < 0.05$ ) larger bread loaves when compared to Quad. Jr. and Sr. Mills (Table 2.8). In addition, bread loaves obtained from Buhler and MIAG mills higher symmetry, crumb grain texture and significantly ( $P < 0.05$ ) higher crumb color scores and lower bread firmness. This indicates that flours with high water absorption resulted in larger bread loaf with good symmetry, desired crumb grain texture and crumb, and softer bread texture. Rogers et al. (1988) have also reported that higher water absorption levels resulted in softer breadcrumb and slower rate of bread firming (Rogers et al., 1988). When evaluating flours obtained from these different mills, flours obtained on Buhler and MIAG mills resulted in bread loaves with desired breadmaking quality characteristics. In contrast, Quad. Jr. and Sr. mill flours resulted in slightly lower bread loaves with low symmetry, crumb grain texture and crumb color when objectively evaluated. Also, these bread loaves were significantly ( $P < 0.05$ ) higher bread firmness. More bran contamination in flours obtained from Quad. Jr. and Quad. Sr. mills could have an impact on dough rheology resulting in lower bread loaf volumes with significantly ( $P < 0.05$ ) lower crumb color scores.

#### 2.5.2. HRS Wheat Quality Evaluation Based on a Scoring System

In the previous section, four different roller mills were compared when used in HRS wheat quality evaluation. Table 2.9 presents the quality scores for individual roller mills.

Table 2.9. The Quality Overall Scoring for Roller Mills\*

Mill Type	Milling Quality Score	Flour Dough Quality Score	Baking Quality Score	Overall Quality Score
Quad. Jr	6.3b	5.7a	6.5b	6.1b
Quad. Sr	6.4b	5.3b	6.6b	6.1b
Buhler	7.3a	4.6c	7.5a	6.4a
MIAG	7.4a	4.8c	7.7a	6.5a

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types.

Quad. Jr. and Sr. mills found to have lower milling quality scores for milling quality evaluation while Buhler and MIAG mills received higher milling quality score. This was expected due to the higher flour yield and flour starch damage; thus, receiving a significantly ( $P < 0.05$ ) milling quality score. The opposite was observed for flour and dough quality scores. Quad. Jr. and Sr. mills received higher flour and dough quality scores owing to the higher farinograph and extensigraph parameters, which resulted in higher flour and dough quality scores. Baking quality score was significantly ( $P < 0.05$ ) higher for Buhler and MIAG, although there was no difference between these mills. This indicates flours milled on these mills generally would produce bread loaves with high loaf volume and desired breadmaking characteristics than Quad. Jr. and Sr. mills. When considering the all the quality scores, the overall quality scores had the same trend for Buhler and MIAG mills. Buhler and MIAG mills had significantly ( $P < 0.05$ ) higher overall quality scores of 6.4 and 6.5, respectively. These scores were higher compared to Quad. Jr. and Sr. mills both receiving overall quality scores of 6.1. This indicates that the overall quality score changes with certain roller mill that is being used for wheat quality evaluation.

While acknowledging the differences between these roller mills for quality evaluation, the objective is now to determine whether ranking of HRS wheat cultivars is affected by the mill type that is used for the evaluation.

In this section, HRS wheat cultivars are compared and ranked for quality evaluation based on a developed overall scoring system. When evaluating these HRS cultivars for quality evaluation, mill type was used as replication. The objective of this section was to determine whether the ranking of Hard Red Spring wheat cultivars for quality evaluation is affected by mill type. Upon evaluating quality characteristics for HRS wheat cultivars, quality scores were assigned as described in the Materials and Methods 2.3.7 section. Table 2.10 shows the milling quality scores for HRS wheat cultivars. Milling quality score was consisted of individual quality parameter scores.

Table 2.10. The Milling Quality Scores for HRS Wheat Cultivars\*

Cultivar	Flour Yield	Flour Ash	Starch Damage	Protein Loss	Milling Quality Score
G-MN Bolles	6.8	8.3	5.3	9.3	7.4
C-ND Glenn	7.0	7.5	4.8	6.8	6.7
G-ND Glenn	7.0	7.5	5.5	5.3	6.5
G-SY Ingmar	7.3	8.0	5.3	5.5	6.7
C-ND Dapps	7.5	8.5	5.8	5.0	7.0
C-ND Faller	7.5	7.8	5.3	5.0	6.6
G-ND Elgin	7.3	8.8	4.0	8.5	7.3
G-ND 817	7.3	9.0	5.3	3.5	6.6
C-ND Elgin	7.5	7.8	5.3	8.0	7.2
C-SD Focus	7.5	7.0	5.0	7.8	6.9
G-Forefront	7.3	9.0	4.0	2.5	6.2
C-ND Prosper	7.5	7.5	5.5	6.8	7.0
LSD**	0.5	1.1	1.4	1.9	0.7

\* Means were calculated across mill types. Means followed by the same letter in the column are not significantly different between mill types.

\*\* Least Significant Different

Wheat cultivar had a significant ( $P < 0.05$ ) effect on the flour yield scores. When averaged across mills, C-ND Dapps, C-ND Faller, C-ND Elgin, C-SD Focus, and C-ND Prosper varieties had high flour yield scores indicating that these cultivars had high flour yield on average of four roller mills. In contrast, G-MN Bolles had the lowest flour yield score. Although wheat cultivars had significant effect, there was very small variation in the flour yield score. All wheat cultivars

except G-MN Bolles received score of 7.0 or above. However, there was large variation in the flour ash for these cultivars. Cultivars G-ND 817 and G-Forefront received high flour ash scores, while C-ND Glenn, G-ND Glenn, and C-ND Prosper cultivars scored the lowest ash scores.

Mill type had a significant ( $P<0.05$ ) effect on the starch damage score; however, there was no significant difference between starch damage for these wheat cultivars. G-ND Elgin and G-SD Forefront cultivars had low starch damage scores, while C-ND Dapps received that highest starch damage score. This is very important as certain amount of starch damage is desirable in breadmaking, and this is to optimize hydration and also to provide a source of fermentable sugars in the production of fermented bread products. Baasandorj et al. (2016) have estimated that the optimum flour starch damage for hard wheat flour was found to be 6.6-8.5%. Therefore, this could be an indication that C-ND Dapps cultivar would have a high score for bread loaf volume. There was a large variation in the protein loss scores for these HRS cultivars. The ANOVA indicated that both mill type and wheat sample had significant ( $P<0.001$ ) effects on the protein loss scores. Thus, large variation in the protein loss scores is a result on both the mill type and wheat sample. The overall milling quality score ranged from 6.2 (G-Forefront) to 7.4 (G-MN Bolles). The milling quality scores of 7 or above were considered “good” milling quality wheat, and these included cultivars G-MN Bolles, C-ND Dapps, G-ND Elgin, C-ND Elgin, and C-ND Prosper. On the contrary, G-Forefront, G-ND Glenn, G-ND 817, and C-ND Faller were considered “fair” milling quality wheat receiving milling quality scores of 6.6 or less.

Flour and dough quality scores were also assigned for HRS wheat cultivars (Table 2.11). It was observed that wheat sample had significant ( $P<0.001$ ) on the wet gluten scores. Wet gluten test indicates the total amount of gluten present in the flour. It was observed that G-MN Bolles, C-ND Glenn, and C-ND Dapps cultivars had high wet gluten scores of 5.8 or above. In

contrast, G-ND Elgin, C-ND Elgin, G-ND Forefront, and C-ND Prosper cultivars had very low wet gluten scores, which indicated that flours for these wheat cultivars had low gluten content. This viscoelastic property of wheat flour dough is important for the breadmaking 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). The low amount of wet gluten, indicated by the low wet gluten, scores in these wheat cultivars could indicate differences in the breadmaking quality.

Table 2.11. Flour and Dough Quality Scores for HRS Wheat Cultivars\*

Cultivar	Farinograph					Extensigraph			Flour and Dough Quality Score
	Wet Gluten	Falling Number	Water Abs.	Peak Time	Stability	Extensibility (135min)	Resistance (135 min)	Area (135min)	
G-MN Bolles	6.0	7.3	5.8	7.8	5.3	5.3	8.8	10.0	6.4
C-ND Glenn	5.8	7.3	5.8	7.8	4.5	6.5	9.8	9.3	6.2
G-ND Glenn	4.5	6.3	6.0	5.5	4.0	6.5	9.3	8.5	5.6
G-SY Ingmar	4.5	6.8	4.8	5.8	5.0	5.3	7.0	8.5	5.5
C-ND Dapps	5.8	5.8	5.5	5.3	4.0	5.5	5.5	6.3	5.1
C-ND Faller	4.8	6.5	6.0	4.8	3.5	6.0	5.0	5.8	5.0
G-ND Elgin	3.8	6.0	5.5	5.3	4.0	5.5	5.0	6.8	5.0
G-ND 817	5.0	6.0	6.3	4.8	3.3	5.8	4.5	5.8	4.9
C-ND Elgin	3.8	6.8	5.8	4.3	3.5	5.5	3.0	4.5	4.7
C-SD Focus	5.0	7.8	4.5	3.5	2.0	5.8	5.5	6.0	4.4
G-Forefront	3.3	6.3	3.8	5.0	3.8	5.8	5.8	7.0	4.6
C-ND Prosper	2.5	6.0	4.8	3.3	2.3	6.3	3.0	3.5	3.7
LSD**	0.5	0.5	0.6	1.4	1.2	0.7	1.7	1.5	0.5

\* Means were calculated across mill types. Means followed by the same letter in the column are not significantly different between mill types.

\*\* Least Significant Different

A similar trend was observed for farinograph stability and extensigraph resistance parameters. Both of these parameters provide information about the dough quality and strength. Hard Red Spring wheat is known for its long farinograph stability and extensigraph resistance to extension parameters. The farinograph stability determines the dough strength by measuring the resistance of dough against the mixing action of blades. Similarly, the extensigraph resistance to extension measures the dough resistance to extension by measuring the force required to stretch the dough with a hook until it breaks. Thus, longer farinograph stability and high resistance to extension (measured by extensigraph) are desirable characteristics of HRS wheat flour.

G-MN Bolles, C-ND Glenn, and G-SY Ingmar cultivars had high farinograph stability, while C-SD Focus, C-ND Prosper, G-ND 817, and C-ND Faller cultivars had low farinograph stability scores. These results indicate that there were differences in the dough strength for these HRS wheat cultivars when averaged across mill types. Similar findings were observed for extensigraph resistance to extension. Cultivars G-MN Bolles, C-ND Glenn, and G-ND Glenn scored very high resistance to extension scores indication that these cultivars had strong gluten strength. In contrast, C-ND Elgin, C-ND Prosper, and G-ND 817 cultivars had very low resistance to extension scores of 4.5 or less (Table 2.11). In terms of flour and dough quality score as a whole, wheat cultivars G-MN Bolles, C-ND Glenn, G-ND Glenn, and G-SY Ingmar received scores of 5.5 or above hence these cultivars were considered “good” flour and dough quality cultivars. In contrast, C-SD Focus, G-SD Forefront, and C-ND Prosper cultivars were considered “fair” flour and dough quality wheat cultivars while receiving scores of 4.5 or less.

Similarly, the baking quality scores were assigned for these HRS cultivars. The ANOVA indicated that wheat sample had a significant ( $P < 0.001$ ) effect on the loaf volume score. It was observed that G-MN Bolles, C-ND Glenn, C-SY Ingmar, and G-ND Dapps cultivars score loaf

volumes scores of 6.5 or above, while C-ND Elgin, C-SD Focus, and C-ND Prosper scored very low bread loaf volume scores (Table 2.12).

Table 2.12. Baking Quality Scores for HRS Wheat Cultivars\*

Cultivar	Baking Abs.	Dough Handling	Loaf Volume	Grain and Texture	Crumb Color	Crust Color	Symmetry	Baking Quality Score
G-MN Bolles	5.8	9.1	7.5	7.6	7.3	9.6	9.1	7.7
C-ND Glenn	5.8	8.6	6.5	7.6	7.7	9.6	8.4	7.3
G-ND Glenn	6.0	9.0	6.3	7.3	7.7	9.6	8.1	7.3
G-SY Ingmar	4.5	9.5	7.3	7.9	7.1	8.8	9.4	7.3
C-ND Dapps	5.0	9.8	6.8	7.6	8.1	9.9	8.8	7.4
C-ND Faller	5.3	9.5	5.8	7.9	8.3	9.6	8.1	7.1
G-ND Elgin	5.3	9.1	6.5	7.6	7.3	9.3	8.9	7.2
G-ND 817	6.0	9.3	6.0	7.2	7.4	9.3	7.9	7.1
C-ND Elgin	5.8	9.3	4.8	7.6	7.1	9.3	8.3	6.7
C-SD Focus	5.0	9.3	5.3	7.7	6.9	8.6	7.8	6.6
G-Forefront	3.3	9.5	6.0	7.6	7.8	8.8	8.1	6.6
C-ND Prosper	5.0	9.1	4.5	7.6	8.1	9.4	6.6	6.4
LSD**	0.8	0.7	0.8	0.6	0.4	0.7	1	0.4

\* Means were calculated across mill types. Means followed by the same letter in the column are not significantly different between mill types.

\*\* Least Significant Different

Baking score consisted of different quality parameters. However, there was small variation in the baking quality score for these HRS wheat cultivars when considering all the baking quality parameters. The baking quality score ranged from 6.4 to 7.7. It was observed that G-MN Bolles had the highest baking quality score of 7.7 making this cultivar “good” baking quality cultivar, while wheat cultivars scoring less than 7.0 were consider “fair” baking quality cultivars. These cultivars were C-ND Elgin, C-SD Focus, G-Forefront, and C-ND Prosper.

Table 2.13 shows the quality scores for 12 HRS wheat cultivars when they are averaged across four roller mill types. This represents a good picture of how these cultivars ranked on different roller mills based on a developed overall scoring system.

Table 2.13. Overall Quality Scores for HRS Wheat Cultivars\*

Cultivar	Wheat Quality Score	Milling Quality Score	Flour and Dough Quality Score	Baking Quality Score	Overall Quality Score
G-MN Bolles	5.8	7.4	6.4	7.7	7.0
C-ND Glenn	7.6	6.7	6.2	7.3	6.9
G-ND Glenn	6.5	6.5	5.6	7.3	6.5
G-SY Ingmar	5.5	6.7	5.5	7.3	6.4
C-ND Dapps	5.7	7.0	5.1	7.4	6.4
C-ND Faller	6.1	6.6	5.0	7.1	6.3
G-ND Elgin	4.9	7.3	5.0	7.2	6.2
G-ND 817	5.9	6.6	4.9	7.1	6.2
C-ND Elgin	6.2	7.2	4.7	6.7	6.1
C-SD Focus	6.8	6.9	4.4	6.6	6.0
G-Forefront	5.1	6.2	4.6	6.6	5.7
C-ND Prosper	5.7	7.0	3.7	6.4	5.6
LSD**	-	0.7	0.5	0.4	0.3

\* Means were calculated across mill types. Means followed by the same letter in the column are not significantly different between mill types.

\*\* Least Significant Different

When considering the ranking of HRS wheat cultivars, the overall quality score was consisted of wheat quality score (15%), milling quality score (15%), and flour and dough quality score (30), and baking quality (40%). These weights were given considering the importance on the end-use quality of HRS wheat. It was observed that ranking of 12 HRS wheat cultivars had the similar trend for flour and dough quality, baking quality, and overall quality scores. This was due to the fact that flour and dough quality, and baking quality scores contributing 70% of the overall quality score.

G-MN Bolles, C-ND Glenn and G-ND Glenn cultivars had overall quality scores of 6.5 or above when averaged across mill types. This indicates that overall quality for these HRS wheat cultivars would be consistently high when used for different roller mills for quality evaluation. Thus, these cultivars would be considered “good” overall quality wheat cultivars. In

contrast, C-SD Focus, G-SD Forefront, and C-ND Prosper cultivars were considered “fair” overall quality wheat cultivars receiving overall quality scores of 6.0 or less.

## 2.6. Conclusion

The current research was carried out to determine whether the overall ranking of Hard Red Spring Wheat cultivars for quality evaluation was affected by mill type. The overall quality scoring system was developed in order to assist in comparing and ranking HRS wheat objectively. Differences in the roller mill types used in the quality evaluation were observed. Quad. Jr. and Sr. mills showed high flour and dough quality scores but low milling, baking, and overall quality scores. In contrast, Buhler and MIAG mills showed high milling, baking, and overall quality scores. Hard Red Spring wheat cultivars were ranked across mill types based on a developed overall scoring system. G-MN Bolles, C-ND Glenn and G-ND Glenn cultivars had overall quality scores of 6.5 or above making these cultivars “good” overall quality cultivars. In contrast, G-MN Bolles, C-ND Glenn and G-ND Glenn cultivars had overall quality scores of 6.5 or above. This indicates that overall quality for these HRS wheat cultivars would be consistently high when used for different roller mills for quality evaluation. Thus, these cultivars would be considered “good” overall quality wheat cultivars. In contrast, C-SD Focus, G-SD Forefront, and C-ND Prosper cultivars were considered “fair” overall quality wheat cultivars receiving overall quality scores of 6.0 or less. Therefore, the overall scoring system was effective in objectively ranking these HRS wheat cultivars. From the results obtained in this study we can conclude that the roller mill type does not affect the overall ranking of HRS wheat cultivars for quality evaluation when using a developed scoring system.

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## **CHAPTER 3. BAKING QUALITY OF HARD RED SPRING WHEAT USING VARIOUS BREADMAKING METHODS AND LOAF SIZES BASED ON A SCORING SYSTEM**

### **3.1. Abstract**

Breadmaking is the ultimate test for Hard Red Spring wheat quality evaluation. Various breadmaking methods, loaf size, and processing conditions are used depending on the objective of the bake test. The current research was carried out to determine whether the overall ranking of Hard Red Spring Wheat cultivars for quality evaluation was affected by breadmaking methods having different fermentation times and loaf sizes. Differences in the straight dough and sponge dough methods were observed in the breadmaking quality evaluation. Straight dough method with 2 hour fermentation resulted in greater bread loaf volume compared to the sponge and dough method for 100g loaf size. However, sponge and dough method was better suited for 1 pound bread loaves resulting in greater loaf volume compared to the straight dough method for same loaf size. In addition, less variability in the bread loaf volume was observed for HRS wheat flours when using the sponge and dough method. In the baking quality evaluation of HRS wheat cultivars, the overall baking quality scores were developed to determine whether the ranking was affected by baking methods. When averaged across various baking methods and conditions, C-ND Elgin, C-SD Focus, C-ND Prosper, G-Forefront, and G-ND 817 cultivars were considered to have “fair” breadmaking quality characteristics, while receiving overall quality scores less than 6. In contrast, cultivars P-ND 817, P-MN Bolles, G-MN Bolles, P-ND Glenn, and G-ND Glenn received overall baking quality scores of 6.5 or above hence these cultivars were considered to have “excellent” baking quality characteristics under different baking conditions. The results in the current research study indicate that although there are differences in the breadmaking methods on the end-use quality evaluation, the ranking of HRS wheat flours (in terms of baking

quality) was not affected by the baking methods and conditions. In other words, cultivars considered to have “fair” quality tended to have low breadmaking quality, while “excellent” baking cultivars would have superior end-use quality regardless of the baking method and processing conditions used.

### 3.2. Introduction

Cereal chemists have been investigating the relationships between different components of wheat endosperm and end-use quality in the past 50 years (Graybosch et al., 1999). Although there are number of flour and dough rheology tests, baking test yields the true evaluation of end-use quality of wheat flour. Breadmaking is the ultimate test for Hard Red Spring wheat quality evaluation. Various breadmaking methods, loaf size, and processing conditions are used depending on the objective of the bake test.

For wheat breeding program, bread loaves are baked based on 25g and 100g flour using the straight-dough method. Straight-dough method is the simplest breadmaking procedure, where all the formula ingredients are mixed into developed dough (Delcour and Hosene, 2010). The U.S. hard wheat breeding programs make early generation selections based on dough rheology test such as mixograph and 25g loaf breadmaking, while 100g loaves are baked using the straight-dough method in later generation selection (Graybosch et al., 1999). In contrast, sponge-and-dough method is widely used in the baking industry. This baking method is the most popular baking process in North America, where two-thirds of the flour, part of the water, and the yeast are mixed (Delcour and Hosene, 2010). This dough is allowed to ferment up to 5h and then combined with the rest of the formula ingredients and mixed into developed dough. Sponge-and-dough baking method is generally considered to have better flavor. In addition, one of the

advantage of this baking method is its tolerance to variations in fermentation and processing time; thus, it's more suitable and preferred baking method in the baking industry.

There have been relatively few comparison studies on breadmaking methods, loaf sizes, and processing conditions. Maeda et al. (2004) evaluated various baking methods for polished whole wheat flours while using four different baking methods: optimized straight dough method, long fermentation method, sponge and dough method, and no time method. However, the dough was made from 300g of flour for each breadmaking method. The authors concluded that straight-dough method with long fermentation was considered suitable for improving the poor dough and baking properties of polished flours (Maeda et al., 2004). Another study was done to compare between the 100g and 25g baking methods for North Dakota Spring wheat using the straight-dough method (Harris and Sanderson, 1938). The authors have concluded that it was not sufficient to accurately predict 100g loaf volume despite the high positive correlation between the two standard methods. A study was done to compare different fermentation times using 100g flour (Finney, 1984).

Although these studies have compared different baking methods and/or fermentation times or loaf sizes, an extensive study combining different breadmaking methods at different fermentation conditions for various bread loaves is needed. This will be very useful to effectively compare various breadmaking methods for wheat quality evaluation. The objective of current research was to determine whether breadmaking methods with different fermentation time and loaf size affect the overall ranking of HRS wheat cultivars in terms of baking quality evaluation. The secondary objective was to develop overall baking quality score to objectively rank these HRS cultivars. Therefore, an overall baking scoring system was developed to assess different baking methods for quality evaluation of these HRS cultivars.

### 3.3. Materials and Methods

#### 3.3.1. Wheat Sample

Five bushels of six Hard Red Spring wheat cultivar composites (WA Glee, ND Elgin, MN Bolles, ND 817, SY Ingmar, and ND Glenn) were obtained from Pacific Northwest (PNW) export region, and another five bushels of 6 HRS wheat cultivar composites (SD Forefront, ND Elgin, MN Bolles, ND 817, SY Ingmar, and ND Glenn) were also obtained from Gulf/Great Lakes (G/GL) export region as part of the 2014 OVA. The composites obtained from 2 export regions are shown in Table 3.1.

Table 3.1. HRS Wheat Cultivar Composite Ratios (%) from Different Locations in North Dakota and Washington for 2 Export Regions\*

Region	Cultivar	Casselton	Crookston	Watertown	Minot	Williston	Washington
PNW	WA Glee	-	-	-	-	-	100
PNW	ND Elgin	20.7	43.7	-	20	15.6	-
PNW	MN Bolles	-	-	-	43.9	56.1	-
PNW	ND 817	-	-	-	64.7	35.3	-
PNW	SY Ingmar	-	-	-	55.5	44.5	-
PNW	ND Glenn	-	-	-	50.0	50.0	-
G/GL	SD Forefront	33.3	33.3	33.4	-	-	-
G/GL	ND Elgin	33.3	33.3	33.4	-	-	-
G/GL	MN Bolles	33.3	33.3	33.4	-	-	-
G/GL	ND 817	33.3	33.3	33.4	-	-	-
G/GL	SY Ingmar	50.0	50.0	-	-	-	-
G/GL	ND Glenn	33.3	33.3	33.4	-	-	-

\* G/GL – Gulf/Great Lakes and PNW – Pacific Northwest

Additional five bushels of 6 HRS wheat cultivars of ND Dapps (2014), ND Elgin (2013), ND Faller (2014), SD Focus (2014), ND Glenn (2012), and ND Prosper (2014) from Casselton location were obtained from the North Dakota State Seed Department, thus making a total of 18 HRS wheat cultivars.

### 3.3.2. Kernel Quality Analysis

The wheat was cleaned on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN) to remove shrunken and broken kernels. Test weights and moisture contents (dockage-free portion) were determined with a GAC 2100 tester (Dickey-John, Auburn, IL, USA). Whole wheat ash and protein content were measured by near-infrared spectroscopy with an Infractec 1241 grain analyzer (Perstorp Analytic, Hoganas, Sweden). Wheat kernel samples (10g) were weighed and prepared after removal of all dockage, shrunken and broken kernels, and other foreign materials. The number of each sample was counted with a model 77 totalizer (Seedburo Equipment, Chicago, IL, USA). Number of counted kernels was converted to 1,000 kernel weight and recorded:

$$1,000 \text{ kernel weight (g)} = (1,000/\text{number of kernels}) \times 10 \text{ g}$$

Wheat kernels were sorted for sizing with a shaker in which a set of Tyler standard sieves (number 7 and 9 [2.92 and 2.24 mm]) was used (Arrow testing sieve shaker, Seedburo Equipment, Chicago, IL, USA). Wheat (100g) was sized on the shaker for 200 s.

Approximately 300 kernels of wheat were prepared for kernel hardness. Samples were poured into the access hopper of the SKCE 4100 device (Perten, Huddinge, Sweden) and analyzed according to AACC International Approved Method 55-31.01. Parameters such as kernel weight (mg), kernel diameter (mm), moisture content (%), and kernel hardness index value were determined. Two hundred grams of wheat samples was sent to the North Dakota Grain Inspection for full-grade grain characteristics.

### 3.3.3. Flour Milling

Wheat samples were tempered to 16% moisture for 18 h before milling. All 18 wheat samples were milled on a MIAG-Multomat laboratory mill (Miag, Braunschweig, Germany).

Approximately 50 kg of wheat samples were milled on MIAG Multomat; the feed rate of wheat to the mill was set at 1360 g/min. The break releases for the first, second, and third breaks were set at 30%, 53%, and 65%, respectively. Flour extractions were determined as the percentage of straight-grade flour produced. Flour was then rebolted through an 84 SS sieve on an Allis-Chalmers rebolter Ser. No. 204 (Allis-Chalmers MFG., Milwaukee, WI, USA) to remove any foreign material. Flour was then blended on a Cross-Flow Blender Serial No. 257063 (Patterson-Kelly Co., East Stroudsburg, PA, USA) for 30 minutes.

### 3.3.4. Baking

Flour samples obtained from the MIAG Multomat mill were baked for 2 different breadmaking methods with various fermentation time and bread loaf sizes (Table 3.2).

Table 3.2. Bread Baking Procedures and Loaf Sizes Used for 18 HRS Flour Samples Obtained from MIAG Multomat Laboratory Mill

Baking Method	Fermentation Time	Loaf Sizes
Straight Dough	2 hour and 3 hour	25 g, 100 g, and 1 lb loaf
Sponge and Dough	4 hour	100g and 1 lb loaf

#### 3.3.4.1. Basic Straight-Dough Method

Flour samples were baked for pup loaf (25 g), 100 g, and 1 lb loaf sizes according to AACCI Approved Method 10-09.01 with the following modifications; fungal  $\alpha$ -amylase (15 SKB) instead of dry malt powder, instant yeast (1.0%) instead of compressed yeast and the addition of 10 ppm ammonium phosphate. After baking, bread loaf volume was measured according to AACCI Approved Method 10-05.01. Both two-hour fermentation with one punch and three-hour fermentation schedule with two punches were 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 symmetry.

#### 3.3.4.2. Sponge-Dough, Pound-Loaf Method

Flour samples were baked according to AACCI Approved Method 10-11.01. This baking method involves a two-step process. In the first step, 60% of the total flour was incorporated at the sponge stage and 40% at the dough stage (second step). Sponge ingredients included 60% of the total flour, 1% instant dry yeast, fungal  $\alpha$ -amylase (15 SKB) and 10 ppm ammonium phosphate. Percentages were based on 300 g of flour at 14% moisture basis. Sponge fermentation time was four hours. Following the sponge stage, the preferment was remixed (D 300 T Mixer, Hobart Corp., Troy, NY, USA) with the remaining ingredients: 40% flour, 40% of the total water, 5% sugar, 2% nonfat dried milk, 2% shortening, 1% salt. The dough was allowed to rest for 30 minutes in a fermentation cabinet (National Mfg. Co., Lincoln, NE) held at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$  at  $86 \pm 5\%$  rh. Dough was molded (experimental moulder, Moline Co., Duluth, MN, USA), and placed in Shogren-type pans. Panned doughs were proofed in the fermentation cabinet for 60 min and baked in a Baxter Rotating Rack Oven Model OV310E (Baxter Manufacturing, Orting WA) for 25 minutes at  $204^{\circ}\text{C}$ . Loaves were cooled for 1 hr, then measured for bread weight and loaf volume according to the AACCI Approved Method 10-05.01. Bread placed in moist environment overnight. Loaves were sliced in a Safety-Slicer Serial No. 78049 (Oliver Machinery Co., Grand Rapids, MI, USA). The bread slicer produced slices of bread 1.25-cm thick from each loaf. 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 symmetry. One-hundred gram and 1 lb. loaf sizes were baked.

#### 3.3.5. Bread Firmness

The texture analysis of bread loaves was done one day after baking. Breads were sliced crosswise using an electric bread slicer. A texture analyzer (Texture Technologies Corp.,

Scarsdale, NY) was used to determine the bread firmness according to AACCI Approved Method 74-09.01.

### 3.3.6. Quality Scoring for HRS Wheat Cultivars on Their Bread Baking Quality Characteristics

The overall bread baking quality score for ranking these 18 HRS cultivars was assigned separately for 3 different loaf sizes: 25g, 100g, and 1 lb. The overall bread baking quality score further consisted of various baking quality tests in which weights were given to calculate individual quality score. Within each quality test, scores between 1 and 10 were assigned for a certain quality test to calculate the overall score, with ten being the best and one being the worst (Tables 3.3-3.5).

Table 3.3. Overall Baking Quality Score Consisting of Various Quality Tests with Weights Assigned for 25g loaf

Score	Mix Time (min)	Baking Absorption (%)	Dough Handling	Oven Spring (cm)	Loaf Volume (cc)	Specific Volume	Crumb Grain	Crumb Color	Crumb Symmetry
1	<1.5	<60	1	<1.1	<120	<5.5	1	1	1
2	1.5-2.0	60-62	2	1.1-1.2	120-130	5.5-6.0	2	2	2
3	2.0-2.5	62-64	3	1.2-1.3	130-140	6.0-6.5	3	3	3
4	2.5-3.0	64-66	4	1.3-1.4	140-150	6.5-7.0	4	4	4
5	3.0-3.5	66-68	5	1.4-1.5	150-160	7.0-7.5	5	5	5
6	3.5-4.0	68-70	6	1.5-1.6	160-170	7.5-8.0	6	6	6
7	4.0-4.5	70-72	7	1.6-1.7	170-180	8.0-8.5	7	7	7
8	4.5-5.0	72-74	8	1.7-1.8	180-190	8.5-9.0	8	8	8
9	5.0-5.5	74-76	9	1.8-1.9	190-200	9.0-9.5	9	9	9
10	>6.0	>76	10	>1.9	>200	>9.5	10	10	10
Weight (%)	5	15	5	10	30	10	10	10	5

Table 3.4. Overall Baking Quality Score Consisting of Various Quality Tests with Weights Assigned for 100g loaf

Score	Mix Time (min)	Baking Absorption (%)	Dough Handling	Oven Spring (cm)	Loaf Volume (cc)	Specific Volume	Crumb Grain	Crumb Color	Symmetry
1	<1.5	<60	1	<1.5	<670	<5.5	1	1	1
2	1.5-2.0	60-62	2	1.5-2.0	670-740	5.5-6.0	2	2	2
3	2.0-2.5	62-64	3	2.0-2.5	740-810	6.0-6.5	3	3	3
4	2.5-3.0	64-66	4	2.5-3.0	810-880	6.5-7.0	4	4	4
5	3.0-3.5	66-68	5	3.0-3.5	880-950	7.0-7.5	5	5	5
6	3.5-4.0	68-70	6	3.5-4.0	950-1020	7.5-8.0	6	6	6
7	4.0-4.5	70-72	7	4.0-4.5	1020-1090	8.0-8.5	7	7	7
8	4.5-5.0	72-74	8	4.5-5.0	1090-1160	8.5-9.0	8	8	8
9	5.0-5.5	74-76	9	5.0-5.5	1160-1230	9.0-9.5	9	9	9
10	>6.0	>76	10	>5.5	>1230	>9.5	10	10	10
Weight (%)	5	15	5	10	30	10	10	10	5

Table 3.5. Overall Baking Quality Score Consisting of Various Quality Tests with Weights Assigned for 1 lb. loaf

Score	Mix Time (min)	Baking Absorption (%)	Dough Handling	Oven Spring (cm)	Loaf Volume (cc)	Specific Volume	Crumb Grain	Crumb Color	Symmetry
1	<1.5	<60	1	<1.5	<2200	<5.5	1	1	1
2	1.5-2.0	60-62	2	1.5-2.0	2200-2300	5.5-6.0	2	2	2
3	2.0-2.5	62-64	3	2.0-2.5	2300-2400	6.0-6.5	3	3	3
4	2.5-3.0	64-66	4	2.5-3.0	2400-2500	6.5-7.0	4	4	4
5	3.0-3.5	66-68	5	3.0-3.5	2500-2600	7.0-7.5	5	5	5
6	3.5-4.0	68-70	6	3.5-4.0	2600-2700	7.5-8.0	6	6	6
7	4.0-4.5	70-72	7	4.0-4.5	2700-2800	8.0-8.5	7	7	7
8	4.5-5.0	72-74	8	4.5-5.0	2800-2900	8.5-9.0	8	8	8
9	5.0-5.5	74-76	9	5.0-5.5	2900-3000	9.0-9.5	9	9	9
10	>6.0	>76	10	>5.5	>3000	>9.5	10	10	10
Weight (%)	5	15	5	10	30	10	10	10	5

### 3.4. Statistical Analysis

The experimental design 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 was used to declare differences between treatments. The experimental design was two-factorial layout with breadmaking methods and wheat cultivars as main factors. Breadmaking methods and wheat cultivars interaction term was used as error term.

### 3.5. Results and Discussion

#### 3.5.1. Comparison of Breadmaking Methods and Processing Conditions for HRS Wheat Cultivars

Wheat flours obtained from the MIAG mill were used for breadmaking experiments. Different breadmaking methods and fermentation time were evaluated for various loaf sizes. Table 3.6 shows the breadmaking characteristics for various baking methods and processing conditions. Breadmaking characteristics were shown to differ among baking method and fermentation time (Table 3.6).

Table 3.6. Breadmaking Characteristics of Various Baking Methods and Loaf Sizes\*

Loaf Size	Baking Method	Fermentation Time	Baking Mix Time	Oven Spring	Loaf Volume	Specific Volume	Crumb Grain	Crumb Color	Firmness
			(min.)	(cm.)	(cc.)				
25g	Straight	2 hour	4.9a	1.4g	217f	7.0c	6.9abc	7.7ab	164b
25g	Straight	3 hour	4.6b	1.6fg	195f	6.7c	7.1ab	7.5c	308a
100g	Straight	2 hour	4.3c	4.5a	1078d	8.2a	7.1a	7.8a	84e
100g	Straight	3 hour	4.3c	4.1b	1003e	7.7b	7.0abc	7.7ab	88de
100g	Sponge	4 hour	2.8e	2.5e	970e	6.9c	6.8c	7.6bc	105cde
Pound	Straight	2 hour	4.0d	3.0d	2489c	5.7e	6.8bc	7.6abc	116cd
Pound	Straight	3 hour	4.3c	3.2c	2550b	5.9de	6.8bc	7.7ab	94cde
Pound	Sponge	4 hour	2.4f	1.7f	2761a	6.1d	6.7c	7.4c	120c

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types.

Baking mix time was shown to vary depending on the baking method. The ANOVA (Table A.14) indicated that both wheat sample and baking method had significant ( $P < 0.001$ ) effects on the mix time. However, it showed that the variation in the baking mix time was due to the baking method, which was shown by the much higher F-value. Baking mix time was significantly ( $P < 0.05$ ) lower for sponge and dough method for different 100g and pound loaves when averaged across wheat samples. The difference in the mix time for these baking method was expected. In the straight dough method, all ingredients are added together and mixed to optimum dough development. In contrast, only part of flour and water, and yeast are mixed but not developed to fully developed dough in the sponge and dough method (Delcour and Hosney, 2010). This dough is fermented for 3-5 hours, and then other ingredients are added and mixed to optimum dough development; thus, the baking mix time is shorter in sponge and dough method. Baking mix time was significantly lower for sponge and dough method for both 100g and pound loaves having mix time of 2.8 and 2.4 min., respectively (Table 3.6).

Oven spring is an important measurement in breadmaking. When the proofed dough is placed in the oven, the dough expands rapidly (Delcour and Hosney, 2010). This phenomenon

is called oven spring. Oven spring is measured by subtracting the differences in the loaf height between end of fermentation and baking. Baking method had significant ( $P < 0.001$ ) effect on the oven spring. Sponge dough method had significantly ( $P < 0.05$ ) lower oven spring compared with straight dough method, and this was consistent across both 100g and pound loaves. Low oven spring is due to the additional mixing in the sponge and dough method, where gas cells produced by the yeast are redistributed and divided into smaller gas cells hence resulting in low dough expansion during baking but with soft bread with fine cell structure (Delcour and Hoseneey, 2010).

The ANOVA (Table A.14) showed that both wheat sample and baking method had significant ( $P < 0.001$ ) effect on the bread loaf volume; however, variation was due to the baking method. When comparing baking methods, although there was no significant ( $P > 0.05$ ) difference, the straight dough method produced smaller loaf compared to sponge and dough method for 100g loaf size (Table 3.6). In contrast, sponge dough method produced significantly ( $P < 0.05$ ) higher bread loaves compared to the straight dough method for pound loaf size. These results indicated that the difference between the baking methods was more apparent for larger loaves than the smaller loaves such as 100g pup loaf. These results are in agreement with Puhr and D'Appolonia (1992), who also reported that sponge and dough method produced larger bread loaves compared to the straight dough method. In addition, smaller bread loaves were obtained as the fermentation time increased for 25g and 100g loaves (Table 3.6), although the difference was not significant. In contrast, bread loaf volumes increased significantly as the fermentation time increased indicating that the difference was greater for larger bread loaves.

During fermentation, gas production and gas retention have an important role when a correctly mixed sponge or dough is fermented (Pylar, 1988). The baker's objective is to control

fermentation where gas production and gas retention are in proper balance. This is important because gas production should reach its maximum rate before retention capacity is fully reached, otherwise too much gas will be lost during fermentation resulting in low dough expansion, which results in smaller bread loaves. On the contrary, if the gas retention reaches to its maximum rate before gas production, then much of the gas is unable to perform its aerating function (Pylar, 1988). However, when both reach to their peaks at the same time, bread with large loaf volume with best grain and texture.

In addition to loaf volume, specific loaf volume is the main quality characteristics of bread (Katina et al., 2006). The loaf specific volume is the ration of bread volume to bread weight, and it is commonly used to assess bread quality (Belz et al., 2012). Specific volume followed the same trend as was seen for bread loaf volume. Lower specific volume was observed with longer fermentation time for both 25g and 100g loaves, while specific volume increased with longer fermentation time for pound loaf breads (Table 3.6). In addition, specific volume was significantly ( $P < 0.05$ ) higher for the straight dough method compared to sponge and dough method. Maede et al. (2004) also reported that the specific volume of polished flour breads was improved when using the straight dough method. However, in the current study, similar results were observed only for the 100g loaf, while higher specific volume was produced for sponge and dough method with pound loaf bread.

Bread texture characteristics such as crumb grain and texture are critical for consumer acceptance (Belz et al., 2012). Sponge and dough method produces bread soft bread with fine cell structure (Delcour and Hosenev, 2010). Longer fermentation time in sponge and dough method allows more time to hydrate and mellow thus resulting in softer crumb (Busken, 2013). Crumb grain evaluation is based on cell size, cell shape, and cell wall thickness (Hayman et al.,

1998). The grain is considered open if it contains intermediate to large cells, while it is considered closed if it has small gas cells. In addition, the cell wall thickness also affects crumb grain. Thin cell walls are predominant in fine crumb grain whereas thick cell walls predominate in a coarse crumb grain (Hayman et al., 1998). Thus, fine crumb grain with small round cells with thin cell walls is considered inferior. Conversely, open grain with large round cells with thick cell walls is also undesirable.

Crumb grain scores were subjectively scored by the baker according to the AACCI official method 10-12.01. The ANOVA indicated that the baking method did not have significant ( $P>0.05$ ), while wheat sample showing significant ( $P<0.001$ ) effect on the crumb grain score. There was no significant ( $P>0.05$ ) difference in the crumb grain score between straight dough and sponge and dough method (Table 3.6). These results are contradicting to what's reported in the previous studies. Crumb firmness is also important bread texture assessment as it is associated with bread freshness (Cauvian and Young, 2007). It was found that baking method had significant ( $P<0.001$ ) effect on the crumb firmness. Sponge dough method resulted in more firm bread compared to the sponge and dough method. More firm bread was obtained for sponge and dough method and this was consistent for both 100g and pound loaves. Fine structure cell structure in sponge and dough method could indicate that bread was denser resulting in firmer crumb structure.

Bartlett's Chi-square test was used to test the homogeneity of the variance. Bartlett's test showed significant differences for baking parameters for different baking methods and processing conditions. Table 3.7 shows the Bartlett's test for baking parameters of different baking methods. Bartlett's test showed significant ( $P<0.0001$ ) difference for the baking mix time. The CV is a relative measure of the variability that's present in the data set. It was observed

that sponge and dough method had lower coefficient of variation (CV) for both 100g and 1 pound loaves (Table 3.7).

Table 3.7. Bartlett's Chi Square Test for Baking Parameters

Baking Parameters		Baking Methods and Fermentation Time								Bartlett's Test	
		25g 2 hr.	25g 3 hr.	100g 2 hr.	100g 3 hr.	100g Sponge	Pound 2 hr.	Pound 3 hr.	Pound Sponge	X <sup>2</sup>	Pr > X <sup>2</sup>
Mix Time	Mean	4.9	4.6	4.3	4.3	2.8	4.0	4.3	2.4	24.7	9E-04
	Std. Dev.	0.4	0.5	0.6	0.6	0.3	0.6	0.7	0.3		
	CV* (%)	8.2	11.7	13.8	14.1	8.9	15.7	15.1	13.2		
Dough Handling	Mean	8.9	8.6	8.9	8.8	8.8	9.0	9.0	8.8	39.1	<.0001
	Std. Dev.	0.4	0.5	0.4	0.5	1.2	0.7	0.7	0.9		
	CV (%)	4.9	5.7	4.2	5.1	13.6	7.9	7.4	10.2		
Oven Spring	Mean	1.4	1.6	4.5	4.1	2.5	3.0	3.2	1.7	15.1	0.034
	Std. Dev.	0.2	0.4	0.5	0.3	0.4	0.5	0.5	0.3		
	CV (%)	17.2	22.9	11.1	7.4	16.2	17.9	14.4	20.3		
Loaf Volume	Mean	217	195	1078	1003	970	2489	2550	2761	103.8	<.0001
	Std. Dev.	18.4	15.4	81.1	93.2	59.8	153.5	125.3	73.3		
	CV (%)	8.5	7.9	7.5	9.3	6.2	6.2	4.9	2.7		
Loaf Volume (Flour)	Mean	8.7	7.8	10.8	10.0	9.7	5.5	5.6	6.1	62.8	<.0001
	Std. Dev.	0.7	0.6	0.8	0.9	0.6	0.3	0.3	0.2		
	CV (%)	8.5	7.9	7.5	9.3	6.2	6.2	4.9	2.7		
Specific Volume	Mean	7.0	6.7	8.2	7.7	6.9	5.7	5.9	6.1	39.6	<.0001
	Std. Dev.	0.6	0.5	0.7	0.7	0.5	0.4	0.3	0.2		
	CV (%)	9.3	7.7	8.3	9.7	6.8	6.4	5.6	3.0		
Firmness	Mean	164	308	84	88	105	116	97	120	142.2	<.0001
	Std. Dev.	46.1	121	16.9	15.9	29.4	29.2	17.6	23.5		
	CV (%)	28.1	39.3	20.0	18.0	27.9	25.3	18.3	19.5		

\*Coefficient of Variation

The low CV values indicate that there was less variability in the mixing for sponge and dough method compared to the straight dough. In the sponge and dough method, only part of the flour, water, and yeast is mixed; however, it is not mixed to optimum dough development. The mixing time is lower for this baking method thus there is less variability in the baking mix time.

Oven spring also showed significant ( $P < 0.05$ ) difference in the Bartlett's test. When comparing baking methods, sponge and dough method had higher CV compared to the straight dough method. However, this does not mean that there was more variation in the oven spring for sponge and dough method. It is because the means were much lower compared to the straight dough method hence resulting in higher CV values. When comparing different fermentation times for the straight dough method, it was observed that lower CV values were found with 3 hour fermentation for both 100g and 1 pound loaves (Table 3.7). This suggests that using straight dough method with 3 hour fermentation is more reproducible, while having less variability in the oven spring. However, low CV value was observed with 2 hour fermentation for 25g loaf. Therefore, these results indicate that 2 hour fermentation may be preferable for smaller loaves, while 3 hour fermentation is suitable for larger loaves (100g and 1 pound) in the straight dough method.

Table 3.7 showed that sponge and dough method had less CV values for bread loaf volume in sponge and dough method compared to the straight and dough method. This suggests that there was less variation in the bread loaf volume when using the sponge and dough method. Hence, sponge and dough method is preferred over straight dough method straight dough method in the industrial production line (Delcour and Hoseney, 2010; Busken, 2013). In contrast, the straight dough method is suited in the wheat breeding programs because this method shows more variability in the bread loaf volume. Thus, it is preferred to use straight dough method to make

early generation selection (Graybosch et al., 1999). Similarly, sponge and dough method had less variation in the bread specific volume having low CV values. This was consistent for both 100g and 1 pound loaves. Again, these results suggest that in terms bread baking quality, the sponge and dough might be preferred over straight dough method having less variation in the baking quality parameters. This is very important in the baking industry because the use of sponge and dough method is more reproducible and consistent between batches.

### 3.5.2. HRS Wheat Breadmaking Quality Evaluation Based on a Scoring System

In the previous section, breadmaking methods were compared for their quality evaluation. Baking quality scoring system was explained in the 3.3.6. Materials and Method section of this chapter. Table 3.8 shows the comparison of various breadmaking methods and processing conditions when evaluated based on the overall baking quality scoring system.

Table 3.8. Baking Quality Scores for Different Baking Methods and Processing Conditions

Fermentation Time	Baking Mix Time	Baking Absorption	Oven Spring	Specific Volume	Loaf Volume	Baking Score
2 hour	8.0a	5.1c	4.2de	6.4c	4.4c	6.2c
3 hour	7.5b	6.5a	5.9bc	5.2de	4.2c	6.2c
2 hour	6.9c	6.6a	7.2a	7.6b	6.8a	7.3a
3 hour	6.8cd	6.4a	6.8ab	6.2c	5.9b	6.7b
Sponge	3.9e	6.3a	3.5e	5.8cd	4.2c	5.9d
2 hour	6.3d	5.6b	4.5de	4.6e	1.8e	5.4e
3 hour	7.1bc	5.7b	4.9cd	5.4d	2.3de	5.8d
Sponge	3.2f	4.7d	1.9f	8.4a	2.8d	6.0cd

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types.

There was significant ( $P < 0.05$ ) difference in the baking mix time for breadmaking methods and loaf sizes. The ANOVA indicated that baking method had significant ( $P < 0.0001$ ) effect on the baking mix time score (Table A.15). It was observed that the straight dough method had longer mix time scores compared to sponge and dough method, and this mix time difference

was consistent across different loaf sizes (Table 3.9). Sponge and dough method had significantly ( $P < 0.05$ ) shorter baking mix scores of 3.9 and 3.2 minutes for both 100g and pound loaves, respectively. This indicates that mix time was shorter for sponge and dough method.

The ANOVA (Table A.15) also indicated that both wheat sample and baking method had significant ( $P < 0.0001$ ) effects on the specific volume and bread loaf volume scores. However, much of the variation was due to breadmaking method, which was indicated by the higher F-value in the ANOVA table. It was observed that sponge and dough method received significantly ( $P < 0.05$ ) low specific volume and bread loaf volume scores for 100g loaf (Table 3.9). In contrast, sponge and dough method had higher specific and bread loaf volume scores for pound loaf. These results indicated that straight dough method would be suited for 100g loaf size, while sponge and dough is preferred method for 1 pound loaf size when evaluating breadmaking quality of HRS wheat samples. In terms of final baking quality scores, straight dough method with 2 hour fermentation had the highest overall baking quality score, which indicates that this processing condition is suited when baking 100g loaf breads. However, when baking breads at 1 pound loaves, it was observed that sponge and dough method received higher score over straight dough method with 2 different fermentation times.

Although there were differences between baking methods and fermentation times for baking quality parameters, the overall all objective of this current research was to determine whether breadmaking methods with different fermentation time and loaf size affect the overall ranking of HRS wheat cultivars. In other words, the objective was to investigate whether the ranking of HRS wheat cultivars for baking quality evaluation was affected by the baking method and processing conditions. To investigate how 18 HRS wheat cultivars were ranked based on their baking quality, the baking quality parameters were averaged across baking methods and

processing conditions. Table 3.9 summarizes the breadmaking quality scores of 18 HRS wheat cultivars.

Table 3.9. The Overall Breadmaking Scores for 18 HRS Wheat Cultivars\*

Cultivar	Mix Time	Baking Absorption	Dough Handling	Oven Spring	Loaf Volume	Specific Volume	Crumb Grain	Crumb Color	Crumb Symmetry
C-ND Elgin	5.5	6.5	8.8	3.0	4.5	3.0	6.6	7.1	6.7
C-SD Focus	5.3	6.3	8.1	4.4	5.0	3.1	6.1	6.7	6.7
C-ND Prosper	5.6	6.1	7.8	5.0	4.8	2.6	6.7	7.9	6.9
G-Forefront	6.0	3.9	8.8	4.9	5.6	3.8	6.7	7.8	7.1
G-ND 817	5.6	6.8	8.9	4.3	5.8	3.4	6.3	7.5	6.8
P-ND Elgin	5.5	5.5	8.4	4.6	6.3	4.0	6.4	7.4	7.3
P-WA Glee	5.3	6.0	9.0	5.0	5.9	3.5	6.8	7.8	7.1
P-SY Ingmar	6.3	4.3	8.9	5.3	6.5	4.9	7.2	7.4	7.2
G-SY Ingmar	6.1	4.5	8.9	4.0	7.0	4.5	7.3	7.4	7.3
C-ND Dapps	6.0	5.9	8.9	5.6	6.0	4.3	6.8	7.8	7.0
C-ND Faller	7.8	6.6	9.1	4.5	5.6	3.5	7.8	8.0	7.4
C-ND Glenn	5.6	6.4	8.9	5.4	6.1	3.8	6.8	8.0	7.0
G-ND Elgin	6.1	6.1	8.5	5.1	6.9	4.3	6.3	7.4	7.1
P-MN Bolles	7.6	6.4	9.4	4.8	6.8	4.6	7.2	7.5	7.4
G-ND Glenn	7.1	6.5	9.0	5.5	6.8	4.5	7.4	7.9	7.3
P-ND 817	6.0	6.1	8.7	5.8	7.6	4.9	6.6	7.8	7.4
G-MN Bolles	7.1	6.4	9.5	5.1	7.3	4.8	7.2	7.8	7.3
P-ND Glenn	7.3	5.3	9.5	5.5	7.4	5.4	8.0	8.0	7.8
LSD (P<0.05)	0.7	0.5	0.6	1.5	1.1	0.8	0.5	0.3	0.5

\* Means were calculated across breadmaking methods. Means followed by the same letter in the column are not significantly different between mill types.

\*\* Least Significant Different

Wheat cultivar had a significant ( $P < 0.0001$ ) effect on the baking mix time indicating that there was variation in the baking mix time. The baking mix time score varied between 5.3 to 7.8 minutes for 18 HRS wheat flours (Table 3.9). When averaged across baking methods and loaf sizes, C-ND Faller, P-MN Bolles, G-MN Bolles, P-ND Glenn, and G-ND Glenn cultivars had mix time scores of 7 or higher. The physical properties of dough come from the interactions between gluten proteins, especially the disulphide-bonded glutenin macropolymer (Wang et al., 2006). The glutenin subunit provides viscous characteristics of the dough, thus resulting in longer dough mixing time. The higher baking mix time indicates that flours obtained from these wheat cultivars may have strong gluten (especially glutenin subunit) which in turn result in longer mixing time. In contrast, C-ND Elgin, P-ND Elgin, C-SD Focus, C-ND Prosper, G-ND 817, P-WA Glee, and C-ND Glenn received baking mix time scores of less than 6, which indicated that flours for these wheat cultivars may have low gluten content (Table 3.9).

Dough handling scores were given by the baker for these wheat cultivars. The ANOVA indicated that only wheat sample had significant effect on the dough handling score (Table A.15), which indicates that the variation in the scores were due to the wheat samples used in this study. It was observed that P-WA Glee, C-ND Faller, P-MN Bolles, G-MN Bolles, P-ND Glenn, and G-ND Glenn cultivars received dough handling scores of 9 or above, which indicate that these cultivars were easy to handle by baker during baking (Table 3.9). However, cultivars such as C-SD Focus, C-ND Prosper, P-ND Elgin, and G-ND Elgin received dough handling scores 8.5 or below, which indicate that dough made from these flour were “sticky” or “slack” when evaluated by the baker.

Oven spring is an important phenomenon that happens during breadmaking. Proofed dough expands during the baking process, and it yields in greater bread loaf volume (Delcours

and Hosenev, 2010). Wheat sample did not have significant ( $P>0.05$ ) effect on the oven spring scores, thus the variation between was more due to the baking method. However, it was observed that C-ND Dapps, P-ND 817, P-ND Glenn, and G-ND Glenn cultivars had oven spring scores of 5.5 when averaged across baking methods, loaf sizes, and fermentation time. These results indicate that these flours yield in greater dough expansion regardless of the baking method and processing conditions. Especially, cultivar Glenn from two different locations showed consistent and high oven spring scores, which might explain why this cultivar is known for its “excellent” end-use quality.

Bread loaf volume is the ultimate measurement for HRS wheat quality evaluation. The ANOVA indicated that wheat sample had a significant ( $P<0.0001$ ) effect on the bread loaf volume. When averaged across baking methods, wheat cultivars P-ND 817, G-MN Bolles, and P-ND Glenn received high bread loaf volume scores of 7 or above. These high scores indicate that flours obtained from these cultivars would result in consistently great bread loaf volume regardless of the baking method and conditions. This is very desirable. In contrast, cultivars C-ND Elgin, C-SD Focus, C-ND Prosper, G-Forefront, G-ND 817, P-WA Glee, and C-ND Faller received bread loaf volume scores of 6 or less. In other words, flours obtained from these wheat cultivars would not result in greater loaf volume under different baking and processing conditions.

The overall baking quality score was developed to assist in ranking and comparing 18 HRS cultivars. The most important breadmaking parameters were considered in the overall scoring for quality evaluation. As mentioned in the Materials and Methods section, these baking parameters were selected and given weights based on the importance of that parameter. For example, bread loaf volume score was given 30%, which means that the overall 30% of the

overall baking quality score would come from the bread loaf volume score. Table 3.10 shows the overall baking quality scores for 18 HRS cultivars averaged across baking methods and conditions.

Table 3.10. The Overall Baking Quality Scores for 18 HRS Cultivars

Cultivar	Baking Score	
C-ND Elgin	5.3	
C-SD Focus	5.5	
C-ND Prosper	5.6	Fair
G-Forefront	5.7	
G-ND 817	5.9	
P-ND Elgin	6.0	
P-WA Glee	6.0	
P-SY Ingmar	6.2	
G-SY Ingmar	6.2	Good
C-ND Dapps	6.2	
C-ND Faller	6.3	
C-ND Glenn	6.3	
G-ND Elgin	6.4	
P-MN Bolles	6.6	
G-ND Glenn	6.7	
P-ND 817	6.8	Excellent
G-MN Bolles	6.8	
P-ND Glenn	6.9	
LSD (0.05)	0.5	

These 18 cultivars were divided into 3 categories based on their overall baking quality scores. Cultivars with overall quality scores of less than 6 (on a 1 to 10 scale) were considered as “fair” quality cultivars for bread baking evaluation. As the baking quality scores were averaged across straight and sponge dough methods, fermentation times, and loaf size, the overall baking quality score provides a very good representation of baking quality of these wheat cultivars. C-ND Elgin, C-SD Focus, C-ND Prosper, G-Forefront, and G-ND 817 cultivars had overall baking quality scores of less than 6; thus, these cultivars were considered “fair” quality cultivars (Table 3.10). These results indicate that these cultivars have acceptable but “fair” breadmaking quality

characteristics under various baking methods. Similarly, cultivars receiving overall baking quality scores between 6.0 and 6.5 were considered to have “good” baking quality characteristics. Such cultivars include: P-ND Elgin, G-ND Elgin, P-SY Ingmar, G-SY Ingmar, P-WA Glee, C-ND Dapps, C-ND Faller, and C-ND Glenn. It was observed that both Elgin and SY Ingmar cultivars from two locations were consistently grouped in this category, which indicates that these two cultivars (Elgin and SY Ingmar) have “good” baking quality characteristics regardless of the growing location. Lastly, cultivars P-ND 817, P-MN Bolles, G-MN Bolles, P-ND Glenn, and G-ND Glenn received overall baking quality scores of 6.5 or above hence these cultivars were considered to have “excellent” baking quality characteristics under different baking conditions. It is important to note that ND 817 cultivar has not been released yet. In addition, ND 817 from two locations had different baking quality results. ND 817 from the Gulf/Great Lakes location was considered to have “fair” baking quality, while ND 817 from the Pacific Northwest location was considered to have “excellent” baking quality. Therefore, there may be effect of growing location on the end-use quality of this cultivar. However, both MN Bolles and ND Glenn cultivars from 2 locations were categorized as “excellent” breadmaking quality cultivars; thus, it can be concluded that these 2 cultivars have very good baking quality characteristics regardless of the baking method, fermentation time as well as loaf sizes.

### 3.6. Conclusion

The current research was carried out to determine whether the overall ranking of Hard Red Spring Wheat cultivars for quality evaluation was affected by breadmaking methods and loaf sizes. The overall baking quality scoring system was developed in order to assist in comparing and ranking HRS wheat objectively. The differences in the straight dough and sponge and dough methods were observed. Straight dough method with 2 hour fermentation resulted in

greater bread loaf volume compared to the sponge and dough method for 100g loaf size. However, sponge and dough method was better suited for 1 pound bread loaves resulting in greater loaf volume compared to the straight dough method for same loaf size. In addition, less variability in the bread loaf volume was observed for HRS wheat flours when using the sponge and dough method. In the baking quality evaluation of HRS wheat cultivars, the overall baking quality scores were developed to determine whether the ranking was affected by baking methods. When averaged across various baking methods and conditions, C-ND Elgin, C-SD Focus, C-ND Prosper, G-Forefront, and G-ND 817 cultivars were considered to have “fair” breadmaking quality characteristics, while receiving overall quality scores less than 6. In contrast, cultivars P-ND 817, P-MN Bolles, G-MN Bolles, P-ND Glenn, and G-ND Glenn received overall baking quality scores of 6.5 or above hence these cultivars were considered to have “excellent” baking quality characteristics under different baking conditions. The results in the current research study indicate that although there are differences in the breadmaking methods on the end-use quality evaluation, the ranking of HRS wheat flours is not affected by the baking methods and conditions. In other words, cultivars considered to have “fair” quality tend to have low breadmaking quality, while “excellent” baking cultivars will have superior end-use quality regardless of the baking method and processing conditions.

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## **CHAPTER 4. EFFECT OF ROLLER MILL TYPE ON SOLVENT RETENTION**

### **CAPACITY OF HARD RED SPRING WHEAT**

#### 4.1. Abstract

Solvent Retention Capacity (SRC) test has been widely accepted and used in the milling industry. SRC test has received great attention due to its simple and rapid procedures, use of small sample (5g), low cost as well as its ability to predict end-use quality. However, its use has been limited only to soft wheat flours. This research investigated the effect of roller mill type on solvent retention capacity of Hard Red Spring wheat. The wheat samples were milled on four roller mills: Quad. Jr., Quad. Sr., Buhler, and MIAG. Both wheat sample and mill type had significant ( $P < 0.001$ ) effect on the water, sodium carbonate, sucrose, and GPI SRC values; however, mill type showed greater effect than did cultivars as indicated by the higher ANOVA F-value. Thus, the variation in these SRC values were more due to the roller mill type. Differences in the SRC results between roller mills were observed. Quad. Jr. and Sr. mills had significantly ( $P < 0.05$ ) lower SRC water, sodium carbonate, and sucrose values, while Buhler and MIAG mills had higher SRC values for these solvents. The results were due to the flour particle size and starch damage differences among these roller mills, indicating that SRC result in much dependent on the roller mill used to produce flour. In addition, lactic acid SRC values were different among mill types. Quad. Jr. mill had significantly ( $P < 0.05$ ) lower lactic acid SRC values, while MIAG mill had the highest lactic acid SRC values. No significant ( $P > 0.05$ ) difference was observed between Quad. Sr. and Buhler mills. These results indicate that gluten strength (in flour) was different among flours obtained from these roller mills when evaluated by the SRC test.

## 4.2. Introduction

The assessment of end-use quality of wheat in a timely manner is very important. Although there are numerous flour and dough rheology tests, baking test is the most reliable and it provides most realistic assessment of wheat quality (Guzman et al., 2015). However, baking tests requires a large amount of time and effort as well as flour. Solvent Retention Capacity (SRC) test developed by Slade and Levine (1994) have been used as a quick quality evaluation test. The SRC test has been widely used by wheat breeders, millers, and bakers, as well as cereal scientists (Kweon et al., 2011). The relationship between flour SRC profiles and finished product quality i.e. cookies, crackers, cakes, noodles, and breads have been widely reported and discussed (Slade and Levine, 1994; Guttieri et al., 2004; Bettge et al., 2002; Gaines, 2004; Tanhehco and Ng, 2008; and Nakamura et al., 2010).

The SRC test addresses the relative contributions of wheat flour components to water absorption using four different solvents. The SRC test is based on the swelling of the wheat flour components in certain solvent solutions: 5% lactic acid for glutenin, 5% sodium carbonate for damaged starch, 50% sucrose for arabinoxylans, and water for all polymers (Kweon et al., 2011). SRC test has been widely accepted and used in the milling industry and wheat quality laboratory because of its simple and rapid procedures, use of small sample (5g), and low cost. In addition, SRC test can be used to predict end-use quality (Kweon et al., 2011). SRC test has received great attention due to these advantages over other quality test; however, its use has been limited only to soft wheat flours. There have been very few studies on the relationship between SRC profiles and Hard Red Spring wheat quality parameters. Hammed et al. (2015) investigated the relationship between SRC profiles and protein molecular distribution and breadmaking functionality of HRS wheat flour. The authors have concluded that the association between SRC

values and quality parameters were different from those for soft wheat flour; hence, more in-depth study is needed. Lindgren and Simsek (2015) have also evaluated HRS millstream fractions using SRC test, and they have concluded that SRC could be used to predict farinograph water absorption and dough quality of flour. In addition, the authors concluded that the findings could display differences in composition and quality between flour mill streams.

The objective of current research study was to determine the effect of roller mill type on solvent retention capacity analysis of Hard Red Spring wheat.

### 4.3. Materials and Methods

#### 4.3.1. Wheat Sample

Five bushels of six Hard Red Spring wheat cultivar composites (SD Forefront, ND Elgin, MN Bolles, ND 817, SY Ingmar, and ND Glenn) were obtained from Gulf/Great Lake Export Region as part of the 2014 Overseas Varietal Analysis (OVA). Additional five bushels of 6 HRS wheat cultivars of ND Dapps (2014), ND Elgin (2013), ND Faller (2014), SD Focus (2014), ND Glenn (2012), and ND Prosper (2014) from Casselton location were obtained from the North Dakota State Seed Department, thus making a total of 12 HRS wheat cultivars (Table 4.1).

Table 4.1. HRS wheat cultivar composite ratios (%) from different locations in North Dakota

Cultivar	Sample Type	Year	Casselton	Crookston	Watertown
			Blending Ratio (%)		
SD Forefront	OVA	2014	33.3	33.3	33.4
ND Elgin	OVA	2014	33.3	33.3	33.4
MN Bolles	OVA	2014	33.3	33.3	33.4
ND 817	OVA	2014	33.3	33.3	33.4
SY Ingmar	OVA	2014	50.0	50.0	-
ND Glenn	OVA	2014	33.3	33.3	33.4
ND Dapps	Experiment Station	2014	100.0	-	-
ND Elgin	Experiment Station	2013	100.0	-	-
ND Faller	Experiment Station	2014	100.0	-	-
SD Focus	Experiment Station	2014	100.0	-	-
ND Glenn	Experiment Station	2012	100.0	-	-
ND Prosper	Experiment Station	2014	100.0	-	-

#### 4.3.2. Kernel Quality Analysis

The wheat was cleaned on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN) to remove shrunken and broken kernels. Test weight and moisture contents (dockage-free portion) were determined with a GAC 2100 tester (Dickey-John, Auburn, IL, USA). Whole wheat ash and protein content were measured by near-infrared spectroscopy with an Infracotec 1241 grain analyzer (Perstorp Analytic, Hoganas, Sweden).

The current standard method of evaluating the percentage of vitreous kernels in the United States was used for determination of DHV kernel content. This was done by manually inspecting a 15-g sample, which was free of shrunken and broken kernels (USDA 1997).

Wheat kernel samples (10g) were weighed and prepared after removal of all dockage, shrunken and broken kernels, and other foreign materials. The number of each sample was counted with a model 77 totalizer (Seedburo Equipment, Chicago, IL, USA). Number of counted kernels was converted to 1,000 kernel weight and recorded.

Wheat kernels were sorted for sizing with a shaker in which a set of Tyler standard sieves (number 7 and 9 [2.92 and 2.24 mm]) was used (Arrow testing sieve shaker, Seedburo Equipment, Chicago, IL, USA). Wheat (100g) was sized on the shaker for 200 s.

Approximately 300 kernels of wheat were prepared for kernel hardness. Samples were poured into the access hopper of the SKCE 4100 device (Perten, Huddinge, Sweden) and analyzed according to AACC International Approved Method 55-31.01. Parameters such as kernel weight (mg), kernel diameter (mm), moisture content (%), and kernel hardness index value were determined. Two hundred grams of wheat samples was sent to the North Dakota Grain Inspection for full-grade grain characteristics.

The ground wheat flour falling number was determined using a Falling Number (Perten Instruments, Springfield, IL, USA) according to AACCI Approved Method 56-81.03.

#### 4.3.3. Flour Milling

Wheat samples were tempered to 16% moisture for 18 h before milling. All 12 wheat samples were milled in four different laboratory mills: Brabender Quadrumat Jr. and Quadrumat Sr. (Brabender Instruments, Hackensack, NJ, USA), Buhler MLU-202 (Buhler Industries, Uzwil, Switzerland), and MIAG-Multomat (Miag, Braunschweig, Germany).

A total of 4 kg of wheat samples were milled on Brabender Quadrumat Jr. according to AACCI Approved Method 26-50.01 and Quadrumat Sr., and Buhler MLU-202 according to AACCI Approved Method 26-21.02. Two hundred gram lots at a time were milled for Quadrumat Jr. and Sr. mills due to the sieving capacity. Approximately 50 kg of wheat samples were milled on MIAG Multomat; the feed rate of wheat to the mill was set at 1360 g/min. The break releases for the first, second, and third breaks were set at 30%, 53%, and 65%, respectively. Flour extractions were determined as the percentage of straight-grade flour

produced. Flours obtained from MIAG mill were then rebolted through an 84 SS sieve on an Allis-Chalmers rebolter Ser. No. 204 (Allis-Chalmers MFG., Milwaukee, WI, USA) to remove any foreign material. Flour was then blended on a Cross-Flow Blender Serial No. L6-0280 (Patterson-Kelly Co., East Stroudsburg, PA, USA) for 30 minutes.

#### 4.3.4. Flour and Dough Quality Analysis

Flour particle size was determined using a RoTap shaker according to AACCI Method 55-60.01 Flour (100g) was weight and sifted on the sieves with screen openings of 250 $\mu$ m, 180 $\mu$ m, 150 $\mu$ m, 125 $\mu$ m, 75 $\mu$ m, and 45 $\mu$ m for 5 minutes. Flour fractions retained on each sieve was weighed and expressed as percentage of flour in each particle size range.

The  $\alpha$ -amylase activity was measured using the Rapid Visco Analyzer (C.W. Brabender Instruments Inc., Hackensack, NJ) according to AACCI Approved Method 22-08.01.

AACCI Approved Method 56-11.02 was used for determination of Solvent Retention Capacity (SRC). Flour sample (5 g) was combined with individually with distilled water, 5% sodium carbonate, 50% sucrose, and 5% lactic acid (25 g of solvent) and shaken every 5 min for 20 min. After shaking, the samples were centrifuged at 1,000 x g for 15 min, and the centrifuge was allowed to stop without breaking. The supernatant was poured off, and the sample tubes were drained at a 90° angle for 10 min. The %SRC was calculated with the following formula:

$$\%SRC = \left( \frac{gel\ wt}{flour\ wt} - 1 \right) \times \left( \frac{86}{100 - \%FM} \right) \times 100$$

where %FM is the flour moisture. From the data obtained from determination of SRC, the gluten performance index (GPI) (Kweon et al. 2011) was calculated according to the following formula:

$$GPI = \frac{lactic\ acid\ SRC}{(sodium\ carbonate\ SRC + sucrose\ SRC)}$$

#### 4.4. Statistical Analysis

The experimental design 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 was used to declare differences between treatments. The experimental design was two-factorial layout with mill type and wheat cultivars as main factors. Mill type and wheat cultivars interaction term was used as error term.

#### 4.5. Results and Discussion

The ANOVA (Table A.17) showed that mill types showed significant ( $P < 0.001$ ) differences on the SRC profiles. The water SRC is related to the overall water holding capacity, which is contributed by wheat flour components such as gluten, damaged starch, and pentosans (Kweon et al, 2011). The mill type influenced water SRC value. Quad. Jr. and Sr. mills had significantly lower water SRC values, while Buhler mill had the highest followed by MIAG water SRC values (Table 4.2).

Table 4.2. Solvent Retention Capacity Profiles of Roller Mills\*

Mill Type	Water (%)	Sodium Carbonate (%)	Lactic acid (%)	Sucrose (%)	GPI (%)
Quad. Jr	64.4c	79.5c	138.8c	98.6b	0.78b
Quad. Sr	65.0c	78.2c	144.4b	99.4b	0.81a
Buhler	76.9a	99.5a	144.3b	119.3a	0.66d
MIAG	74.6b	96.4b	152.6a	119.6a	0.71c

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types

The difference in the water SRC could be due to the damaged starch in the flours obtained from these roller mills. Kweon et al. (2011) reported that native wheat starch could hold 0.3-0.45g of water per gram of dry starch whereas damaged starch (from mechanical stress during milling) can hold 1.5-2g of water per dry starch. This indicates that starch damage in the

flour influences the flour water absorption measured by the SRC test. Starch damage in flours obtained from these roller mills, which may have influenced the water SRC profiles. Baasandorj et al. (2015) have reported significant differences in the flour starch damage content; 5.0% for Quad. Jr., 4.1% for Quad. Sr., and 6.1% for Buhler Mills, respectively. Therefore, starch damage differences could explain the differences observed in water SRC values for these roller mills.

In addition to damaged starch, flour particle size may have contributed to the differences in the water SRC values. One of the key differences in wheat flours produced by different milling techniques is the different particle size obtained (Maldonado and Rose, 2013). Therefore, the particle size distribution affects functionality as well as baking quality. Figure 4.1 illustrates the flour particle size distribution for different roller mills.

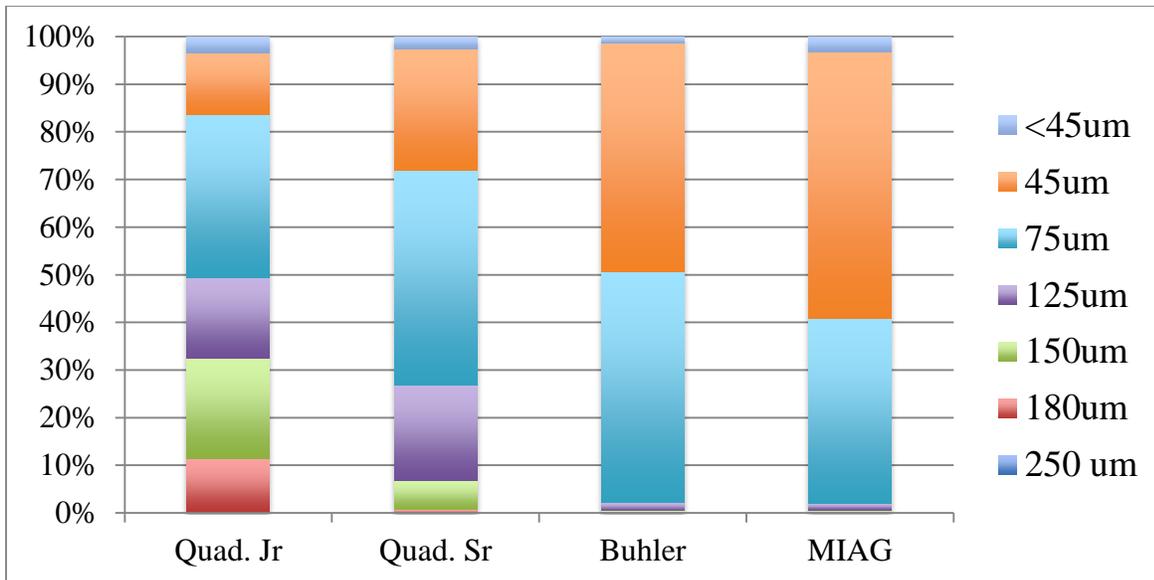


Figure 4. 1. Flour Particle Size Distribution for Roller Mills at Different Sieve Openings

The flour particle size distribution varied among mill types owing to the milling process associated in each mill. Flours produced from Quad. Jr. and Sr. mill had non-uniform and much coarser particle size, while Buhler and MIAG mills produced very uniform and fine flours (Figure 4.1). This indicates that coarser flour produced from Quad. Jr. and Sr. mills resulted in

slow rate of water hydration. In contrast, flours produced from Buhler and MIAG mills resulted in significantly higher ( $P<0.05$ ) water SRC values, indicating that the fine flour particles resulted in faster water uptake. Therefore, a combination of high starch damage and fine flour particle size produced in Buhler and MIAG mills explain the higher water SRC values.

However, water is not the best solvent for functional flour polymers (Kweon et al., 2011). The other three solvents are used to exaggerate the contribution of one functional flour component, compared with its contribution to swelling in water. Sodium carbonate SRC is related to the damaged starch. Sucrose SRC gives an indication of flour arabinoxylan characteristics, while lactic acid SRC is associated with gluten strength of flour.

The ANOVA (Table A.17) showed that mill type had significant ( $P<0.001$ ) effect on the sodium carbonate SRC values. It was observed that both Quad. Jr. and Sr. mills had significantly ( $P<0.05$ ) lower sodium carbonate SRC values, while Buhler mill had the highest value. This indicates that there were differences in the flour starch damage, which could be a result of milling principles of these roller mills. Low flour starch damage content in Quad. Jr. and Sr. mills may have resulted in low sodium carbonate SRC values, while high starch damage in Buhler and MIAG mills resulted in high sodium carbonate SRC values (Table 4.2). Kweon et al. (2009) also reported an increased sodium carbonate SRC value due to increased starch damage, which resulted from decreased particle size by pin-milling.

Both mill type and wheat sample significant ( $P<0.001$ ) effect on the SRC lactic acid values; which indicated that there were differences in the SRC lactic acid values across mill types when average across wheat cultivars. Quad. Jr. mill had significantly ( $P<0.05$ ) lower SRC lactic acid value of 138.8%, while MIAG mill had the highest value of 152.2%. Both Quad. Sr. and Buhler mill had 144.4% and 144.3% SRC lactic acid values. These results indicate that

flours obtained from these roller mills differed in lactic acid SRC values, in which milling process influenced the gluten strength in these flours. In addition, mill type had significant ( $P < 0.05$ ) effect on the sucrose SRC values. Both Quad. Jr. and Sr. mills had significantly ( $P < 0.05$ ) lower sucrose SRC values of 98.6% and 99.4% respectively and there was no difference between these mills. In contrast, Buhler and MIAG mill had significantly higher sucrose SRC values of 119.3% and 119.6% compared to Quad. Jr. and Sr. mills. These results indicated that flour arabinoxylan characteristics were different among mill types, as sucrose SRC gives an indication of the flour arabinoxylan (Duyvejonck et al., 2011). Lastly, gluten performing index (GPI) was also calculated for these roller mills (Table 4.1). SRC GPI value describes the overall performance of flour glutenin (Kweon et al., 2011). SRC GPI ranged from 0.66 to 0.81 for these roller mills, indicating that there was a difference in the milling machine. SRC GPI was significantly ( $P < 0.05$ ) different for each mill type; Quad. Sr. had the highest (0.86) GPI while Buhler mill had the lowest (0.66). It was also observed that smaller mills (Quad. Jr. and Sr.) had higher SRC GPI when compared to Buhler and MIAG mills. This is because of the significant lower sodium carbonate and sucrose values observed for Quad. Jr. and Sr. mills. These lower values would result in higher GPIs observed for these mill types, as the provided equation (section 4.3.4) illustrates how GPI is calculated.

Correlation analyses were conducted among SRC values for different roller mills. High and positive significant ( $P < 0.05$ ) correlations were observed among different SRCs (Table 4.3). Water SRC showed very high and positive correlation with ( $P < 0.001$ ) sodium carbonate SRC, and this was consistent across all mills. Ram et al. (2005) also reported high and positive correlations between water and sodium carbonate SRCs in their study. This very high correlation between water and lactic acid SRCs indicated that the major factor determining water absorption

is starch damage. Sucrose SRC showed significant ( $P < 0.01$ ) and positive correlations with water, sodium carbonate, and lactic acid SRCs (Table 4.3). These positive correlations were consistent across mill types, except there was no significant ( $P < 0.05$ ) correlation with sucrose and sodium carbonate SRCs on Quad. Sr. mill.

Table 4.3. Correlation Coefficients for Solvent Retention Capacity Across Roller Mills

Mill Type	SRC Parameters	Water	Sodium Carbonate	Lactic Acid	Sucrose	GPI
Quad. Jr.	Water	1.00	0.95***	0.56ns	0.79**	0.15ns
	Sodium Carbonate	0.95***	1.00	0.39ns	0.74**	-0.07ns
	Lactic Acid	0.56ns	0.39ns	1.00	0.83***	0.88***
	Sucrose	0.79**	0.74**	0.83***	1.00	0.48ns
	GPI	0.15ns	-0.07ns	0.88***	0.46ns	1.00
Quad. Sr.	Water	1.00	0.88***	0.29ns	0.61*	-0.06ns
	Sodium Carbonate	0.88***	1.00	0.02ns	0.43ns	-0.34ns
	Lactic Acid	0.29ns	0.02ns	1.00	0.73**	0.92***
	Sucrose	0.61*	0.43ns	0.73**	1.00	0.42ns
	GPI	-0.06ns	-0.34ns	0.92***	0.42ns	1.00
Buhler	Water	1.00	0.94***	0.39ns	0.76**	-0.24ns
	Sodium Carbonate	0.94***	1.00	0.36ns	0.74**	-0.29ns
	Lactic Acid	0.39ns	0.36	1.00	0.79**	0.77**
	Sucrose	0.76**	0.74**	0.79**	1.00	0.25ns
	GPI	-0.24ns	-0.29ns	0.77**	0.25ns	1.00
MIAG	Water	1.00	0.85***	0.42ns	0.75**	-0.12ns
	Sodium Carbonate	0.85***	1.00	0.46ns	0.83***	-0.15ns
	Lactic Acid	0.42ns	0.46ns	1.00	0.80**	0.80**
	Sucrose	0.75**	0.83***	0.80**	1.00	0.29ns
	GPI	-0.12ns	-0.15ns	0.80**	0.29ns	1.00

\*, \*\*, and \*\*\* represent significance at 0.05, 0.01, and 0.001, respectively. ns – not significant  
GPI, Gluten Performance Index

Lastly, lactic acid showed very high and positive correlations with GPI, and it was consistent across these roller mills. Hammed et al. (2015) have also reported very high correlations between lactic acid SRCs and GPI for HRS wheat flours from various growing locations. This is expected because lactic acid SRC indicates flour protein content, whereas GPI indicates the overall performance of glutenin (Kweon et al., 2011). In other words, SRC lactic acid is influenced by protein content, while GPI is more affected by protein quality. Although

there were differences among mill types for SRC values, the correlations between SRC values were consistent across mill types. This indicates that there were differences between SRC due to mill type effect; however, these correlations among SRC values would still be consistent regardless of the mill type. In addition, lactic acid SRCs showed very high and positive with bread loaf volume compared to GPI. SRC lactic acid and bread loaf volume correlations were consistent across mills: Quad. Jr. ( $r = 0.83$ ,  $P < 0.001$ ), Quad. Sr. ( $r = 0.94$ ,  $P < 0.001$ ), Buhler ( $r = 0.72$ ,  $P < 0.01$ ), and MIAG ( $r = 0.93$ ,  $P < 0.001$ ) (Data Presented in Chapter 2). Therefore, lactic acid SRC could be used as an important parameter to evaluate early generation wheat breeding lines as an effort to replace breadmaking process.

It has been suggested that SRC test can be conducted by Rapid Visco Analyzer (RVA) (Dang and Bason, 2006). The RVA test measures the starch performance by heating and followed by cooling a starch mixture under shear stress (Fujiwara et al., 2016). Starch absorbs water and swells upon heating and shear stress, and this increases the viscosity above the pasting temperature and eventually reaching a peak viscosity. When wheat flour starch is heated upon addition of water, the viscosity increases with gradual increase in temperature (Delcour and Hosney, 2010). This results in loss of granular birefringence thus starch gelatinizes. The gelatinization temperature for wheat starch is 50-57°C. Starch granules rupture and allow amylose to leach out into the surrounding solution with continued heating (Fujiwara et al., 2016). This causes a reduction, or “breakdown”, in viscosity. As the temperature decreases, the mixture starts to form gel and viscosity starts to increase again. This is referred to as “set back.” To investigate the relationship RVA was performed on the flour samples obtained from four roller mills (Table 4.4).

Table 4.4. Pasting Properties of Flours from Four Roller Mills

Mill Type	Peak Viscosity	Trough	Breakdown	Final Viscosity	Setback	Peak Time	Pasting Temp.
	(cP)	(cP)	(cP)	(cP)	(cP)	(min.)	(°C)
Quad. Jr	2880a	1632a	1249a	2834a	1202a	6.17a	67.3a
Quad. Sr	2826a	1575b	1251a	2794a	1219a	6.19a	67.3a
Buhler	2401c	1318c	1083c	2424c	1107c	6.18a	67.6a
MIAG	2461b	1323c	1138b	2484b	1161b	6.17a	69.6a

\* Means were calculated across wheat cultivars. Means followed by the same letter in the column are not significantly different between mill types

Mill type had a significant ( $P < 0.001$ ) effect on the peak viscosity parameter. Quad. Jr. and Sr. mills had significantly ( $P < 0.05$ ) higher peak viscosity compared to Buhler and MIAG mills. Consequently, Buhler had significantly ( $P < 0.05$ ) higher peak viscosity than MIAG mill. The flour particle size may have impacted the differences in the peak viscosity in these mills. The particle size distribution was different among these roller mills, as reported in the Chapter 2. The particle size distribution of the samples affects RVA results; a longer time is required for larger particles to be fully wetted (Crosbie and Ross, 2015 RVA handbook). The settling of the particles changes the effective concentration thus strongly affects viscosity. Coarser flour particles obtained from Quad. Jr. and Sr. mills may have resulted in significantly ( $P < 0.05$ ) higher peak viscosity for these mills. In contrast, Buhler and MIAG mills had lower peak viscosity indicating that more fine and uniform flour particles size resulted in lower peak viscosity. A similar trend was observed for final viscosity. Quad. Jr. and Sr. mills had significantly higher final viscosity, while Buhler and MIAG mills had lower RVA final viscosity. However, Buhler mill had significantly ( $P < 0.05$ ) lower final viscosity than the MIAG mill. Therefore, mill type had a significant effect on the RVA results, which indicates that various roller mills have an effect on the pasting properties of the flours obtained.

Correlation analyses were conducted between RVA and SRC values for different roller mills. This was done to study whether SRC can be conducted by RVA, as previously suggested by Dang and Bason (2006). It was observed that overall there was no relationship between RVA and SRC parameters (Table 4.5).

Table 4.5. Correlation Coefficients for RVA and SRC Parameters Across Roller Mills

Mill Type	Parameters	Water	Sodium Carbonate	Lactic Acid	Sucrose	GPI
Quad. Jr	Peak Viscosity	0.52ns	0.37ns	0.10ns	0.21ns	-0.06ns
	Trough	-0.05ns	-0.13ns	-0.40ns	-0.35ns	-0.37ns
	Breakdown	0.72**	0.57ns	0.38ns	0.49ns	0.14ns
	Final Viscosity	-0.25ns	-0.32ns	-0.38ns	-0.39ns	-0.27ns
	Set Back	-0.47ns	-0.52ns	-0.23ns	-0.34ns	-0.04ns
Quad. Sr.	Peak Viscosity	0.65*	0.68*	0.18ns	0.58*	-0.12ns
	Trough	0.39ns	0.37ns	-0.18ns	0.23ns	-0.37ns
	Breakdown	0.63*	0.68*	0.34ns	0.63*	0.05ns
	Final Viscosity	0.24ns	0.17ns	-0.15ns	0.13ns	-0.26ns
	Set Back	-0.04ns	-0.15ns	-0.07ns	-0.05ns	-0.03ns
Buhler	Peak Viscosity	0.65*	0.57ns	0.33ns	0.64*	-0.10ns
	Trough	0.25ns	0.19ns	-0.02ns	0.19ns	-0.20ns
	Breakdown	0.72**	0.66*	0.46ns	0.75**	0.01ns
	Final Viscosity	0.04ns	0.01ns	0.02ns	0.13ns	-0.04ns
	Set Back	-0.23ns	-0.23ns	0.08ns	0.03ns	0.18ns
MIAG	Peak Viscosity	0.16ns	0.18ns	0.01ns	0.16ns	-0.14ns
	Trough	-0.17ns	-0.19ns	-0.31ns	-0.27ns	-0.22ns
	Breakdown	0.36ns	0.19ns	0.23ns	0.44ns	-0.03ns
	Final Viscosity	-0.25ns	-0.29ns	-0.20ns	-0.24ns	-0.05ns
	Set Back	-0.31ns	-0.36ns	-0.06ns	-0.17ns	0.14ns

\*, \*\*, and \*\*\* represent significance at 0.05, 0.01, and 0.001, respectively. ns – not significant  
GPI, Gluten Performance Index

There was low and positive correlations between RVA peak viscosity and SRC water and sucrose values for Quad. Sr. and Buhler mills. Similarly, RVA breakdown was positively correlated with SRC water, sodium carbonate, and sucrose values. However, in the current study there was no significant ( $P < 0.05$ ) and strong correlations between RVA and SRC parameters. These findings are not in agreement with a previous study by Lindgren and Simsek (2015), who

reported that flour pasting profile parameters showed significant correlations with SRC values for millstream samples. This could be due to the wheat samples used in this study; wheat samples had significant ( $P < 0.05$ ) effect on the most of the RVA parameters thus contributing to large variation. SRC test is based on the energetics, which is related to polymer-solvent compatibility (Kweon et al., 2011). Thus, SRC method avoids the kinetic effects, which would be incorrectly introduced by rheological methods such as RVA. Kweon et al. (2011) thus have concluded that the SRC method cannot be conducted by RVA. In the current study, there was no strong relationship between SRC and RVA parameters across four roller mills, which is in agreement with Kweon and others (2011). However, this is not in agreement with Lindgren and Simsek (2015), who reported that SRC values had strong correlations with pasting parameters for millstream samples.

#### 4.6. Conclusion

The current research was carried out to determine the effect of roller mill type on solvent retention capacity (SRC) test for Hard Red Spring wheat. The mill type had significant ( $P < 0.001$ ) effect on the SRC results. There were differences in the SRC results between roller mills. Quad. Jr. and Sr. mills had significantly ( $P < 0.05$ ) lower SRC water, sodium carbonate, and sucrose values, while Buhler and MIAG mills had higher SRC values for these solvents. These results were due to the flour particle size and starch damage differences among these roller mills, indicating that SRC result is much dependent on the roller mill used to produce flour. In addition, lactic acid SRC values were different among mill types. Quad. Jr. mill had significantly ( $P < 0.05$ ) lower lactic acid SRC values, while MIAG mill had the highest lactic acid SRC values. However, there was no significant ( $P > 0.05$ ) difference between Quad. Sr. and Buhler mills. These results indicate that gluten strength (in flour) was different among flours obtained from

these roller mills when evaluated by the SRC test. Solvent Retention Capacity (SRC) test has received great attention due to its simple and rapid procedures as well as the ability to predict end-use quality for different solvents used in the test. SRC test has been established and widely accepted in the milling industry for soft wheat flours, while SRC test is relatively new for Hard Red Spring wheat flours. The results from this research study indicated that SRC values for different solvents were significantly different across various roller mills. This indicated that SRC results are much dependent on the roller mill that is being used for quality evaluation. This is very important for the milling industry and wheat quality labs, as there can be different rollers mills used in the quality evaluation for HRS wheat. Therefore, the selection of roller mill to produce flour can have a significant impact on the SRC results due to the significant differences observed in this study. Therefore, selecting a certain mill type for SRC test for quality evaluation is crucial knowing that the differences exist between various roller mills.

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## **CHAPTER 5. PHYSICOCHEMICAL PROPERTIES OF HARD RED SPRING WHEAT**

### **MIAG MILLSTREMS**

#### 5.1. Abstract

Depending on the size and complexity of the roller mill, there can be many flour millstreams. The miller can produce wide range of flours of different quality and refinement. The miller's objective is to then combine different flour streams in order to meet the flour that is needed for the end-use product. The current research was carried out to investigate various MIAG Multomat millstreams for their physicochemical characteristics. A total of five HRS wheat cultivars from Gulf/Great Lakes and Pacific Northwest regions were milled on a MIAG-Multomat pilot mill. About 200g of stock were collected from each millstream and were used for quality analysis. The ANOVA indicated that main effects wheat cultivar, millstreams, and interactions between the main effects showed significant differences on the flour quality tests. These results indicate that there was a varietal difference for quality parameters as well as growing regions. When comparing flour millstreams, reduction millstreams accounted for 47.6% of the total flour yield, while break and sizing millstreams combined 20.2% of the total flour yield. Break millstreams had average ash content of 0.77%, while reduction streams (excluding the 5<sup>th</sup> middling stream) was 0.48%, which significantly lower than the break millstreams. It was also found that the most refined (reduction) streams have the brightest color, while high ash (break) streams had the darkest color. The break flours along with tail cyclone flour had very high protein content average of 17.0%, while reduction millstreams had much lower protein content average of 13.5%. Break millstreams had significantly lower starch damage, while reduction millstreams had greater flour starch damage Reduction millstreams were slightly higher AX content compared to the break millstreams. It was found that both B5 and M5 streams

had higher AX content compared to other break and reduction millstreams. This indicates that streams containing higher ash content yield higher AX%. The knowledge of wheat kernel distribution in different millstreams as well as the flour composition in these millstreams can provide millers with very important information to optimize the functionality of flour blends.

## 5.2. Introduction

Milling is simply a size reduction of wheat into more fine ground flour. The wheat flour milling process involves series of break, middling reduction, and sifting operations (Wang and Flores, 2000). The main objective of milling is to separate bran and germ from endosperm as cleanly as possible, while reducing the endosperm into finer particles. Roller milling is the traditional process used for flour milling, where both break (corrugated) and reduction (smooth) rolls are employed for particle size reduction. Break rolls open the wheat kernel and is to scrape off much of the endosperm from the outer layers, while reduction rolls gradually reduce the endosperm into flour. After each roll passage, the material or stock is sifted, and the flour passing through the fine sieves is collected, and is given the name and number of the corresponding roll passage. Depending on the size and complexity of the roller mill, there can be many flour or millstreams and flour is obtained in each millstream.

A commercial hard wheat mill can produce 30 or more flour mill streams (Machet, 2005). Therefore, the miller can produce wide range of flours of different quality and refinement. The miller's objective is to then combine different flour streams in order to meet the flour that is needed for the end-use product. Generally, the flour components can vary in the quantity and quality of the protein, ash content, flour color, flour water absorption, particle size, starch damage, and dough rheological properties. However, the breadmaking test ultimately determines the end-use quality of a flour.

The physical and chemical composition of wheat kernel is very heterogeneous; therefore, different flour millstreams can vary in composition and quality. Protein and ash concentration increases from the center of endosperm to the outer layers of the wheat kernel (Posner, 2005). Millstream analysis is therefore very important in the milling industry as to routinely check mill operation and efficiency. Typical millstream analysis includes: moisture, ash content and protein content. The evaluation of these parameters has been subject of many studies; however, the pentosan composition has been also a subject of interest (Wang et al., 2006).

There have been relatively few studies on millstream evaluation. Most of these studies have been conducted on a smaller Buhler MLU 202 laboratory mill. These studies have reported that flours produced from break and reduction side had different flour quality characteristics. However, there has not been a recent and extensive research study, which evaluated MIAG Multomat millstreams for quality evaluation. The millstream evaluation of MIAG Multomat would mimic the commercial flour millstreams. The current research is aimed at investigating various MIAG Multomat millstreams for their physicochemical characteristics when evaluating HRS wheat cultivars. Therefore, the objective of this study was to evaluate MIAG millstreams of HRS wheat cultivars for their physicochemical characteristics.

### 5.3. Materials and Methods

#### 5.3.1. Wheat Sample

Five bushels of 5 Hard Red Spring wheat genotype composites (ND Elgin, MN Bolles, ND 817, SY Ingmar, ND Glenn) were obtained from Pacific Northwest (PNW) and Gulf/Great Lakes export regions as part of the 2014 Overseas Varietal Analysis (OVA). The composites obtained from 2 export region are shown in table 5.1.

Table 5.1. HRS Wheat Cultivar Composite Ratios (%) from Different Locations in North Dakota and Washington for Two Export Regions\*

Region	Cultivar	Casselton	Crookston	Watertown	Minot	Williston
PNW	ND Elgin	20.7	43.7	-	20	15.6
PNW	MN Bolles	-	-	-	43.9	56.1
PNW	ND 817	-	-	-	64.7	35.3
PNW	SY Ingmar	-	-	-	55.5	44.5
PNW	ND Glenn	-	-	-	50.0	50.0
G/GL	ND Elgin	33.3	33.3	33.4	-	-
G/GL	MN Bolles	33.3	33.3	33.4	-	-
G/GL	ND 817	33.3	33.3	33.4	-	-
G/GL	SY Ingmar	50.0	50.0	-	-	-
G/GL	ND Glenn	33.3	33.3	33.4	-	-

\* G/GL – Gulf Great Lakes and PNW – Pacific Northwest

### 5.3.1. Kernel Quality Analysis

The wheat was cleaned on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN) to remove shrunken and broken kernels. Test weights and moisture contents (dockage-free portion) were determined with a GAC 2100 tester (Dickey-John, Auburn, IL, USA). Whole wheat ash and protein content were measured by near-infrared spectroscopy with an Infractec 1241 grain analyzer (Perstorp Analytic, Hoganas, Sweden). Wheat kernel samples (10g) were weighed and prepared after removal of all dockage, shrunken and broken kernels, and other foreign materials. The number of each sample was counted with a model 77 totalizer (Seedburo Equipment, Chicago, IL, USA). Number of counted kernels was converted to 1,000 kernel weight and recorded:

$$1,000 \text{ kernel weight (g)} = (1,000/\text{number of kernels}) \times 10 \text{ g}$$

Wheat kernels were sorted for sizing with a shaker in which a set of Tyler standard sieves (number 7 and 9 [2.92 and 2.24 mm]) was used (Arrow testing sieve shaker, Seedburo Equipment, Chicago, IL, USA). Wheat (100g) was sized on the shaker for 200 s.

Approximately 300 kernels of wheat were prepared for kernel hardness. Samples were poured into the access hopper of the SKCS 4100 device (Perten, Huddinge, Sweden) and analyzed according to AACC International Approved Method 55-31.01. Parameters such as kernel weight (mg), kernel diameter (mm), moisture content (%), and kernel hardness index value were determined. Two hundred grams of wheat samples were sent to the North Dakota Grain Inspection for full-grade grain characteristics.

### 5.3.2. Flour Milling

Wheat samples were tempered to 16% moisture for 18 h before milling. All 10 wheat samples were milled on a MIAG-Multomat laboratory mill (Miag, Braunschweig, Germany). Approximately 50 kg of wheat samples were milled on MIAG Multomat; the feed rate of wheat to the mill was set at 1360 g/min. The break releases for the first, second, and third breaks were set at 30%, 53%, and 65%, respectively. Flour extractions were determined as the percentage of straight-grade flour produced. Flour was then rebolted through an 84 SS sieve on an Allis-Chalmers rebolter Ser. No. 204 (Allis-Chalmers MFG., Milwaukee, WI, USA) to remove any foreign material. Flour was then blended on a Cross-Flow Blender Serial No. 257063 (Patterson-Kelly Co., East Stroudsburg, PA, USA) for 30 minutes. Percent yield based on wheat and total product was calculated for each millstreams.

### 5.3.3. Millstream Collection

Approximately 200g of sample were collected from the millstreams during milling (Table 5.2).

Table 5.2. The MIAG Multomat Millstream Names and Respective Stream Numbers\*

Stream Name	Stream #
1st Break	1
2nd Break I	2
Break Dust	3
Sizing I	4
2nd Break II	5
3rd Break	6
Sizing II	7
5th Break	8
4th Break	9
1st Middlings	10
2nd Middlings	11
3rd Middlings	12
4th Middlings	13
6th Middlings	15
Tail Flour	16
Tail Cyclone Flour	22
5th Middlings	14
Low Grade	17
Low Quality	18
Tail Shorts	19
Head Shorts	20
Bran	21
Tail Cyclone Shorts	23

\* Millstreams in highlighted color make the straight grade flour.

#### 5.3.4. Proximate Analysis

Moisture content of each sample was determined with air-oven drying at 135°C according to AACCI Approved Method 44-19.01. Ash content of each flour sample was determined according to AACCI Approved Method 08-01.01. Flour (3g) was weighed and placed in an ash crucible. Flour ash contents of each sample were expressed as a percentage of the initial sample weight. Flour protein content was determined according to AACCI Approved Method 46-30.01 with a LECO FP 528 nitrogen/protein analyzer (LECO, St. Joseph, MI, USA).

Starch damage in the flour millstreams were determined with a Megazyme starch damage assay procedure according to AACCI Approved Method 76-31.01

Flour color scores in the millstreams were determined by light reflectance according to AACCI Approved Method 14-22.01 with a Minolta color difference meter (CR 310, Minolta Camera, Osaka, Japan).

Flour Particle size distribution test was conducted on a vibratory sieve shaker (Retsch, Germany). The particle size distribution was based on the weight percentage retained on stacked sieves of 600, 500, 425, 250, 150, 100, 50 and <50  $\mu\text{m}$ .

#### 5.3.5. Determination of Total Arabinoxylans (TOT-AX) and Arabinose to Xylose Ratio (A/X) of Flour Mill Streams Using Gas Chromatography (GC)

The arabinoxylan content and arabinose to xylose ratio were determined by preparation of alditol acetates and analysis by gas chromatography with flame ionization detection (GC-FID). The samples were weighed (7mg) into glass screw top test tubes. Sample hydrolysis was conducted at 121°C for 1 hour with trifluoroacetic acid (2M). After hydrolysis the internal standard (m-inositol, 75  $\mu\text{l}$ , 10mg/ml) was added to each tube and the samples were dried under nitrogen at 55°C. The samples were reduced by addition of ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) and sodium borohydride in DMSO (20mg/ml). The samples were incubated at 40°C for 90 minutes and the reaction was terminated by addition of 6 drops of glacial acetic acid. The acetylation was conducted at room temperature for 10 minutes by addition of 1-methylimidazol (0.1ml, as a catalyst) and acetic anhydride (0.5ml). The acetylation was terminated by addition of water (4ml) and the samples were partitioned against methylene chloride (1ml) two times. The methylene chloride fractions were pooled and dried under nitrogen at 45°C and redissolved in 1 ml of acetone before analysis by GC-FID (Blakeney et al., 1983).

Analysis by GC-FID was conducted using an Agilent (Agilent Technologies, Santa Clara, CA) 7890A gas chromatograph with flame ionization detector. A Supelco (Supelco, Bellefonte, PA) SP-2380 fused silica capillary column (30m x 0.25 μm x 2 μm) was used for separation. The system parameters were as follows: 0.8 ml/min flow rate, 82737 Pa flow pressure, 230°C injector temperature, 100°C initial oven temperature, 250°C detector temperature and the carrier gas was helium (Blakeney et al. 1983; Mendis and Simsek 2015). The total arabinoxylan content was calculated using the following formula: % arabinoxylan = (% arabinose + % xylose) \* 0.88 and the arabinose to xylose ratio was calculated by dividing percent arabinose by percent xylose (Henry 1986; Mendis and Simsek, 2015).

#### 5.4. Statistical Analysis

The experimental design 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 was used to declare differences between treatments. The experimental design was three-factorial arrangement with location, cultivar and millstream as main factors. Location, cultivar, and millstream interaction term was used as error term.

#### 5.5. Results and Discussion

##### 5.5.1 Effects of Location, Wheat Cultivar, and Millstreams on Quality Characteristics

In the MIAG Multomat roller mills a total of 23 millstreams were collected and analyzed, as previously described in the materials and methods. Table 5.3 shows the ANOVA for the flour yield (%) based on the total wheat that was milled.

Table 5.3. The ANOVA for Flour Yield (%) Based on the Total Milled Wheat

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	0.3	1.1	0.296
Cultivar (VAR)	4	0	0.11	0.979
Stream (STR)	22	202.1	827.48	<.0001
LOC*VAR	4	0	0.2	0.939
LOC*STR	22	0.6	2.53	0.001
VAR*STR	88	0.3	1.28	0.123

Both millstream, and location x millstream interaction had significant ( $P < 0.001$ ) effect on the flour yield. This indicates that the amount of flour produced in each millstream was different. This result is expected, as the reduction millstreams produce more flour than the break side (Machet, 2005). This is because in the break side the objective is to open up the wheat kernel and produce middlings, while the objective in the reduction side is to reduce the middlings into more fine flour. However, location and cultivar did not have significant ( $P > 0.05$ ) effect on the total flour produced. This means that the cultivars used in this study did not differ in the flour produced for different millstreams. In addition, location did not have significant ( $P > 0.05$ ) effect on the flour yield produced in millstreams. This indicates that these five cultivars from Gulf/Great Lakes and Pacific Northwest regions did not differ in the flours produced in various millstreams. Although location x millstream interaction was significant ( $P < 0.001$ ), the variation in the flour yield was mostly due to the significant difference in the millstreams due to a higher F-value (Table 5.3).

In the break side of the mill there was lower amount of flour that was produced while there was significantly ( $P < 0.05$ ) greater flour was obtained in the reduction sides, which are indicated by 1<sup>st</sup> to 6<sup>th</sup> middlings (except 5<sup>th</sup> middlings). The smaller break flour yield is desired in HRS wheat milling, because it indicates that the break roller mill produces the more middlings which are the broken endosperm pieces and free of bran fragments (Baasandorj et al., 2015). These middlings are then reduced into finer flour in the reduction side of the flour mill. The

amount of the flour produced in the break sides is controlled and influenced by the break releases. Roll gaps are adjusted to set the break releases by the miller during the milling process. The break release is reported as percentage of the original material going through a certain 20W sieve (Posner and Hibbs, 2005). Therefore, the adjustment of mill break release affects the total results and balance. In the current study, the break releases for the first, second, and third breaks were set at 30%, 53%, and 65%, respectively.

Middling streams 1, 2, and 3 produced more than 10% of the flour yield, where middling millstream 3 (M3) produced the greatest flour yield (Figure 5.1). Nelson and McDonald (1977) also reported that first middling (M1) gave the highest yield followed by M3 when milling HRS wheat cultivars using a same MIAG Multomat mill.

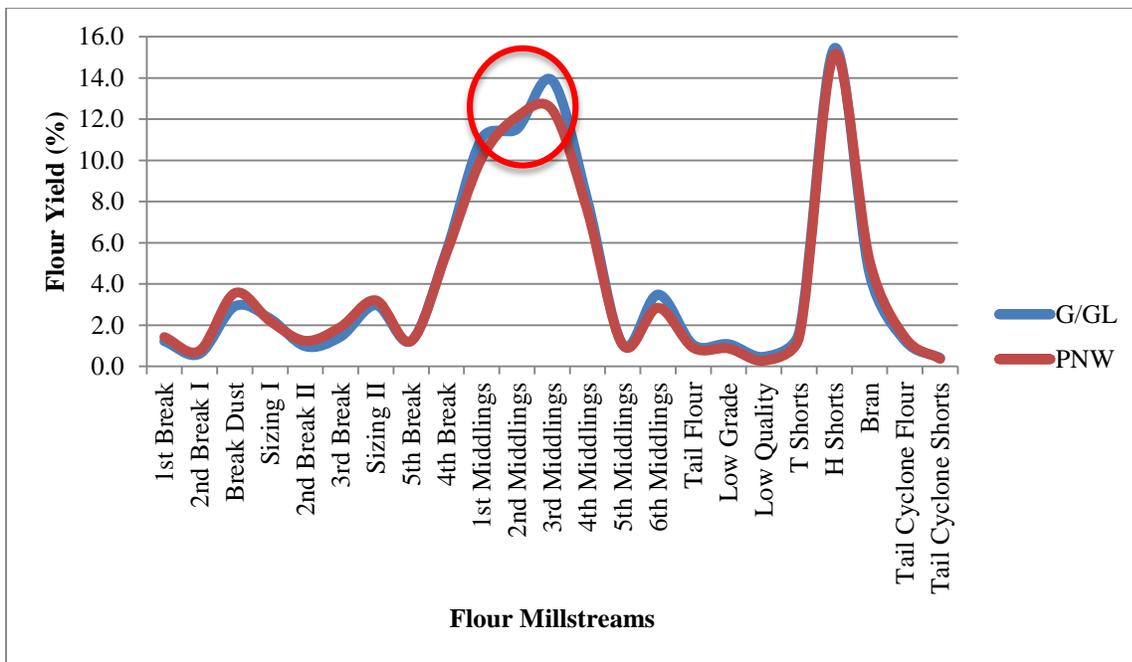


Figure 5.1. Millstream Flour Yield (%) for Two Growing Regions: Gulf/Great Lakes and Pacific Northwest (PNW)

The ANOVA also indicated that cultivar, millstream, location x cultivar, location x millstream, and cultivar x millstream interactions had significant effect on the flour ash content

(Table 5.4). This indicates that there was difference in the flour ash content between cultivars, millstreams, and interactions between the main effects.

Table 5.4. The ANOVA for Flour Ash Content (%)

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	0	0.6	0.4522
Cultivar (VAR)	4	0.1	6.3	0.0002
Stream (STR)	22	23.4	1204.4	<.0001
LOC*VAR	4	0.2	11	<.0001
LOC*STR	22	0.1	3.7	<.0001
VAR*STR	88	0	1.6	0.019

As shown in table 5.4, the most variation in the flour ash content was found for the different millstreams followed by the wheat cultivars for the main effects. In addition, the interactions between location, cultivar, and millstreams. The very high significant difference among millstreams was expected, as various millstreams have the different parts of the wheat kernel hence resulting in different flour ash content. However, there was no significant ( $P>0.05$ ) difference between growing locations thus the flour ash content was not different between Gulf/Great Lakes and Pacific Northwest locations. Wheat cultivar had a significant ( $P<0.001$ ) effect on the flour ash content. In addition, location x cultivar interaction was significant. Figure 5.2 illustrates the flour ash content for wheat cultivars when averaged across various millstreams.

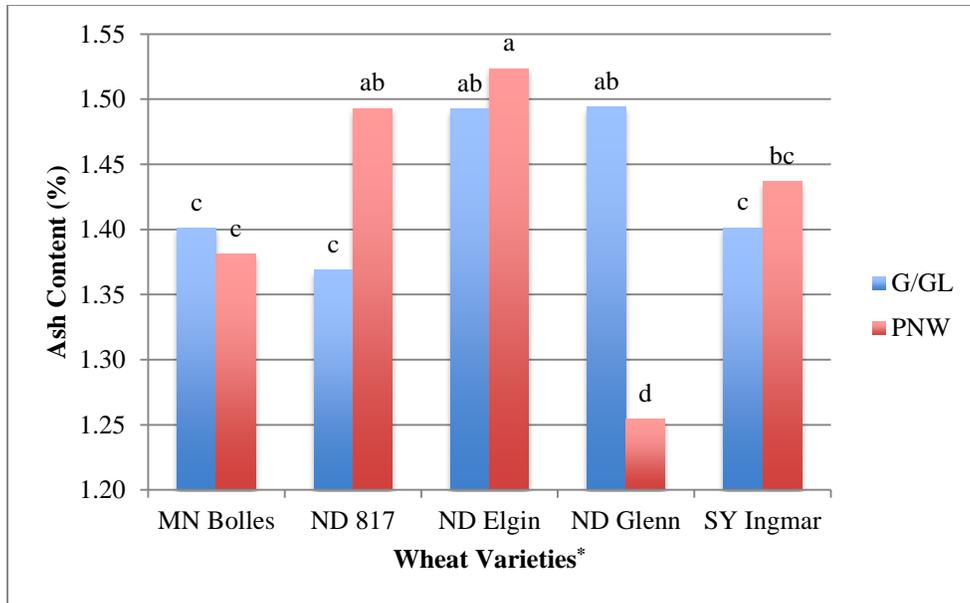


Figure 5.2. Mean of Millstream Flour Ash content for 5 HRS Wheat Cultivars for Two Growing Regions

\* Means followed by the same letter in the column are not significantly different between mill types.

ND-Elgin wheat cultivar had the highest flour ash for both growing regions, followed by SY Ingmar and MN Bolles cultivars. However, there was a large variation in the ash content between two growing regions for ND-817 and ND-Glenn cultivars. ND 817 cultivar from Gulf/Great Lakes (G/GL) region had ash content of 1.37% while Pacific Northwest (PNW) had higher ash content of 1.49%. In contrast, ND Glenn from PNW had low ash value of 1.25% while G/GL region had higher ash content of 1.49%. These differences could be due to the composite make-ups. The wheat cultivars for G/GL region consisted of Casselton, Crookston, and Watertown locations, while the cultivars for PNW region consisted of Minot and Williston locations (Table 5.1). In other words, PNW region consisted of western part of ND locations, while G/GL region consisted of eastern part of the ND locations. Therefore, there were differences in the ash content for these 5 wheat cultivars between 2 regions (G/GL and PNW), especially for ND-817 and ND-Glenn.

The ANOVA also indicated that wheat cultivars and cultivar x stream interaction were significant for flour ash combined (Table 5.4). Figure 5.3 shows the ash content of various millstreams for these 5 cultivars.

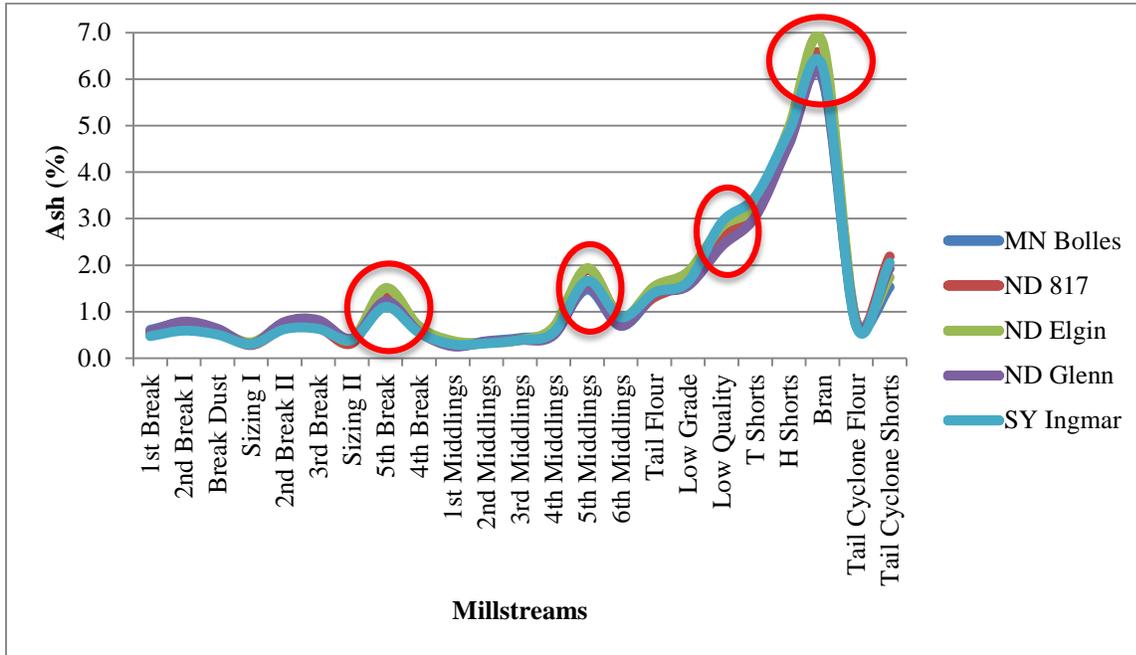


Figure 5.3. Ash Content of Various Millstreams for Five HRS Wheat Cultivars

The ash content of certain millstreams did vary among wheat cultivars. It was observed that the most of the variation in ash content was observed for streams that are high in ash content for these wheat cultivars. The millstreams that showed more variation in ash content for these cultivars included: 5<sup>th</sup> break (5B), 5<sup>th</sup> middling (5M), low quality flour (LQ), and bran streams. The 5B stream has the highest ash content among break stream flours, while 5M stream has the highest flour ash content compared to other middling streams. Similarly, wheat cultivars also had difference in the ash content for low quality flour and bran streams.

Flour protein content was also influenced by location, wheat cultivars, and millstream. The ANOVA table shows the significant effects and interactions between the main effects (Table 5.5).

Table 5.5. The ANOVA for Protein Content

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	17.9	201.2	<.0001
Cultivar (VAR)	4	39	439	<.0001
Stream (STR)	22	42.4	477.1	<.0001
LOC*VAR	4	4.8	53.9	<.0001
LOC*STR	22	0.4	4.9	<.0001
VAR*STR	88	0.9	10.2	<.0001

The main effects had significant ( $P < 0.0001$ ) effects on the flour protein. In addition, location x cultivar, location x millstream, and cultivar x millstream interactions were all significant. This indicates that protein content in millstreams were different as well as between locations and among 5 wheat cultivars. Figure 5.4 shows the protein content for 23 millstreams for 2 growing regions.

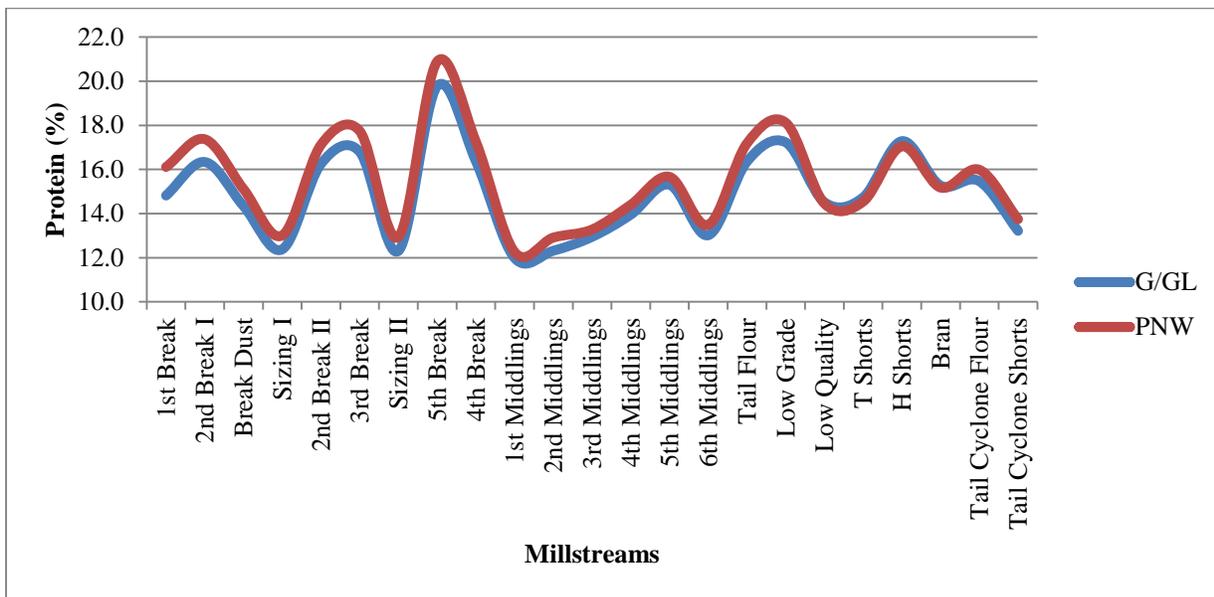


Figure 5.4. Millstream Protein Content for Two Growing Regions

PNW had more protein content for all 23 millstreams compared to G/GL region. This indicates that there was locational difference in the protein content. As stated previously, PNW region wheat cultivar samples were consisted from Minot and Williston locations for all 5 wheat cultivars (except for ND-Elgin), while G/GL region was consisted of Casselton, Crookston, and

Watertown locations. These results indicate that growing environment was different between these locations, as the protein content was significantly ( $P < 0.0001$ ) different for two regions. When comparing wheat cultivars, the protein content was different among 5 wheat cultivars. Figure 5.5 clearly illustrates the difference in the protein content for wheat cultivars across all millstreams.

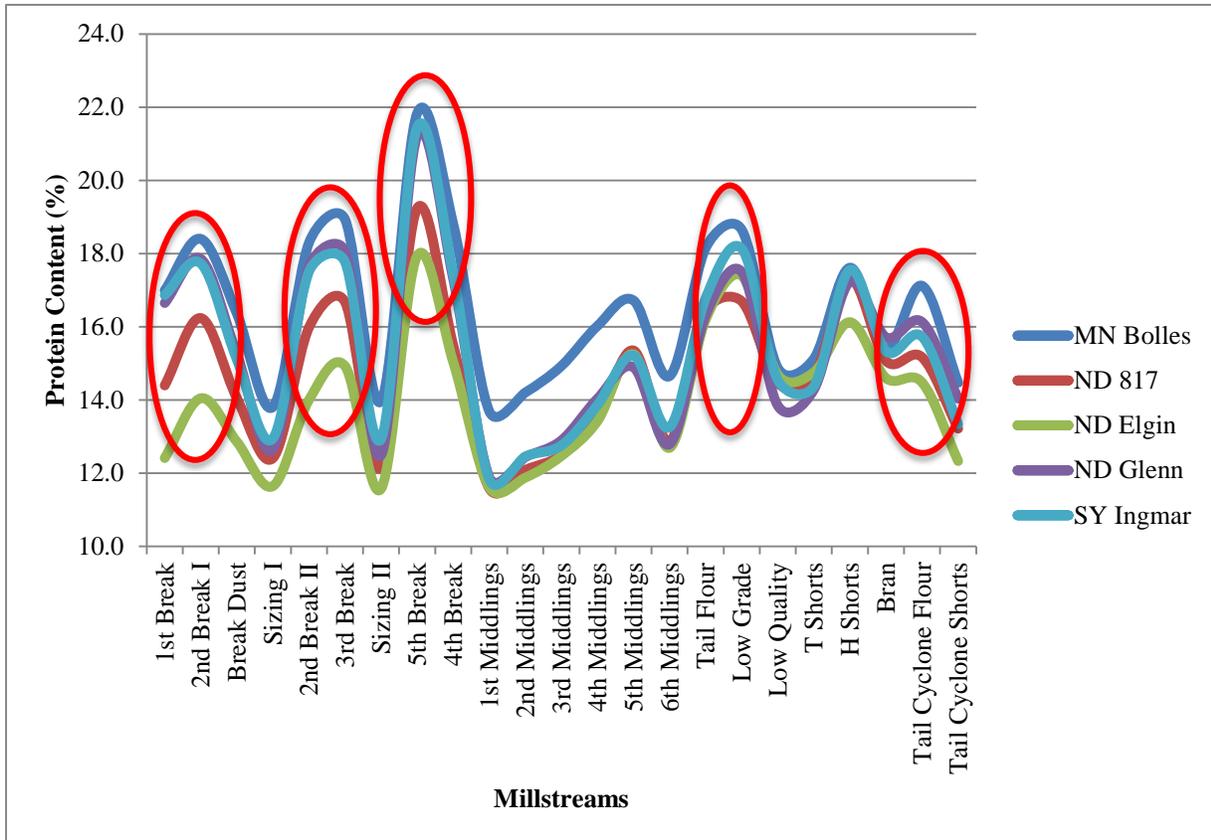


Figure 5.5. Millstream Protein Content for 5 Wheat Cultivars

Flour protein content varied among wheat cultivars. It could be seen that wheat cultivars had difference in the protein content, which was consistent across all millstreams. However, the difference was much greater for millstreams that are high in protein content for these wheat cultivars. This trend was clearly seen and consistent across millstreams such as 2<sup>nd</sup> Break I, 2<sup>nd</sup> Break II, 3<sup>rd</sup> Break, 5<sup>th</sup> Break, Tail Flour, Low Grade Flour, and Tail Cyclone Flour. For example, ND-Elgin had the lowest protein content for these millstreams followed by ND 817,

while MN-Bolles wheat cultivar had the highest protein content followed by ND-Glenn samples. These differences could be due the wheat protein for these wheat cultivars. Overall across millstreams, MN-Bolles had significantly higher protein content of 16.5% followed by ND-Glenn and SY-Ingmar with 15.4%. In contrast, ND-Elgin and ND-817 cultivars had protein content of 14.0% and 14.7%, respectively. Therefore, the protein content difference for these cultivars were consistently seen across millstreams, especially millstreams that are high in protein content.

Flour color for was determined for these wheat cultivars. The ANOVA indicated that only millstream had significant ( $P < 0.0001$ ) effect on flour color  $L^*$  value, which measures the lightness or brightness in flour (Table 5.6). This is expected because not only there are flour millstreams but also millstreams that are not included in the straight-grade flour. Millstreams that are high bran content are generally darker in color resulting in lower  $L^*$  values. In addition, location x millstream showed significant ( $P < 0.0001$ ) effect on the  $L^*$  value.

Table 5.6. ANOVA for Color ( $L^*$ ) Values

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	0.8	1.5	0.2310
Cultivar (VAR)	4	1.1	2	0.0999
Stream (STR)	22	971	1734.8	<.0001
LOC*VAR	4	0.5	1	0.4395
LOC*STR	22	1.9	3.3	<.0001
VAR*STR	88	0.4	0.7	0.9289

Figure 5.6 shows the  $L^*$  color values content for 2 different regions.

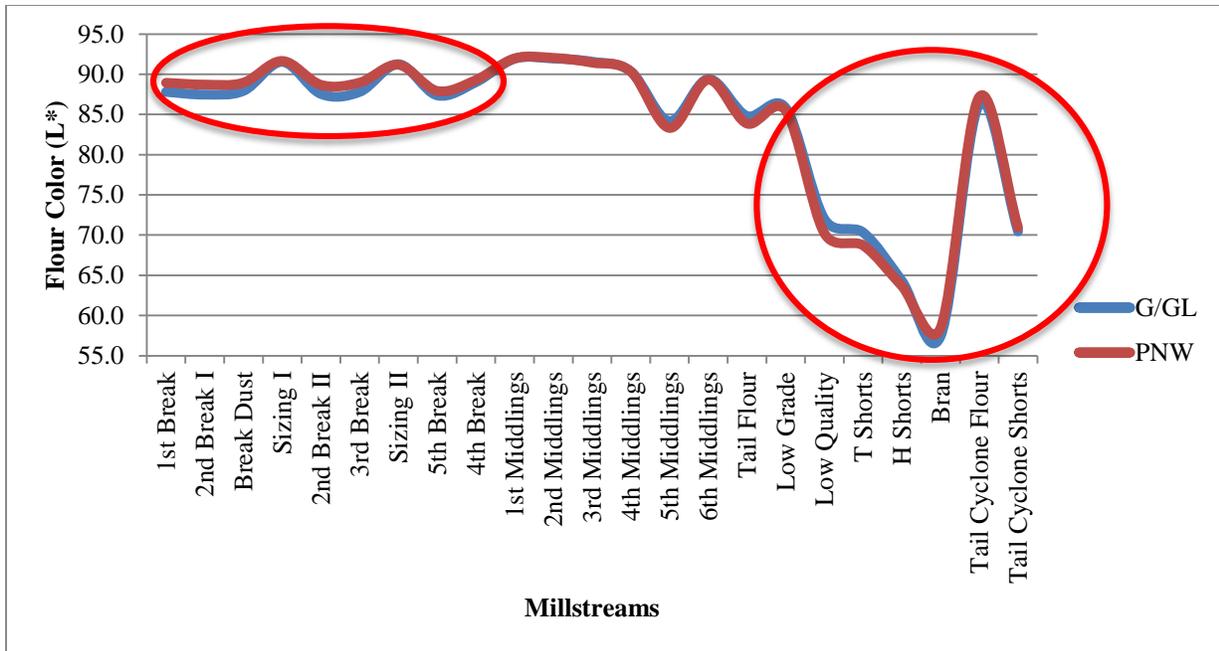


Figure 5.6. Millstream Flour L\* Color Values for G/GL and PNW Regions

G/GL region had lower L\* values for break millstreams compared to PNW region. This low L\* means that flours in break millstreams for G/GL was duller compared to PNW region (Figure 5.6). However, there was no difference in the L\* values for these 2 regions in the middlings millstreams. In addition, there was a significant and negative correlation ( $P < 0.001$ ,  $r = -0.95^{***}$ ) between millstream ash content and flour color L\* values. These result indicate that flour color L\* value decreased as the ash content increased in certain millstreams. The negative association between L\* value and ash content was clearly especially for break millstreams, low quality flour, and bran millstreams. As mentioned previously, G/GL region had lower L\* values for break millstreams. Consequently, these break millstreams high higher ash content compared for G/GL compared to PNW region (Figure 5.7).

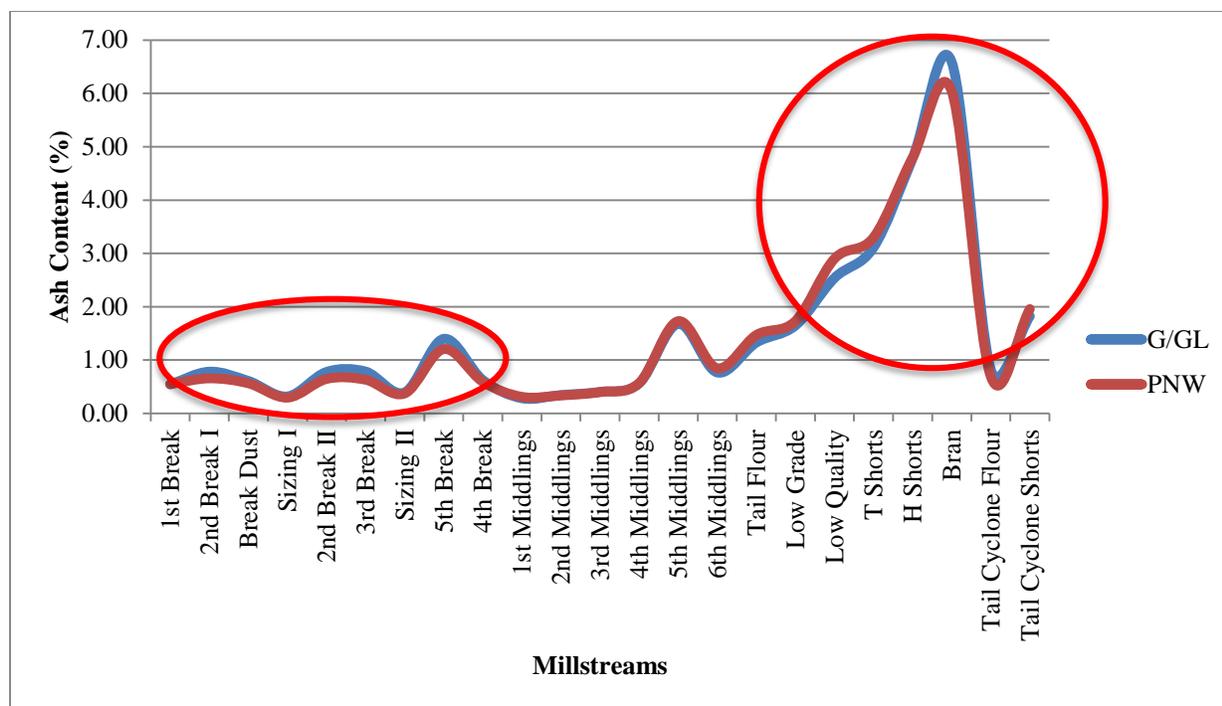


Figure 5.7. Millstream Flour Ash Content for G/GL and PNW Regions

In addition, this negative association was clearly observed for tail shorts and bran millstreams. High ash content in G/GL region had lower  $L^*$  values. The main effects location, cultivar, and millstream also had significant ( $P < 0.0001$ ) effects on the starch damage. In addition, the location x cultivar, location x millstream, and cultivar x millstream showed significant ( $P < 0.01$ ) effects on the starch damage (Table 5.7).

Table 5.7. ANOVA for Starch Damage

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	44.9	134.3	<.0001
Cultivar (VAR)	4	6.4	19.1	<.0001
Stream (STR)	22	72.9	218	<.0001
LOC*VAR	4	9.6	28.8	<.0001
LOC*STR	22	0.8	2.3	0.0037
VAR*STR	88	0.6	1.7	0.0056

These results indicate that there was difference in the starch damage between 2 regions, as well as among wheat cultivars and flour millstreams. All 5 wheat cultivars from G/GL region

had higher damaged starch content compared to PNW region (Figure 5.8). On average, ND-817 had significantly ( $P<0.05$ ) higher starch damage followed by ND-Glenn and ND-Elgin, while MN-Bolles and SY-Ingmar cultivars had significantly lower starch damage.

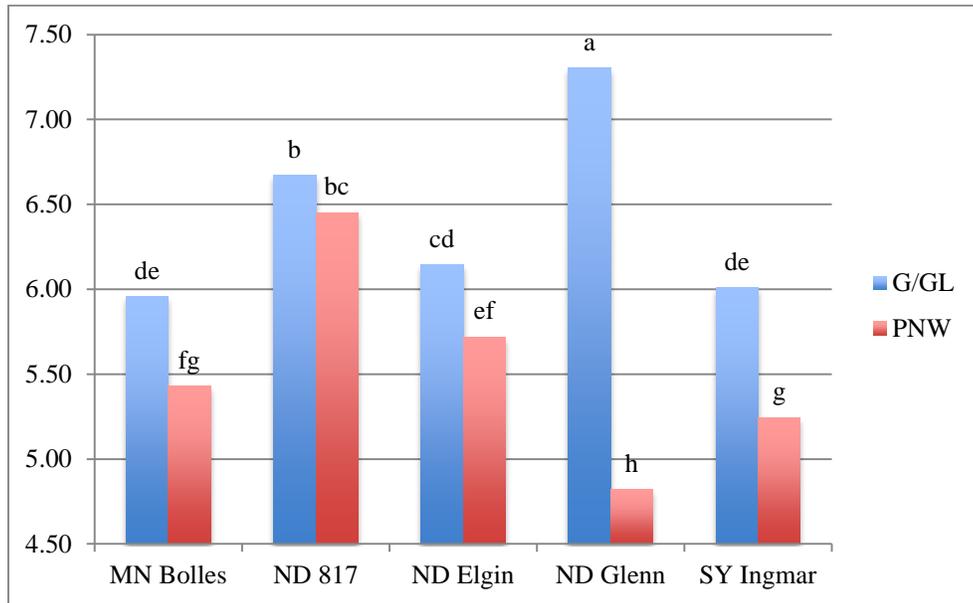


Figure 5.8. Starch Damage for Wheat Cultivars from Two Growing Regions

\* Means followed by the same letter in the column are not significantly different between mill types.

Starch damage was also determined for each millstream for two growing regions, as the location x millstream interaction had significant ( $P<0.0001$ ) effect on the starch damage. Starch damage was consistently greater for all millstreams for G/GL growing region (Figure 5.9).

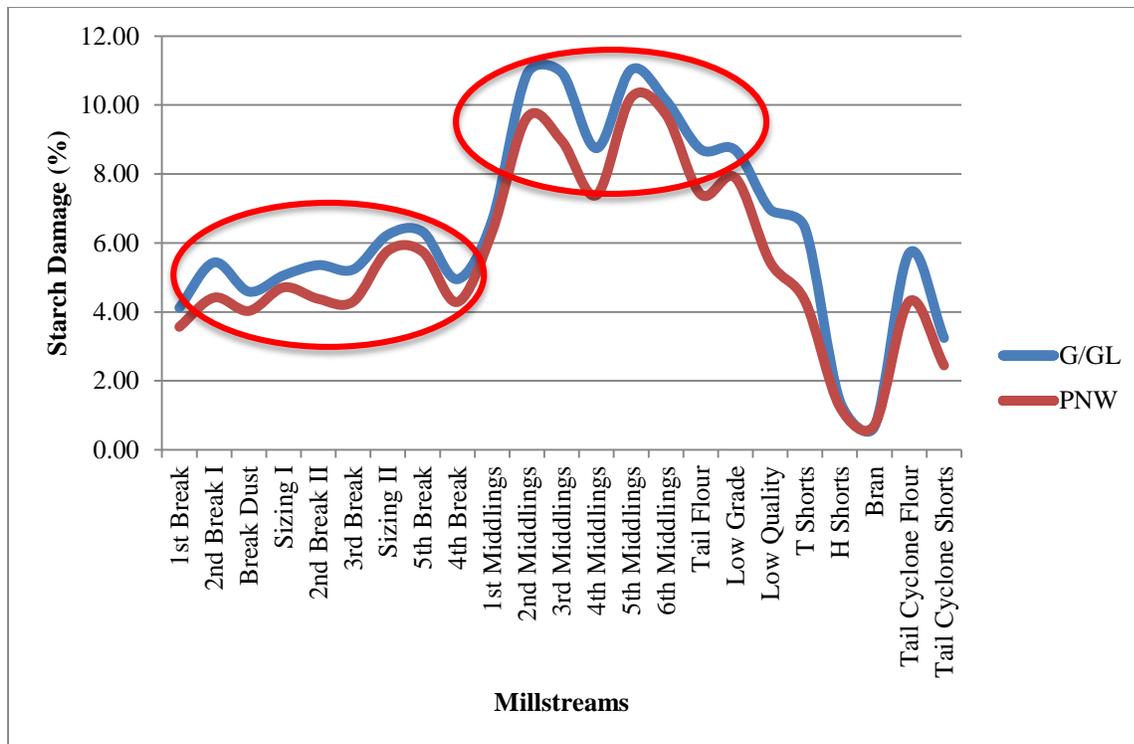


Figure 5.9. Millstream Starch Damage for Two Growing Regions

Break millstreams had significantly ( $P < 0.05$ ) lower flour starch damage, while reduction or middlings millstreams had higher starch damage. This result was consistent for both G/GL and PNW regions. Starch damage is influenced by the roller surface of the mill (Machet, 2005). Low starch damage in the break millstreams is expected because corrugated rolls in the break side impart low starch damage. In contrast, these intermediate stocks from the break millstreams go through many smooth reductions rolls, which result in higher flour starch damage content. These findings in the current study are in agreement with Black et al. (1981) and Holas and Tipples (1978), who reported that break flours had lowest starch damage values. More recently, Lindgren and Simsek (2015) also reported that reduction millstreams had significantly ( $P < 0.05$ ) higher flour starch damage.

Lastly, arabinoxylan content was determined for individual millstreams. Similar to starch damage, the main effects location, cultivar, and millstream had significant ( $P < 0.05$ ) effect on the arabinoxylan content (Table 5.8).

Table 5.8. The ANOVA for Arabinoxylan Content (%)

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	103.6	16.5	0.0001
Cultivar (VAR)	4	19.7	3.1	0.0182
Stream (STR)	22	783.3	125	<.0001
LOC*VAR	4	25.2	4	0.0049
LOC*STR	22	14.6	2.3	0.0028
VAR*STR	88	10.9	1.8	0.0048

The interactions between the main effects also showed significant difference for arabinoxylan content. These results indicated that arabinoxylan content was different among 2 regions as well as wheat cultivars and different millstreams. Figure 5.9 shows the arabinoxylan content for all millstreams for G/GL and PNW regions.

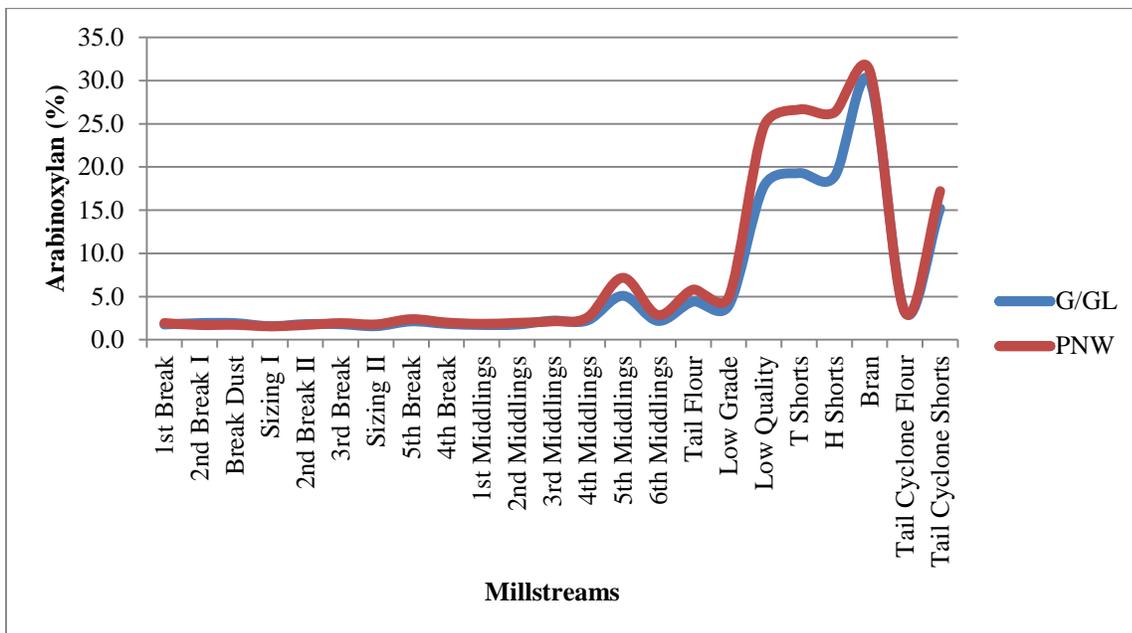


Figure 5.10. Millstream Arabinoxylan Content (%) for Two Regions

PNW region had higher arabinoxylan content in various millstreams, especially for 5<sup>th</sup> middlings, tail flour, low quality flour, tail shorts, head shorts, and bran millstreams (Figure 5.9). These millstreams contain high in aleurone layer, which will increase arabinoxylan content (Lindgren and Simsek, 2015). For example, 5<sup>th</sup> middlings, tail flour, and low grade flours have much higher arabinoxylan content compared to other flour millstreams (break and middlings millstreams). These millstreams also had higher ash content. There was a strong association between ash and arabinoxylan content ( $P < 0.001$ ,  $r = 0.94$ ), which indicates that streams with higher ash content had greater arabinoxylan content. It was also found that cultivar x millstream interaction had significant ( $P < 0.01$ ) effect on the arabinoxylan content. Figure 5.10 shows the arabinoxylan content (%) for wheat cultivars across millstreams.

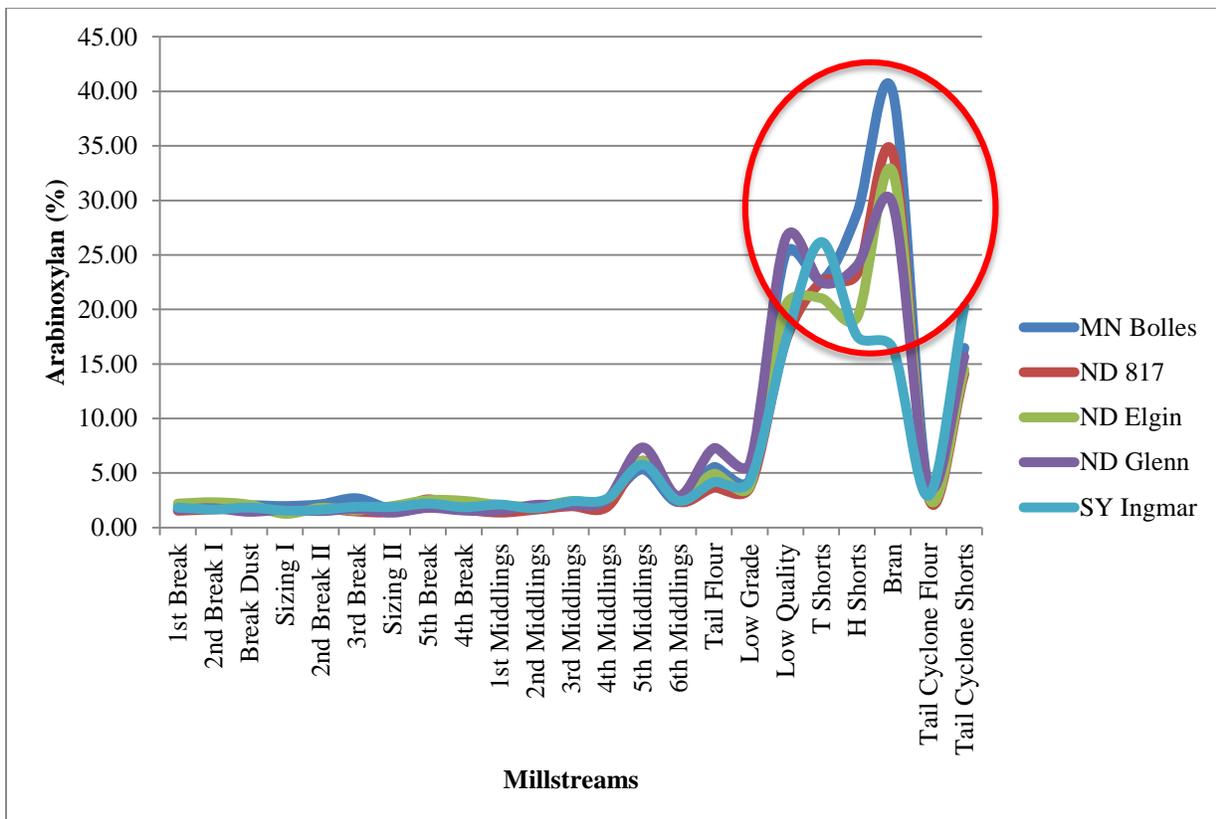


Figure 5.11. Millstream Arabinoxylan Content (%) for Various HRS Wheat Cultivars

As can be seen from the figure 5.10, the latter millstreams showed much variation in the arabinoxylan content. These millstreams are very high in ash content thus are not included in the straight grade flour. Large variation in the arabinoxylan content for these millstreams might have led to a significant difference for the cultivar x millstream interaction. However, there was not much variation in the arabinoxylan content among wheat cultivars for the millstreams that are include in the straight grade flour. This indicates that during the milling process the endosperm reduced by the break and reduction rolls is the farthest from the aleurone layer, thus resulting in lower arabinoxylan for these millstreams (Lindgren and Simsek, 2015).

#### 5.5.2. The Evaluation of Flour Millstreams on Flour Quality Characteristics

In the previous section, the effects of location, cultivar, and millstreams were discussed. The main effects and their interaction showed significant effect on quality characteristics. However, in this section the evaluation of these millstreams will be discussed when averaged across locations and wheat cultivars. MIAG Multomat roller mill produces total of 23 different millstreams. The variation in composition of the different millstreams has been the subject of many studies. Depending on the stage of the milling process the millstreams have varying total flour yield, ash and protein content, starch damage, and arabinoxylan content (Machet, 2005). Table 5.9 shows the flour quality parameters for MIAG millstreams when averaged across location and cultivars.

Table 5.9. Flour Quality Characteristics for MIAG Millstreams

Stream	Flour Yield	Ash Content	Protein Content	Color			Starch Damage	AX
	(%)	(%)	(%)	L*	a*	b*	(%)	(%)
1st Break	1.3	0.55	15.5	88.4	-0.73	9.2	3.8	1.9
2nd Break I	0.7	0.72	16.9	88.1	-0.68	9.4	4.9	1.8
Break Dust	3.2	0.59	14.7	88.5	-0.76	9.9	4.3	1.8
Sizing I	2.3	0.31	12.7	91.6	-1.38	9.8	4.9	1.6
2nd Break II	1.1	0.71	16.7	88.1	-0.70	9.6	4.9	1.8
3rd Break	1.7	0.71	17.3	88.4	-0.72	10.1	4.8	1.9
Sizing II	3.1	0.39	12.6	91.2	-1.26	9.5	6.0	1.7
5th Break	1.2	1.30	20.3	87.7	-0.52	9.8	6.0	2.3
4th Break	5.6	0.61	16.8	89.1	-0.88	10.0	4.6	1.9
1st Middlings	10.6	0.30	12.1	92.0	-1.34	9.0	6.5	1.8
2nd Middlings	11.8	0.34	12.6	92.0	-1.26	8.5	10.3	1.9
3rd Middlings	13.2	0.40	13.1	91.5	-1.17	8.8	10.0	2.2
4th Middlings	7.8	0.57	14.2	90.3	-0.95	9.0	8.1	2.4
5th Middlings	1.1	1.71	15.5	83.7	0.96	10.0	10.6	6.1
6th Middlings	3.2	0.81	13.3	89.4	-0.58	8.8	9.9	2.5
Tail Flour	1.0	1.40	16.8	84.3	0.46	10.1	8.1	5.1
Low Grade	1.0	1.69	17.7	85.6	0.07	9.7	8.3	4.4
Low Quality	0.4	2.72	14.5	71.1	3.64	11.8	6.2	21.2
Tail Shorts	1.5	3.21	14.6	69.5	4.08	12.1	5.4	23.0
Head Shorts	15.3	4.79	17.2	64.0	5.08	13.1	1.3	22.6
Bran	4.8	6.31	15.2	58.0	7.15	14.8	0.7	30.6
Tail Cyclone Flour	1.3	0.76	15.7	86.7	-0.35	9.9	5.0	3.2
Tail Cyclone Shorts	0.4	1.89	13.5	70.7	3.27	12.3	2.8	16.2
LSD* (P<0.05)	0.4	0.12	0.3	0.7	0.15	0.2	0.5	2.2
LSD (P<0.01)	0.6	0.16	0.4	0.9	0.21	0.3	0.7	2.9

\* LSD – Least Significant Difference

Total flour yield produced in millstreams were significantly different across millstreams. Total flour yields on the reduction sides are always greater than that of the break side (Machet, 2005). Reduction or middlings millstreams accounted for 47.6% of the total flour yield, while break and sizing millstreams combined 20.2% of the total flour yield. The smaller break flour yield is desired in HRS wheat milling, because it indicates that the break roller mill produces the more middlings which are the broken endosperm pieces and free of bran fragments (Baasandorj

et al., 2015). In addition, the amount of flour produced from break and reduction side of the mill is very important because the adjustment of mill break releases affects the total results and mill balance (Posner and Hibbs, 2005). When comparing middlings (M) millstreams, middlings 1, 2, and 3 accounted for the most of the flour yield produced in middlings streams, accounting for 35.6% (Table 5.9). Nelson and McDonald (1977) also reported that M1, M2, and M3 streams accounted for the most of flour produced in the reduction side when milling HRS wheat cultivars on the MIAG-Multomat mill. However, these authors reported that first middling (M1) gave the highest flour yield (12.7-14.7%) followed by M3 (11.0-12.9%). In the current study, M3 gave the highest flour yield (13.2%) followed by M2 (11.2%) (Table 5.9). This difference could be due to the varietal difference as well as the pilot mill flour extraction rate.

Holas and Tipples (1978) also reported that M2 had the highest yield (16.5%) followed by M3 (14.0%) and M1 (11.5%) when milling No. 1 Canadian Western Red Spring (CWRS) on a commercial scale Buhler mill. Black et al. (1981) also that highest proportion of flour was produced on the reduction rolls, with M1 and M2 accounting for about 30% when they milled CWRS wheat on the Grain Research Laboratory Pilot Mill. In addition, Martin and Dexter (1991) also reported that M1 always gave the highest flour yield of 20% when milling red spring wheat on a Buhler laboratory mill. The variation in the total flour yield in these middlings streams could be due to the different types of mills and wheat cultivars with different grain hardness. However, middlings millstreams always produced the highest flour yield regardless of the mill type and wheat cultivars in these studies, which is in agreement of the results found in the current study.

It is well established that both ash and protein content have increasing gradients from the inner to the other endosperm. The flour obtained from the outer layers of the wheat kernel has

different composition than the flour obtained from the center of the wheat kernel during the early stages of milling (Orth and Mander, 1975). The flour that comes from the center is the whitest and lowest in protein and ash content, while flour from the other part of the kernel is the darkest and highest protein and ash contents. Later reduction flours are higher in protein and ash content than the early reduction flours (Machet, 2005). Therefore, during the milling process, the increase in the flour ash content occurs normally in the latter stages of the reduction process where total flour is increased by regrinding and re-sieving bran rich streams. This is because the incorporation of relatively small quantity of bran particles (including aleurone cells) account for considerable increase in ash content (Machet, 2005).

Ash content was significantly different between break and reduction millstreams. Break millstreams (1B, 2B, 3B, 4B, and 5B) had average ash content of 0.77%, while reduction or middling streams had lower flour ash content of 0.69%. However, it is important to note that 5<sup>th</sup> middlings stream had very high ash content of 1.71% hence this stream was not included in the straight-grade flour (Table 5.9). The ash content for reduction streams (excluding the 5<sup>th</sup> midds.) was 0.48%, which significantly lower than the break millstreams. The lower flour ash in reduction millstreams was expected because flours in these streams are re-sifted. It was also observed that sizing I (S1) and 1<sup>st</sup> middlings (M1) millstreams had the lowest ash content of 0.31 and 0.30%, respectively. This is because these millstreams are because sizing flours and first reduction flours are the most highly refined flour millstreams (Izydorczyk et al., 2003). In other words, these millstreams are the least contaminated by non-endosperm material. Black et al. (1981) and Preston and Dexter (1994) also reported that M1, M2, S1, and S2 streams had the lowest ash contents of all millstreams when milling CWRS wheat on a pilot mill. As previously mentioned, 5<sup>th</sup> middlings had very high flour ash content of 1.71 thus this stream was not

included. Because of the flour yield for this stream was relatively low (1.1%) (Table 5.9), the decision was made to not include 5<sup>th</sup> middlings in the straight grade flour due to the very high flour ash content. Also, 6<sup>th</sup> middlings stream also had high ash content (0.86%) but was included in the straight grade flour because this stream accounted for 3.2% of the total flour yield (Table 5.9). Wang and Flores (2000) also found the ash content to be high for last reduction and bran flour millstream but concluded that the ash content of the straight-grade flour was not greatly affect because flour yield for these streams were low.

The protein content increases from the center of the wheat kernel to the outer layers. The protein gradient in the wheat kernel results in different qualities and quantities of protein in various millstreams (Wang and Flores, 2000). The protein content was significantly ( $P < 0.05$ ) different among various millstreams (Table 5.9). There was a wide variation in the protein content from 12.6% to 20.3% when averaged across locations and wheat cultivars. The break flours along with tail cyclone flour had very high content with average of 17.0%, while reduction millstreams had much lower protein content average of 13.5%. Low protein content in reduction millstreams is expected because these millstreams are derived from stocks consisting largely of lower protein inner endosperm particles, which possess lower protein content than the break flours (Nelson and McDonald 1977; Prabhasankar et al., 2000).

Protein content gradually increased from 15.5% to 20.3% for 1<sup>st</sup> to 5<sup>th</sup> break millstreams (Table 5.9). These results are in agreement with Nelson and McDonald (1977), Endo et al. (1987), Prabhasankar et al. (2000), who also found that protein content increased from the first to the last break millstream. Black et al. (1981) also reported that four break flours along with bran flour had very high protein content for CWRS wheat. Similarly, the protein content for reduction millstream also increased from first to fifth reduction millstreams, 12.1% to 15.5% respectively

(Table 5.9). Lastly, sizing flours I and II also had very low protein content of 12.7% and 12.6%, respectively. Although there was large variation between break and reduction millstreams, the increasing protein content in latter break and reduction flour was largely attributed to a concentration of sub-aleurone endosperm of high protein content in these millstreams (Machet, 2005).

Flour color has been extensively studied as a means to flour refinement. In general, there is a negative association between flour ash content and the flour color measured by brightness ( $L^*$ ) value (Machet, 2005). In the current study, there was a very strong negative association between flour ash content and brightness value ( $P < 0.001$ ,  $r = -0.95$ ). Figure 5.12 illustrates the association between flour ash content and flour color brightness value for different millstreams.

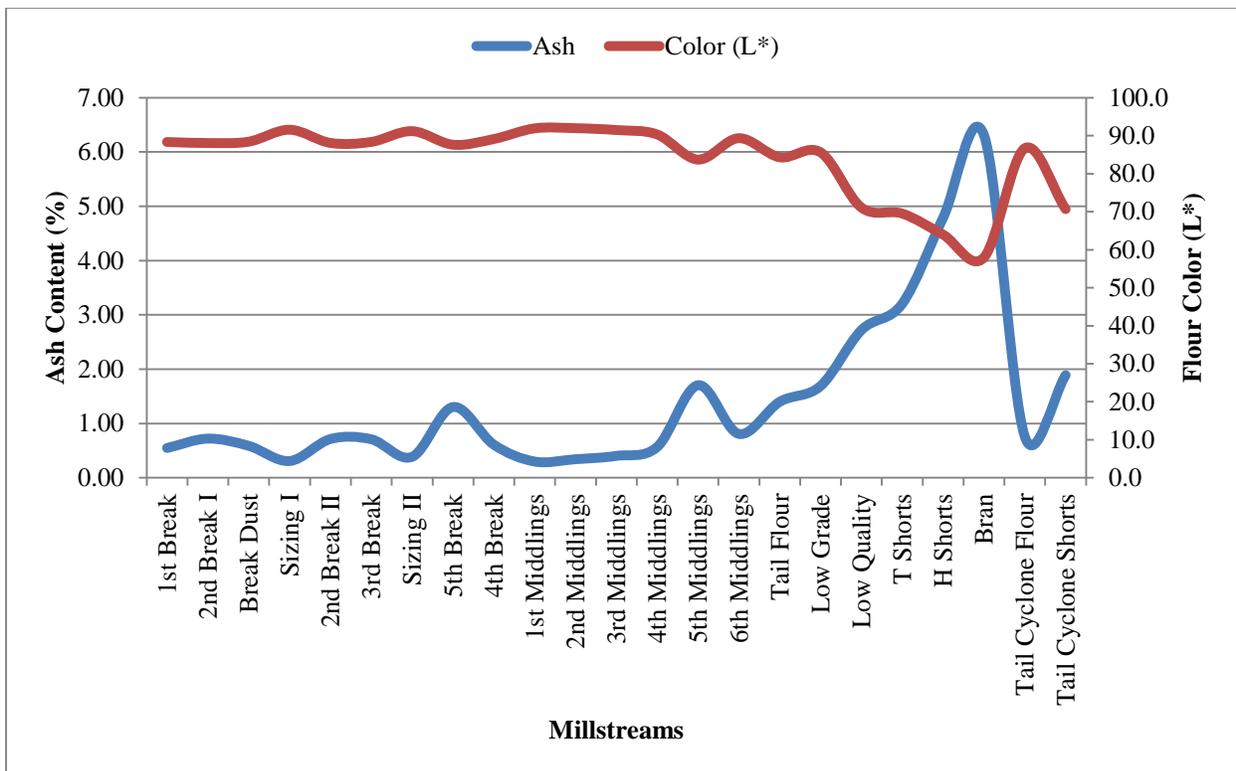


Figure 5.12. The Association Between Flour Ash Content and Brightness ( $L^*$ ) Value for Millstreams

Break flours had lower brightness average value of 88.3, while reduction millstreams had higher brightness value of 89.8. Because 5<sup>th</sup> middlings was not included in the straight grade flour, the reduction millstreams had higher brightness of 91.0 value when excluding the 5<sup>th</sup> middlings stream. The break millstreams had higher ash content of 0.77 (Table 5.9) thus resulting in duller looking flour with 89.8 brightness value. Conversely, reduction millstreams had lower ash content average of 0.48 with brightness value of 91.0. These results indicate that the most refined streams have the brightest color, while high ash streams have the darkest color. These results are in agreement with Wang and Flores (2000), who found that M1, M2, M3 flours were brighter compared with other millstream flours. Therefore, these results indicate that reduction flours are more desirable compared to break millstreams due to the low ash content and brighter color.

Among the factors that increase starch damage in an individual roller mill are finer, flatter corrugations, endosperm hardness, higher roll differentials, higher roll temperature, and finer aperture on sieves (Posner, 2009). In contrast, the factors that decrease starch damage are larger-diameter rolls on reductions, longer roll surfaces, lower differentials on smooth rolls, and less pressure between grinding rolls. In the break system of the roller mill, the corrugated rolls are employed thus impart low starch damage. However, in the reduction system smooth rolls impart higher flour starch damage. Therefore, the break millstreams possess the lowest starch damage values, while reduction millstreams tend to have higher starch damage content (Machet, 2005). Starch damage was determined for different millstreams. It was found that break millstreams had significantly lower starch damage, while reduction or middlings millstreams had greater flour starch damage (Table 5.9). Figure 5.13 illustrates the starch damage for different millstreams.

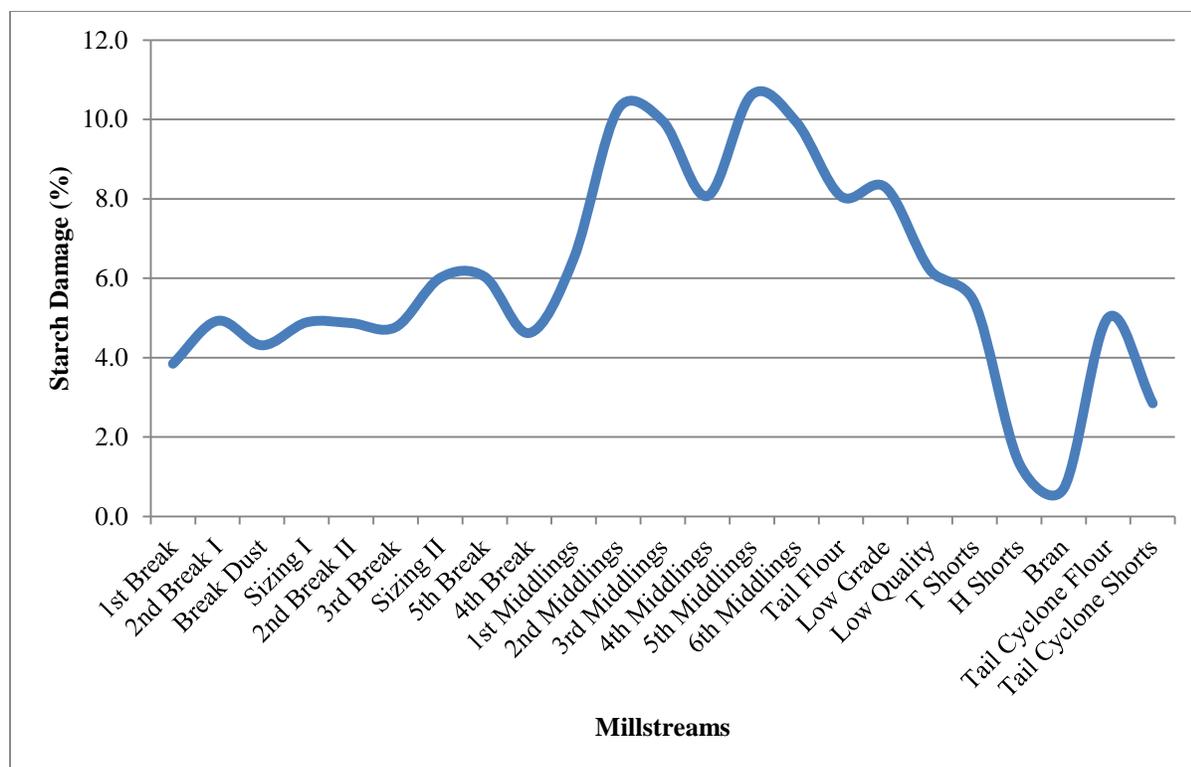


Figure 5.13. Millstream Starch Damage (%)

Starch damage in the break millstreams ranged from 3.84% to 6.03%. This was significantly ( $P < 0.05$ ) lower compared to the reduction millstreams (Figure 5.13). The reduction millstreams varied from 6.50% to 10.62%. These results indicate the break flours have lower flour starch damage, while reduction flours have greater flour starch damage. These results are in agreement with Black et al. (1981) and Holas and Tipples (1978), who reported that break flours had the lowest starch damage values. It was also reported that the highest starch damage values were associate with M5 and M6 flours (Wang and Flores, 1999). In the current study; however, M2 and M5 were found to have the greatest starch damage values of 10.26% and 10.62%, respectively (Table 5.9).

The variation in the starch damage between break and reduction millstreams could be partly explained by the particle size differences in these millstreams. It was found that starch

damage was negatively correlated with coarser particles, while fine particles had positive correlation with starch damage (Table 5.10).

Table 5.10. The Correlation Coefficients between Sieve Openings and Flour Quality Characteristics

Sieve Opening (um)	Ash Content (%)	Protein Content (%)	Color (L*)	Starch Damage (%)	Arabinoxylan (%)
600	0.88***	0.06ns	-0.73***	-0.53***	0.68***
500	0.52***	0.10ns	-0.56***	-0.43***	0.47***
425	0.52***	0.13*	-0.55***	-0.41***	0.48***
250	0.56***	0.04ns	-0.67***	-0.03***	0.66***
150	0.28***	-0.15*	-0.54***	-0.29***	0.50***
100	0.06ns	0.22**	-0.11ns	0.2***	0.10ns
50	-0.82***	0.03ns	0.91***	0.51***	-0.87***
<50	-0.67***	-0.37***	0.71***	0.23***	-0.62***

\*, \*\*, and \*\*\* represent significance at 0.05, 0.01, and 0.001, respectively. ns – not significant

These results indicate that coarser particle size flour had less starch damage; however, finer particle size flour were associated with greater starch damage. Therefore, flours obtained from the break millstreams had low starch damage values compared with high starch damage values found in the reduction millstreams. This could indicate that flour particle size in these millstreams could be different, in which could explain the differences in starch damage observed for various millstreams. The relationship between flour particle size and starch damage is shown in Figure 5.14. Although the correlation between flour particle size and starch damage (Table 5.10), it was observed that low starch damage values in break millstreams were associated with greater percentage of coarse flour particle size, while high starch damage values in reduction millstreams were associated with higher percentage of flours retained over 50um and less 50um sieves.

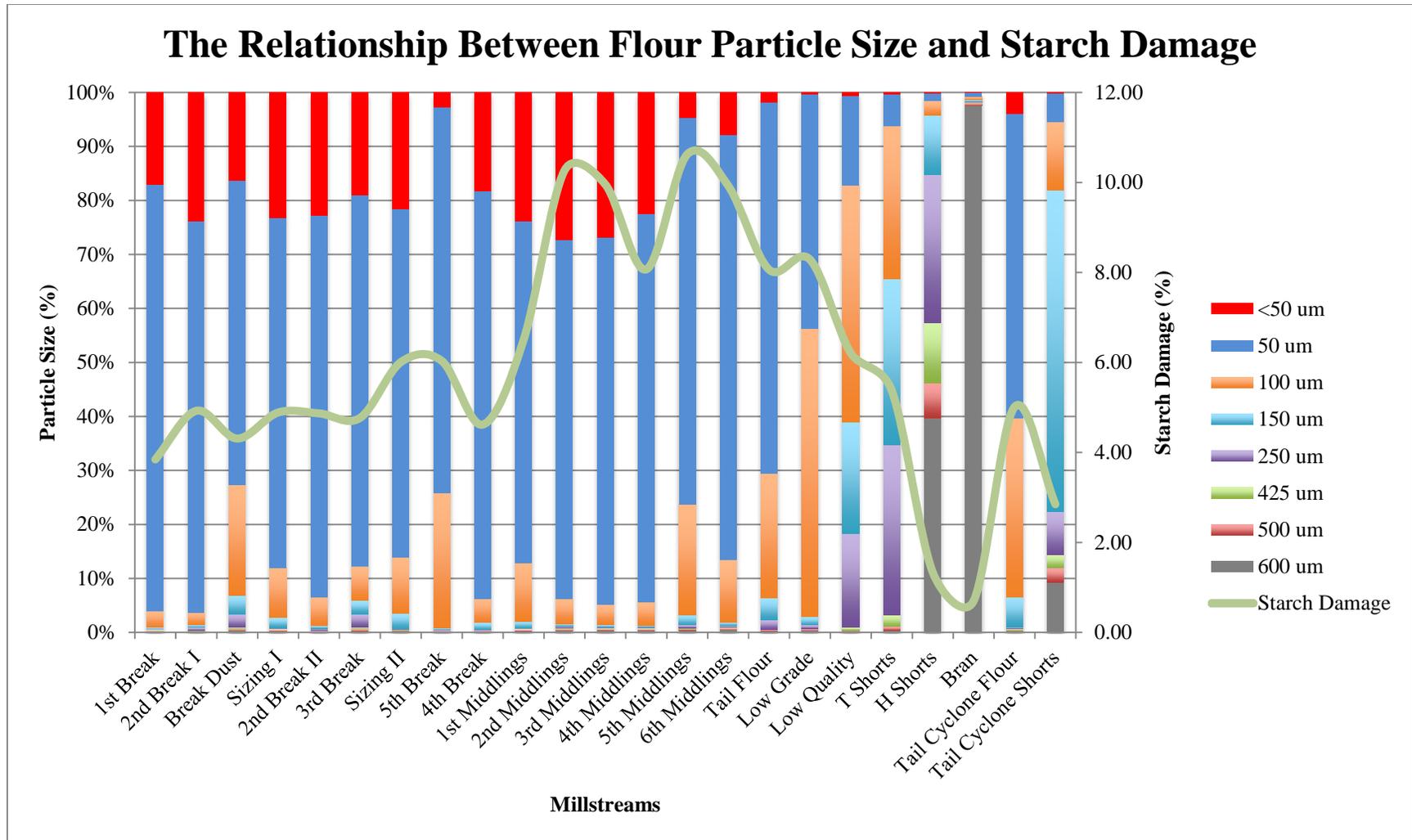


Figure 5.14. The Particle Size Distribution for Different Millstreams

These results indicate that damaged starch of flour fractions decreased with increase in flour particle size, while starch damage increased with decrease in particle size. The results are in agreement with Wang and Flores (2000), who also reported that starch damage had negative association with flour particle size.

Arabinoxylans (AX) are the major non-starch polysaccharides (NSP) in the cell walls of wheat endosperm and bran (Wang et al., 2006). Similar to mineral and protein, arabinoxylans are not distributed uniformly in the wheat kernel. Although the arabinoxylan content is relatively low (2-3%, w/w) in the straight grade flour, these polymer play important role in breadmaking (Wang et al., 2006). It has been reported that water-extractable AX (WE-AX) increased dough foam structure resulting in higher bread loaf volumes with finer and more homogeneous crumb (Courtin and Delcour, 2002). In contrast, water-unextractable AX (WU-AX) can form physical barriers for the gluten network during dough development thus lowering dough foaming structures. Therefore, WU-AX result in lower loaf volumes with coarse crumb and higher crumb firmness. Although AX content is relatively low in the straight grade flour, different millstreams vary in the arabinoxylan content (Wang et al., 2006).

The arabinoxylan content was determined for different millstreams to investigate the variation of AX between break and reduction millstreams. The arabinoxylan content (%) varied from 1.76% to 2.52% in both break and reduction millstreams with the exception of 5<sup>th</sup> reduction or middlings stream (Table 5.9 and Figure 5.15). M5 stream was found to have very high AX of 6.13%. The break millstreams had lower AX average content of 1.91%, while reduction millstreams had an average AX of 2.15% excluding the 5<sup>th</sup> middlings stream. Therefore, the reduction millstreams were slightly higher AX content compared to the break millstreams. It was found that both B5 and M5 streams had higher AX content compared to other break and

reduction millstreams. This indicates that streams containing higher ash content yield higher AX%. There was a very high and positive correlation between ash content and AX content ( $P < 0.001$ ,  $r = 0.88$ ). B5 millstream had ash content of 1.30% yielded AX of 2.26%, while M5 stream ash content of 1.71% yielded AX content of 6.13% (Table 5.9). Wang et al. (2006) also reported that M1 and M2 streams yielded lower total AX content, while M5, M6, and bran flour (higher ash content streams) yielded higher total AX content.

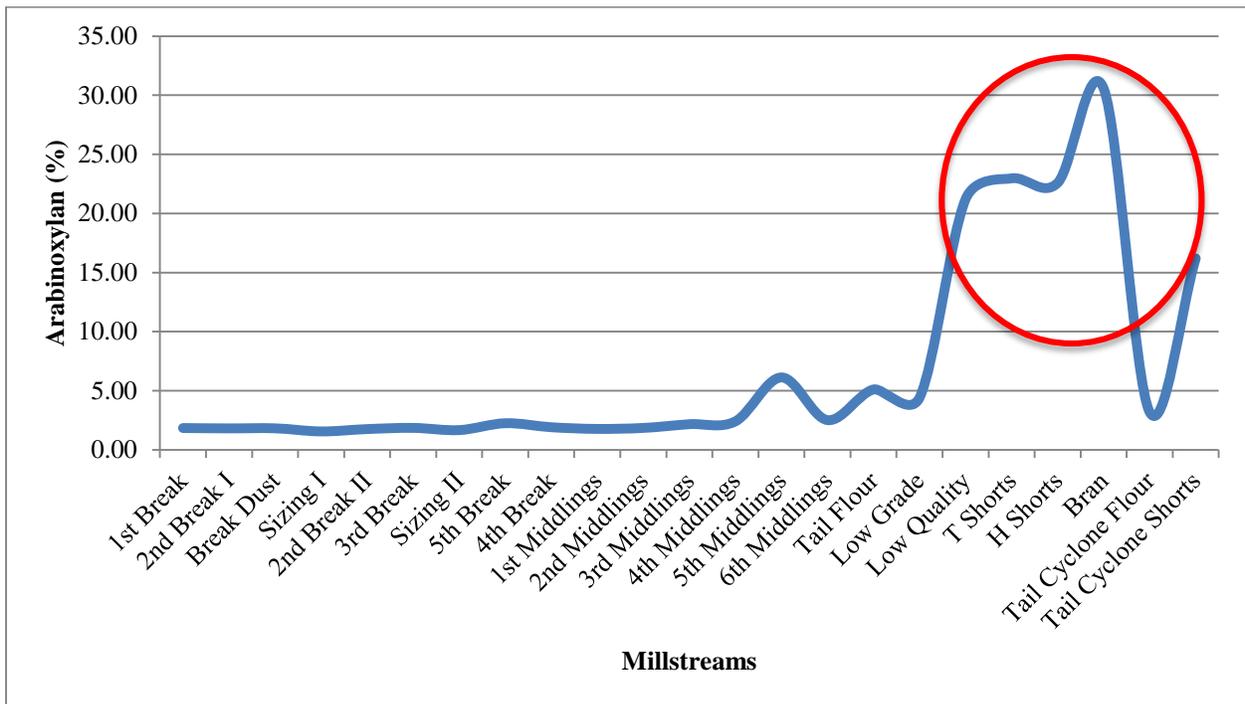


Figure 5.15. The Arabinoxylan Content for Different Millstreams

In addition to break and reduction millstreams, the latter streams found to have significantly ( $P < 0.05$ ) higher AX content (Figure 5.14). It was observed that low quality flour, shorts, and bran streams had much higher AX% content. High AX% in these streams could be due to the very high ash content in these streams. Ciacco and D'Appolonia (1982) and Delcour et al. (1999) have also reported that arabinoxylan content changes from inner to the outer layers of the wheat kernel with the aleurone layer of bran having the highest concentration. Wang et al.

(2006) also found that there was very strong positive correlations between ash content and total AX% when the ash content was above 0.6%.

## 5.6. Conclusion

The current research was carried out to investigate various MIAG Multomat millstreams for their physicochemical characteristics when evaluating five HRS wheat cultivars from two regions. Main effects wheat cultivar, millstreams, and interactions between the main effects showed significant differences on the flour quality tests. These results indicate that there was a varietal difference for quality parameters as well as growing regions. When comparing flour millstreams, reduction millstreams accounted for 47.6% of the total flour yield, while break and sizing millstreams combined 20.2% of the total flour yield. Break millstreams had average ash content of 0.77%, while reduction streams (excluding the 5<sup>th</sup> midds.) was 0.48%, which significantly lower than the break millstreams. The most refined (reduction) streams have the brightest color, while high ash (break) streams had the darkest color. The break flours along with tail cyclone flour had very high content with average of 17.0%, while reduction millstreams had much lower protein content average of 13.5%. Break millstreams had significantly lower starch damage, while reduction millstreams had greater flour starch damage Reduction millstreams were slightly higher AX content compared to the break millstreams. Both B5 and M5 streams had higher AX content compared to other break and reduction millstreams. This indicates that streams containing higher ash content yield higher AX%. The knowledge of wheat kernel distribution in different millstreams as well as the flour composition in these millstreams can provide millers with very important information to optimize the functionality of flour blends.

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## **CHAPTER 6. BREADMAKING CHARACTERISTICS AND PROTEIN MOLECULAR WEIGHT DISTRIBUTION OF HARD RED SPRING WHEAT MIAG MILLSTREAMS**

### 6.1. Abstract

Wheat kernel is heterogeneous in physical and chemical composition. As a result different millstreams vary in the flour quality and composition thus have an effect on the end-use quality. The current research was carried out to investigate various MIAG Multomat millstreams for their baking quality characteristics when evaluating five HRS wheat cultivars from two regions. Main effects wheat cultivar, millstreams, and interactions between the main effects showed significant ( $P < 0.05$ ) differences on the breadmaking quality characteristics. These results indicate that there was a varietal difference for quality parameters as well as growing regions. When comparing flour millstreams, reduction millstream flours had higher baking absorption due to higher starch damage and arabinoxylan content. Break flours produced larger bread loaves (234 cc), while reduction flours resulted in smaller bread loaves (188 cc). Greater starch damage and arabinoxylan content in reduction flours had negative effect on the bread loaf volume. In addition to breadmaking, protein molecular weight distribution (MWD) as well as molecular weights were determined for protein fractions. SDS-extractable gliadin SDS-unextractable polymeric proteins (HMW and LMW-glutenins) were found to be higher in break flours explaining the larger bread loaves obtained for break millstreams. Reduction flours had very high molecular weights for HMW and LMW-glutenin subunits ( $1.6 \times 10^7$  Da and  $2.3 \times 10^6$  Da respectively) in the SDS-extractable protein fraction. In addition, HMW-glutenin in SDS-unextractable was negatively correlated with bread loaf volume ( $P < 0.01$ ,  $r = -0.70$ ). This indicated that very high HMW-glutenin has inferior effect on the bread loaf volume. These results explain the differences observed in the bread loaf volume between break and reduction

millstreams. Break flours produced larger loaves owing to lower molecular weights for both HMW and LMW-glutenins, while reduction flours produced smaller loaves owing to a higher molecular weights for these glutenin subunits. The knowledge of wheat kernel distribution in different millstreams as well as the flour protein composition on end-use quality in these millstreams can provide millers with very important information to optimize the functionality of flour blends.

## 6.2. Introduction

Wheat milling is a key source of variation in flour quality for breadmaking in addition to growing environment and wheat cultivar. A commercial hard wheat mill can produce 30 or more flour mill streams (Machet, 2005). Because wheat kernel is heterogeneous in physical and chemical composition, different millstreams vary in the flour quality and composition. The flour components in various millstreams can vary in the quantity and quality of the protein, ash content, flour color, flour water absorption, particle size, starch damage, and dough rheological properties. However, the breadmaking test ultimately determines the end-use quality of a flour. Since protein content and composition are the major factors determining breadmaking quality, there have been extensive studies on these relationships.

In terms of dough rheology, the mixing characteristics are one of the most important parameter. The mixograph has been widely used in cereal science research as it has been proven be a tool in research and in flour quality evaluation, especially sample size is limited (Machet, 2005). Despite its use in flour quality evaluation, the use of mixograph for millstream analysis has been limited. Machet (2005) has reported that there was wide range of protein content and refinement in various millstreams let to large differences in dough mixing properties.

Wheat flour proteins contain mixtures of glutenins, gliadins, albumins, and globulins (Mendichi et al., 2008). Glutenins are polymeric proteins in which individual subunits are linked by disulphide bonds, while gliadins are monomeric proteins that consist of single chain polypeptides that contribute to viscous properties of dough (Field et al., 1983a,b; Eliasson, 1993). Glutenins have been described as “nature’s largest polymers” and they are the main components responsible for differences in end-use quality among different cultivars (Weegels et al., 1996). More specifically, high molecular weight (HMW) glutenin subunits have been widely studied because of the relationship between these proteins and wheat quality characteristics.

Glutenin subunits have been studied due to their relationship with bread baking characteristics; however, more emphasis on the high molecular weight glutenin subunits (HMW-GS) (Mendichi et al, 2008). There is a strong correlation between baking quality and glutenin polymers (Field et al., 1983ab; Gupta et al., 1993). The molecular weight and size of these two glutenin polymers can be determined by SE-HPLC with Multi Angle Light Scattering (MALS) detector, as these glutenin polymers have very broad distribution of molecular and size. The combination of MALS with an SEC system is very powerful and reliable technique for determining the MWD of macromolecules.

The MALS technique has been long used to determine the molecular weight distribution (MWD), size and confirmation of both synthetic and natural polymers. Bean and Lockhart (2001) investigated the characterization of wheat gluten protein using the MALS in conjunction with SE-HPLC. Mendichi et al. (2008) also concluded in their study that size exclusion chromatography with MALS technique was shown to be a useful distinguishing glutenin polymers coming from different wheat cultivars. However, the authors have added that it was important to choose the appropriate experimental conditions. MALS coupled to SEC can provide

absolute molar mass information at every point of the eluting sample (Wyatt Technology, 2012). This allows identification of the protein and its association state and to also detect traces of higher order aggregates. In addition, MALS combined with UV and RI detection is a powerful tool to characterize protein conjugates.

The objective of this research was to investigate millstreams that contribute to straight grade flour on their mixing and breadmaking quality characteristics as well as protein molecular weight distribution (PWD) and molecular weight analysis.

### 6.3. Materials and Methods

#### 6.3.1. Wheat Sample

Five bushels of 5 Hard Red Spring wheat genotype composites (ND Elgin, MN Bolles, ND 817, SY Ingmar, ND Glenn) were obtained from Pacific Northwest (PNW) and Gulf/Great Lakes export regions as part of the 2014 Overseas Varietal Analysis (OVA). The composites obtained from 2 export region are shown in table 6.1.

Table 6.1. HRS Wheat Cultivar Composite Ratios (%) from Different Locations in North Dakota and Washington for 2 Export Regions \*

Region	Cultivar	Casselton	Crookston	Watertown	Minot	Williston
PNW	ND Elgin	20.7	43.7	-	20	15.6
PNW	MN Bolles	-	-	-	43.9	56.1
PNW	ND 817	-	-	-	64.7	35.3
PNW	SY Ingmar	-	-	-	55.5	44.5
PNW	ND Glenn	-	-	-	50.0	50.0
G/GL	ND Elgin	33.3	33.3	33.4	-	-
G/GL	MN Bolles	33.3	33.3	33.4	-	-
G/GL	ND 817	33.3	33.3	33.4	-	-
G/GL	SY Ingmar	50.0	50.0	-	-	-
G/GL	ND Glenn	33.3	33.3	33.4	-	-

\* G/GL – Gulf Great Lakes and PNW – Pacific Northwest

### 6.3.2. Kernel Quality Analysis

The wheat was cleaned on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN) to remove shrunken and broken kernels. Test weights and moisture contents (dockage-free portion) were determined with a GAC 2100 tester (Dickey-John, Auburn, IL, USA). Whole wheat ash and protein content were measured by near-infrared spectroscopy with an Infractec 1241 grain analyzer (Perstorp Analytic, Hoganas, Sweden). Wheat kernel samples (10g) were weighed and prepared after removal of all dockage, shrunken and broken kernels, and other foreign materials. The number of each sample was counted with a model 77 totalizer (Seedburo Equipment, Chicago, IL, USA). Number of counted kernels was converted to 1,000 kernel weight and recorded:

$$1,000 \text{ kernel weight (g)} = (1,000/\text{number of kernels}) \times 10 \text{ g}$$

Wheat kernels were sorted for sizing with a shaker in which a set of Tyler standard sieves (number 7 and 9 [2.92 and 2.24 mm]) was used (Arrow testing sieve shaker, Seedburo Equipment, Chicago, IL, USA). Wheat (100g) was sized on the shaker for 200 s.

Approximately 300 kernels of wheat were prepared for kernel hardness. Samples were poured into the access hopper of the SKCE 4100 device (Perten, Huddinge, Sweden) and analyzed according to AACC International Approved Method 55-31.01. Parameters such as kernel weight (mg), kernel diameter (mm), moisture content (%), and kernel hardness index value were determined. Two hundred grams of wheat samples were sent to the North Dakota Grain Inspection for full-grade grain characteristics.

### 6.3.3. Flour Milling

Wheat samples were tempered to 16% moisture for 18 h before milling. All 10 wheat samples were milled on a MIAG-Multomat laboratory mill (Miag, Braunschweig, Germany).

Approximately 50 kg of wheat samples were milled on MIAG Multomat; the feed rate of wheat to the mill was set at 1360 g/min. The break releases for the first, second, and third breaks were set at 30%, 53%, and 65%, respectively. Flour extractions were determined as the percentage of straight-grade flour produced. Flour was then rebolted through an 84 SS sieve on an Allis-Chalmers rebolter Ser. No. 204 (Allis-Chalmers MFG., Milwaukee, WI, USA) to remove any foreign material. Flour was then blended on a Cross-Flow Blender Serial No. 257063 (Patterson-Kelly Co., East Stroudsburg, PA, USA) for 30 minutes. Percent yield based on wheat and total product was calculated for each millstreams.

#### 6.3.4. Millstream Collection

Approximately 200g of sample was collected from the millstreams during milling.

Table 6.2. The MIAG Millstreams for Straight-Grade Flour with Respective Stream Numbers

Stream Name	Stream #
1st Break	1
2nd Break I	2
Break Dust	3
Sizing I	4
2nd Break II	5
3rd Break	6
Sizing II	7
5th Break	8
4th Break	9
1st Middlings	10
2nd Middlings	11
3rd Middlings	12
4th Middlings	13
6th Middlings	15
Tail Flour	16
Tail Cyclone Flour	22

### 6.3.5. Mixing Characteristics

The mixogram was obtained with a 10 g bowl mixer according to AACCI Approved Method 54-40.02. Flour (10g) was mixed with the optimum amount of water for 8 min. The optimum amount of water was determined by the protein content according to the following formula listed in the AACCI Approved Method: % absorption = (1.5% x % protein) + 43.6.

The mixograph pattern was evaluated with MIXSMART computer software (version 3.40, National Manufacturing Division, TMCO, Lincoln, NE, USA). Computer-analyzed parameters of the mixograph included midline peak time (MPT), midline peak width, midline peak height, and left slope and tail slope of envelope.

### 6.3.6. Breadmaking

Flour samples (25g) were baked according to AACCI Approved Method 10-09.01 with the following modifications; fungal  $\alpha$ -amylase (15 SKB) instead of dry malt powder, instant yeast (1.0%) instead of compressed yeast and the addition of 10 ppm ammonium phosphate. After baking, bread loaf volume was measured according to AACCI Approved Method 10-05.01. 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 symmetry.

### 6.3.7. SE-HPLC and MALS of Proteins

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 analysis of protein MWD. 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 2,500 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  $\mu\text{m}$  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 in the residue was solubilized by sonication. 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  $\times$  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  $\times$  4.5 mm, BIOSEP SEC S4000, Phenomenex, Torrance, CA) with a guard cartridge (Ohm et al., 2009). Injection volume was 10  $\mu\text{L}$ . 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). Absorbance area values were calculated for four main fractions of SE-HPLC chromatograms: F1 (3.9-4.4 min), F2 (4.4-5.1 min), F3 (5.1-6.0 min), and F4 (6.0-7.1 min) and converted to percentage values based on flour weight (14% mb) (Park et al. 2006; Baasandorj et al. 2015a). Primary components of each fraction are known to be high molecular weight protein (HMW) polymeric protein for F1; low molecular weight (LMW) polymeric protein for F2; gliadins for F3; and albumins and globulins for F4 (Larroque et al., 1997; Morel et al., 2000; Ohm et al., 2009).

Protein molecular weights in these fractions (F1 to F4) for both SDS-extractable and SDS-unextractable fractions were determined using the high performance size exclusion chromatography (HPSEC) with multi angle light scattering (MALS).

#### 6.4. Statistical Analysis

The experimental design 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 was used to declare differences between treatments. The experimental design was three-factorial layout with location, cultivar and millstream as main factors. Location, cultivar, and millstream interaction term was used as error term.

#### 6.5. Results and Discussion

##### 6.5.1. Effects of Location, Wheat Cultivar, Millstreams on Breadmaking Characteristics and Protein Molecular Weight Distribution

A total of 16 millstreams shown in table 6.2 were used for the mixing and breadmaking quality characteristics as well as protein molecular weight distribution analysis. These 16 millstreams are blended to make up the straight grade flour. The objective of this experiment was to investigate these 16 individual millstreams on mixing and breadmaking quality characteristics as well as protein molecular weight distribution. It was observed that both wheat cultivar and millstreams had significant ( $P < 0.0001$ ) effects on mixograph peak time (MPT), while location did not have significant ( $P > 0.05$ ) effect (Table 6.3). In addition, location x cultivar interaction was found to be significant ( $P < 0.0001$ ). However, large variation in the MPT was due to the wheat cultivar, which was indicated by the greater F-value.

Table 6.3. The ANOVA for Mixograph Peak Time

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	0.5	1.23	0.2715
Cultivar (VAR)	4	29.5	72.55	<.0001
Stream (STR)	15	8.4	20.76	<.0001
LOC*VAR	4	3.0	7.49	<.0001
LOC*STR	15	0.4	1.1	0.3744
VAR*STR	60	0.6	1.54	0.0481

Mixograph peak time (MPT) is closely related to the mixing time required to reach optimum dough strength for breadmaking (Baasandorj et al., 2015a). Weak flours produce dough that breaks down and has little tolerance to variation in mixing. In contrast, strong dough produced from hard flours requires long mixing times. As mentioned, MPT varied among wheat cultivars for Gulf/Great Lakes (G/GL) and Pacific Northwest (PNW) regions (Figure 6.1).

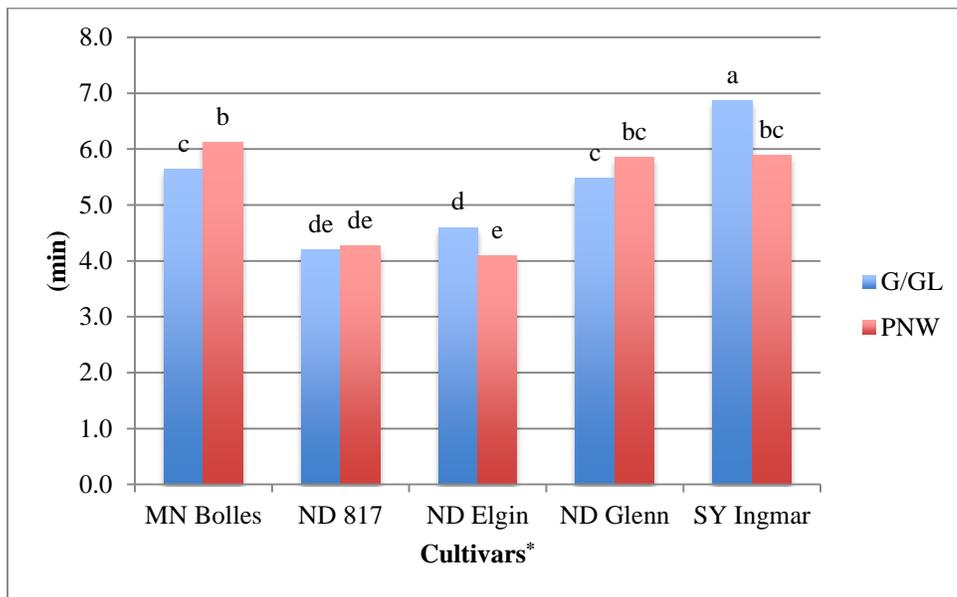


Figure 6.1. Mixograph Peak Time (MPT) for Wheat Cultivars

\* Means followed by the same letter in the column are not significantly different between mill types.

SY-Ingmar cultivar had the highest average MPT of 6.4 min, and this high MPT for this cultivar was consistent across this cultivar. MN-Bolles and ND-Glenn cultivars had MPT of 5.9 min and 5.7 min, respectively. In contrast, ND-817 and ND-Elgin cultivars had significantly

( $P < 0.05$ ) lower MPT of 4.2 min and 4.3 min, respectively. These results indicate that flours produced from SY-Ingmar, MN-Bolles, and ND-Glenn cultivars were very strong, while ND-817 and ND-Elgin cultivars produced weak flours.

Similar results were observed for the baking mix time. Location, wheat cultivars as well as millstreams had significant effect on the baking mixing time (Table 6.4). However, unlike the mixograph peak time, much of the variation was due to the location and wheat cultivar.

Table 6.4. The ANOVA for Baking Mix Time

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	1.71	25.05	<.0001
Cultivar (VAR)	4	0.73	10.72	<.0001
Stream (STR)	15	0.15	2.14	0.0197
LOC*VAR	4	0.03	0.5	0.7373
LOC*STR	15	0.08	1.17	0.3223
VAR*STR	60	0.09	1.32	0.1439

There was a significant ( $P < 0.05$ ) difference in baking mix time for 2 regions. G/GL region had baking mix time of 4.7 min while PNW region had 4.5 min. Figure 6.2 illustrates the baking mix time for five wheat cultivars across these two regions.

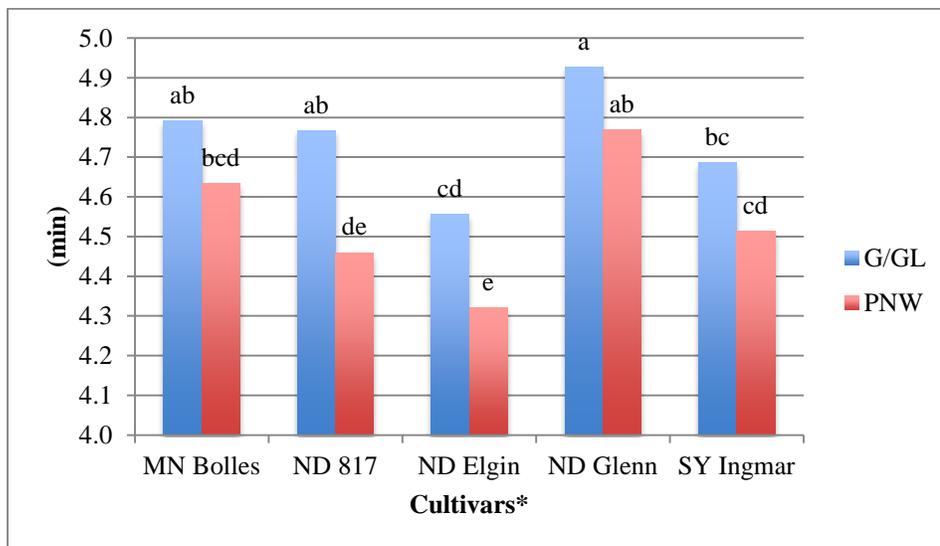


Figure 6.2. The Baking Mix Time for Wheat Cultivars for Two Regions

\* Means followed by the same letter in the column are not significantly different between mill types.

Baking mix time was higher for G/GL region compared to PNW region, and this was consistent for all 5 wheat cultivars. Longer in the baking time for G/GL region indicates that wheat samples from Casselton, Crookston, and Watertown locations produced strong wheat flours with longer baking mix time. In contrast, the same wheat cultivars from Minot and Williston locations produced weak flours with shorter baking mix time for PNW region. It was also observed that ND-Glenn cultivar had significantly ( $P<0.05$ ) higher baking mix time of 4.8 min, followed by MN-Bolles, ND-817, and SY-Ingmar cultivars with 4.7 min, 4.6 min, and 4.6 min, respectively. In contrast, ND-Elgin cultivar had significantly ( $P<0.05$ ) lower baking mix time of 4.4 min, which indicates that this cultivar produced weak flour.

Similar to baking mix time, the location, wheat cultivars, and millstreams had significant ( $P<0.05$ ) effects on the baking water absorption (Table 6.5). In addition, location x cultivar interaction was significant ( $P<0.05$ ).

Table 6.5. The ANOVA for Baking Absorption

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	107.3	80.8	<.0001
Cultivar (VAR)	4	5.4	4.1	0.0055
Stream (STR)	15	23.1	17.4	<.0001
LOC*VAR	4	4.2	3.2	0.0191
LOC*STR	15	2.1	1.6	0.1041
VAR*STR	60	1.1	0.8	0.81

These results indicate that baking water absorption was different among G/GL and PNW regions as well as wheat cultivars and millstreams. A similar trend was also seen for baking absorption. A significantly ( $P<0.05$ ) higher baking absorption was found for G/GL (68.2%), while PNW region resulted in baking absorption of 66.2%. Water absorption is one of the most important quality determinants in breadmaking (Morgan et al., 2000). High protein wheat, such

as HRS wheat generally has higher water absorption capacity and greater loaf volume potential compared to other wheat (Carson and Edwards, 2009). Figure 6.3 illustrates the baking absorption values for 5 wheat cultivars from 2 growing regions.

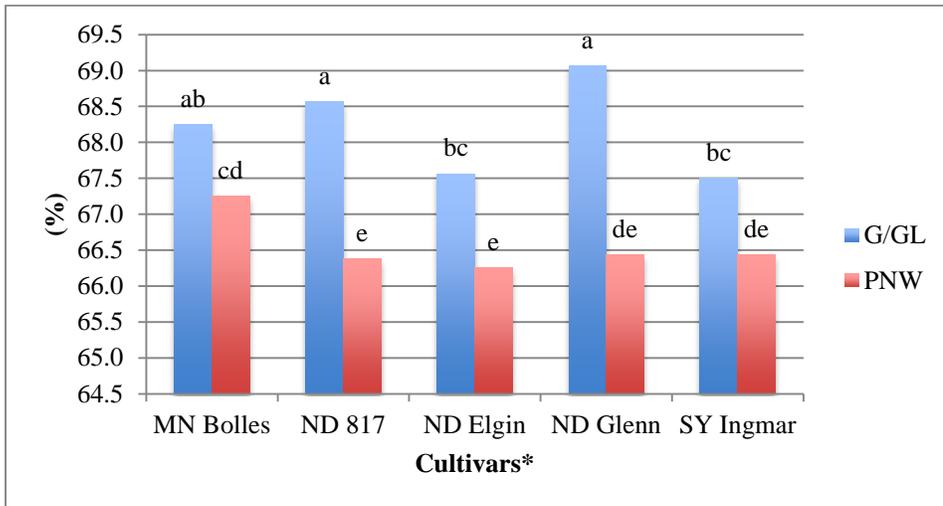


Figure 6.3. The Baking Water Absorption for Wheat Cultivars from Two Growing Regions  
 \* Means followed by the same letter in the column are not significantly different between mill types.

There was a difference in the baking absorption between G/GL and PNW regions, and this was observed for all 5 wheat cultivars. When comparing wheat cultivars, MN-Bolles and ND-Glenn cultivars had significantly ( $P < 0.05$ ) higher values of 67.8% each respectively. In contrast, ND-Elgin and SY-Ingmar cultivars had significantly ( $P < 0.05$ ) lower baking absorption values of 66.9% and 67.0%, respectively. These differences in the baking absorption between 2 region as well as 5 wheat cultivars could indicate that bread loaf volumes might be different for regions and these wheat cultivars. Conversely, the ANOVA indicated that the main effects location, cultivar, and millstreams had significant ( $P < 0.001$ ) effects on the bread loaf volume (Table 6.6).

Table 6.6. The ANOVA for Bread Loaf Volume

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	3102.0	12.88	0.0007
Cultivar (VAR)	4	6605.3	27.44	<.0001
Stream (STR)	15	7329.4	30.44	<.0001
LOC*VAR	4	1123.7	4.67	0.0024
LOC*STR	15	233.0	0.97	0.4989
VAR*STR	60	463.4	1.92	0.0061

This indicates that bread loaf volume was different between 2 regions as well as wheat cultivars and millstreams. In addition, it was also observed that location x cultivar and cultivar x stream interaction was significant ( $P < 0.01$ ). Similar to baking absorption, bread loaf volume was found to be greater for all wheat cultivars for G/GL region.

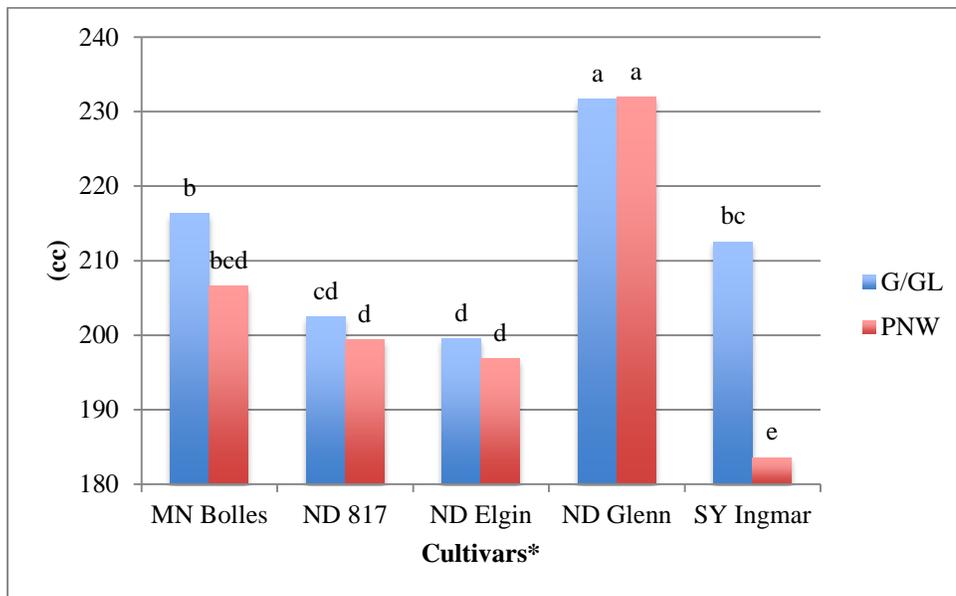


Figure 6.4. Bread Loaf Volume for Wheat Cultivars for Two Regions

\* Means followed by the same letter in the column are not significantly different between mill types.

ND-Glenn cultivar had significantly ( $P < 0.05$ ) higher loaf volume (232cc) followed by MN-Bolles (212cc). In contrast, ND 817, ND Elgin, and SY-Ingmar cultivars had significantly ( $P < 0.05$ ) bread loaf volumes of 201cc, 198cc, and 198cc, respectively. As mentioned, the cultivar x millstream interaction was significant ( $P < 0.05$ ), which indicates that flours produced

from these 5 wheat cultivars had different bread loaf volumes across millstreams. A similar trend was observed for these wheat cultivars across different millstreams (Figure 6.5-6.8)

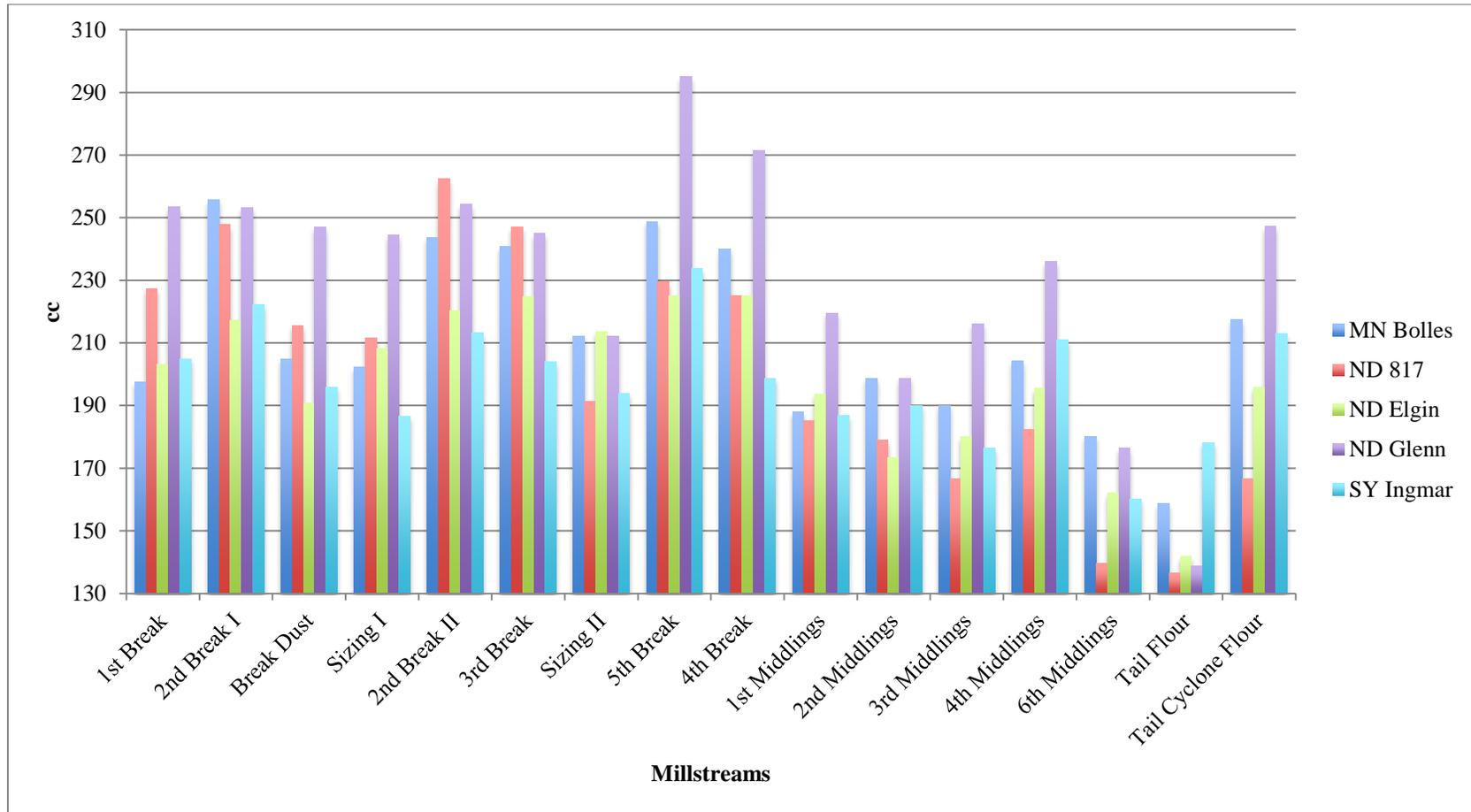


Figure 6.5. Bread Loaf Volumes for Wheat Cultivars Across Millstreams



Figure 6.6. Bread Pictures of Various MIAG Millstreams for ND-Glenn (Top), ND-Elgin (Middle Top), MN-Bolles (Middle Bottom), and ND-817 (bottom) cultivars



Figure 6.7. Side View Bread Pictures of Various MIAG Millstreams for ND-Glenn (top) and ND-Elgin (bottom)

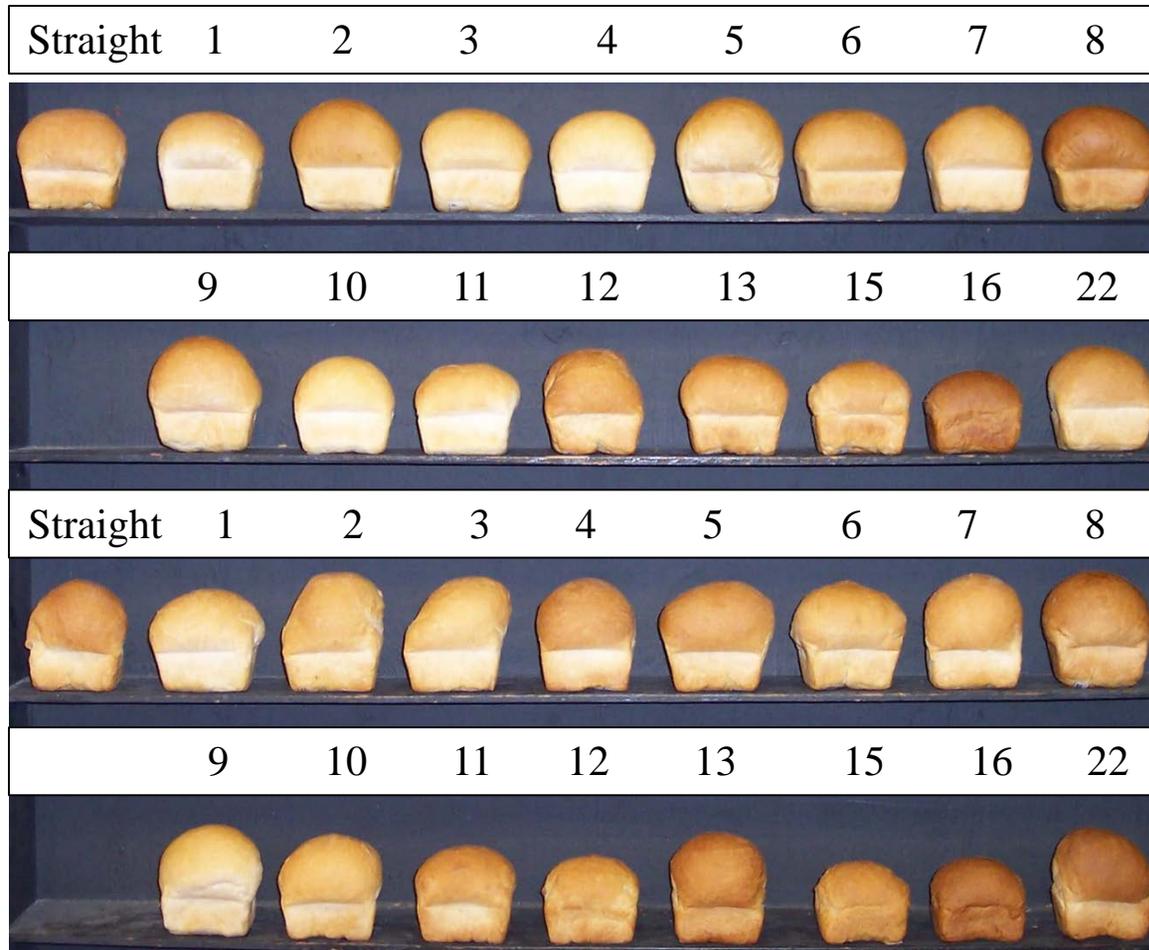


Figure 6.8. Side View Bread Pictures of Various MIAG Millstreams for MN-Bolles (top) and ND-817 (bottom)

Across millstreams it was observed that ND-Glenn and MN-Bolles had greater bread loaf volumes for most of the millstreams with the exception of 2<sup>nd</sup> Break II and Tail flour (Figure 6.5-6.8). In contrast, ND-Elgin and SY-Ingmar had consistently smaller bread loaf volume across millstreams. The ANOVA also indicated that the main effects also had significant ( $P < 0.01$ ) effects on the bread specific volume (Table 6.7).

Table 6.7. The ANOVA for Bread Specific Volume

Source	DF	Mean Square	F Value	Pr > F
Location (LOC)	1	3.93	11.8	0.0011
Cultivar (VAR)	4	8.84	26.56	<.0001
Stream (STR)	15	9.53	28.64	<.0001
LOC*VAR	4	1.65	4.97	0.0016
LOC*STR	15	0.36	1.07	0.4033
VAR*STR	60	0.60	1.8	0.012

G/GL region also found to have significantly higher bread loaf volume (7.1) compared to PNW (6.8). The difference was not apparent as seen for baking absorption and bread loaf volume between these 2 regions. However, there was significant ( $P < 0.0001$ ) difference for wheat cultivars. ND-Glenn cultivar had significantly ( $P < 0.05$ ) higher specific volume of 7.9 followed by MN-Bolles (7.0), while ND-817, ND-Elgin, and SY-Ingmar cultivars had significantly ( $P < 0.05$ ) lower bread specific volumes of 6.8, 6.7, and 6.5, respectively.

Flour polymeric and monomeric protein fractions were analyzed by SE-HPLC. Absorbance area values were calculated for four main fractions of SE-HPLC chromatograms: F1 (3.9-4.4 min), F2 (4.4-5.1 min), F3 (5.1-6.0 min), and F4 (6.0-7.1 min) and converted to percentage values based on flour weight (14% mb) (Park et al. 2006; Baasandorj et al., 2015a). Primary components of each fraction are known to be high molecular weight protein (HMW) polymeric protein for F1; low molecular weight (LMW) polymeric protein for F2; gliadins for F3; and albumins and globulins for F4 (Larroque et al., 1997; Morel et al., 2000; Ohm et al.,

2009). Table 6.8 shows SDS-extractable and SDS-unextractable protein fraction for 2 regions and wheat cultivars. The ANOVA indicated that location and cultivar had significant ( $P < 0.0001$ ) effects on the SDS-extractable and SDS-unextractable protein fractions.

Table 6.8. SDS-Extractable and SDS-Unextractable Protein Fractions of 2 Regions and Wheat Cultivars

Region/Cultivar	SDS-Extractable (% Flour)				SDS-Unextractable (% Flour)			
	F1	F2	F3	F4	F1	F2	F3	F4
G/GL	0.21	0.29	1.08	5.85	0.44	0.75	1.45	1.31
PNW	0.17	0.47	1.27	6.28	0.32	0.98	1.56	1.23
LSD* ( $P < 0.05$ )	0.01	0.01	0.02	0.07	0.02	0.03	0.03	0.04
MN-Bolles	0.19	0.37	1.37	6.59	0.50	0.90	1.76	1.50
ND-817	0.22	0.36	1.03	5.93	0.45	0.88	1.32	1.22
ND-Elgin	0.16	0.33	1.04	5.18	0.28	0.73	1.41	1.18
ND-Glenn	0.16	0.40	1.22	6.26	0.33	0.93	1.56	1.26
SY-Ingmar	0.19	0.44	1.23	6.37	0.34	0.90	1.47	1.19
LSD ( $P < 0.05$ )	0.01	0.02	0.03	0.11	0.02	0.04	0.04	0.06

\*LSD – Least Significant Difference

PNW region had significantly ( $P < 0.05$ ) higher SDS-extractable flour protein fractions (%) for F2, F3, and F4. This indicates that flours produced from G/GL region had greater amount of LMW-glutenin, gliadins, and albumins and globulins. However, G/GL region had higher SDS-extraction protein fraction F1, which indicated that this flours obtained from this region had greater amount of HMW-glutenin. For SDS-unextractable flour protein fractions, G/GL region had higher F1 and F4 fractions, while PNW region had greater amount of F2 and F3. It was also observed that wheat cultivars had significant ( $P < 0.0001$ ) effect on the both SDS-extractable and SDS-unextractable flour protein fractions. This indicates that wheat cultivars had different protein composition.

For SDS-extractable fractions, ND-817 had significantly ( $P < 0.05$ ) higher F1 fraction, which is mainly composed of HMW-glutenin whereas ND-Elgin and ND-Glenn had lower F1 protein fractions. The F2 fraction, which is mainly composed of LMW-glutenin, was found to be

greater for ND-Glenn and SY-Ingmar cultivars, while it lower for MN-Bolles, ND-817, and ND-Glenn cultivars (Table 6.8). The F3 fraction is mainly composed of gliadins. It was observed that MN-Bolles, ND-Glenn, and SY-Ingmar cultivars had greater amount of gliadins, while ND-817 and ND-Elgin cultivars had significantly ( $P < 0.05$ ) lower gliadins, which could be responsible for quantitative variation of flour protein. Baasandorj et al. (2015b) reported that gliadin was the main protein fraction responsible for quantitative variation of flour protein, and it showed significant and positive correlations with bread loaf volume. The F4 protein fraction is mainly composed of albumins and globulins. It was observed that F4 fraction was found to be higher for MN-Bolles, ND-Glenn, and SY-Ingmar cultivars, while ND-817 and ND-Elgin cultivars had lower F4 fraction.

For SDS-unextractable fractions, G/GL region had significantly ( $P < 0.05$ ) greater F1 fraction, which is mainly composed of HMW-glutenins. The SDS-unextractable HMW polymeric protein was found to have positive association with mixing characteristics (Baasandorj et al., 2015a), dark hard vitreous (DHV) kernel affecting water absorption (Baasandorj et al., 2015b), and with farinograph water absorption and peak time, and bread loaf volume (Baasandorj et al., 2016). Therefore, high SDS-unextractable HMW polymeric protein found in G/GL region could explain the greater bread loaf volume of 213cc compared to 204cc for PNW region, although there was very weak but positive correlation ( $P < 0.05$ ,  $r = 0.18$ ). Similar trend was found for wheat cultivars. MN-Bolles and ND-817 had greater HMW polymeric proteins, while ND-Elgin had the lowest HMW polymeric protein (Table 6.8). This could explain the differences in the bread loaf volumes for these cultivars. However, F1 fraction was found to be lower for ND-Glenn cultivar, which had the highest bread loaf volume of 232cc. Therefore,

SDS-unextractable HMW polymeric protein may not be solely responsible for bread loaf volume variation when comparing millstreams for end-use quality analysis.

The molecular mass for these 4 fractions was then determined by high performance size exclusion chromatography (HPSEC) with multi angle light scattering (MALS). The ANOVA indicated that location, cultivar, and millstreams had significant ( $P < 0.001$ ) effects on the molecular mass 4 protein fractions. It was observed that G/GL region had significantly higher molecular mass in 4 protein fractions for both SDS-extractable and –unextractable protein.

Table 6.9. The Molecular Mass for SDS-extractable and –unextractable Protein Fractions for 2 Growing Regions and Wheat Cultivars

Region/Cultivar	SDS-Extractable (Da)				SDS-Unextractable (Da)			
	F1	F2	F3	F4	F1	F2	F3	F4
G/GL	2.0x10 <sup>7</sup> a	4.3x10 <sup>6</sup> a	1.2x10 <sup>6</sup> a	6.3x10 <sup>5</sup> a	2.7x10 <sup>6</sup> a	7.2x10 <sup>5</sup> a	5.2x10 <sup>5</sup> a	6.4x10 <sup>5</sup> a
PNW	1.2x10 <sup>7</sup> b	2.2x10 <sup>6</sup> b	9.4x10 <sup>5</sup> b	1.8x10 <sup>5</sup> b	1.7x10 <sup>6</sup> b	5.3x10 <sup>5</sup> b	4.5x10 <sup>5</sup> b	6.1x10 <sup>5</sup> a
MN Bolles	2.8x10 <sup>7</sup> a	4.8x10 <sup>6</sup> a	1.3x10 <sup>6</sup> a	1.2x10 <sup>6</sup> a	3.2x10 <sup>6</sup> a	7.3x10 <sup>5</sup> a	4.8x10 <sup>5</sup> b	7.9x10 <sup>5</sup> a
ND 817	2.1x10 <sup>7</sup> b	5.2x10 <sup>6</sup> a	1.5x10 <sup>6</sup> a	2.7x10 <sup>5</sup> b	2.7x10 <sup>6</sup> b	8.0x10 <sup>5</sup> a	6.0x10 <sup>5</sup> a	6.6x10 <sup>5</sup> b
ND Elgin	9.1x10 <sup>6</sup> d	1.8x10 <sup>6</sup> c	6.9x10 <sup>5</sup> c	1.8x10 <sup>5</sup> b	1.7x10 <sup>6</sup> c	5.2x10 <sup>5</sup> b	4.2x10 <sup>5</sup> c	5.0x10 <sup>5</sup> c
ND Glenn	1.0x10 <sup>7</sup> d	2.0x10 <sup>6</sup> bc	7.3x10 <sup>5</sup> c	1.7x10 <sup>5</sup> b	1.5x10 <sup>6</sup> c	5.1x10 <sup>5</sup> b	4.7x10 <sup>5</sup> bc	6.7x10 <sup>5</sup> b
SY Ingmar	1.4x10 <sup>7</sup> c	2.5x10 <sup>6</sup> b	1.1x10 <sup>6</sup> b	2.2x10 <sup>5</sup> b	1.7x10 <sup>6</sup> c	5.4x10 <sup>5</sup> b	4.6x10 <sup>5</sup> bc	4.6x10 <sup>5</sup> c

\* Means were calculated across millstreams. Means followed by the same letter in the column are not significantly different between mill types

The molecular weight was significantly ( $P < 0.05$ ) for F1 fraction (Table 6.9). This fraction is mainly composed of HMW-glutenin subunits. Glutenins are polymeric proteins, which consists of individual subunits that are linked by disulphide bonds (Field et al., 1983), and these polymers are thought to have high molecular weight ranging from few hundred thousand to many millions (Mendichi et al., 2008). The F1 fraction had molecular weight of 2.0 million Da for G/GL region, while PNW region had lower molecular weight (1.2 million) for F1 fraction for SDS-extractable protein (Table 6.9). A similar trend was also observed for SDS-unextractable proteins. In addition, G/GL region had higher molecular weight for F3 fraction compared to PNW region. The F3 fraction, primarily composed of gliadins, was higher for G/GL region compared to PNW region for both SDS-extractable and –unextractable proteins.

Wheat cultivars also had different molecular weights for protein fraction for both SDS-extractable and –unextractable proteins. It was observed that MN-Bolles and ND-817 cultivars had significantly ( $P < 0.05$ ) higher molecular weight for HMW-glutenin protein fraction (F1) for both SDS-extractable and –unextractable proteins (Table 6.9). In contrast, ND-Elgin and ND-Glenn wheat cultivars had significantly lower molecular weight for F1 fraction for both SDS-extractable and –unextractable proteins. There was a significant and negative association between SDS-unextractable HMW-glutenin fraction with bread loaf volume. In other words, very high molecular mass had inferior effect on the bread loaf volume ( $P < 0.001$ ,  $r = -0.70$ ). However, there seems to be conflicting association between the molecular weight of HMW-glutenin subunit and bread loaf volume. It was mentioned earlier that both MN-Bolles and ND-Glenn cultivars had significantly ( $P < 0.05$ ) higher bread loaf volume compared to other cultivars. MN-Bolles had very high molecular weights for HMW and LMW-glutenins as well as gliadins, while ND-Glenn had the lowest molecular weights in these fractions. Therefore, the molecular

weight or mass in these fractions alone could not explain the variation in the bread loaf volume, as the relationship between protein fractions (both amount and molecular weight) appears to be more complex. Furthermore, bread loaf volume appears to be dependent not just amount and molecular weight of these protein fractions but also other quality factors such as protein content, starch damage, water absorption, and arabinoxylan content.

#### 6.5.2. The Evaluation of Millstreams of Breadmaking Quality Characteristics and Protein Molecular Weight Distribution

In the previous section, the effects of location and wheat cultivar were discussed. The ANOVA showed that these main effects had significant ( $P < 0.001$ ) effect on breadmaking quality characteristics as well as protein molecular weight (PWD) distribution. However, in this section the effects of millstreams will be investigated on quality parameters when averaged across growing region and wheat cultivars. Table 6.10 shows the baking quality evaluation for different millstreams. Mixograph peak time (MPT) is close related to the mixing time required to reach optimum dough strength for breadmaking (Baasandorj et al., 2015a). Weak flours produce dough that breaks down and has little tolerance to variation in mixing. In contrast, strong dough produced from hard flours requires long mixing times. It was observed that break millstreams had significantly ( $P < 0.05$ ) higher MPT of 6.2 min, while middlings millstreams had an average MPT of 4.8. This means that break flours took longer mixing time to reach optimum dough strength, which indicates that flours produced from break streams. In contrast, reduction or middlings millstreams were weaker compared to break millstream flours owing to a shorter MPT. The wide range of protein content and refinement provided by millstreams could explain the large differences in dough mixing properties (Machet, 2005). It was found that break millstream flours had higher protein content and coarser particles size compared to reduction

millstreams. High protein content and larger particle size in break flours may explain the longer MPT, as water penetrates slower for larger particles resulting in longer dough development time. Flour protein content showed significant and positive correlation with MPT ( $P < 0.001$ ,  $r = 0.45$ ). High protein content in break millstreams resulted in longer dough development time compared to shorter MPT observed for reduction millstreams. In addition, there was a difference in MPT among break millstreams as well as reduction millstreams. Early break millstream flours had longer MPT compared to later break millstream flours. Break millstreams 1-4 had slightly higher mixing time compared to later millstreams 4<sup>th</sup> and 5<sup>th</sup> breaks (Table 6.10).

Table 6.10. Mixing and Breadmaking Quality Characteristics of Different Millstreams

Millstream	Mixograph Peak Time	Baking Mix Time	Baking Abs.	Bread Loaf Volume	Specific Volume	Oven Spring
1st Break	6.6	4.5	65.6	217.4	7.3	2.0
2nd Break I	6.3	4.8	65.9	239.3	8.1	2.3
Break Dust	5.5	4.6	65.7	210.9	7.1	2.1
Sizing I	5.3	4.7	65.2	210.6	7.2	1.9
2nd Break II	6.4	4.6	66.6	238.8	8.1	2.2
3rd Break	6.2	4.8	66.6	232.3	7.9	2.2
Sizing II	4.7	4.4	66.8	204.7	7.0	1.8
5th Break	5.9	4.6	67.6	246.5	8.1	2.0
4th Break	5.8	4.7	66.8	232.2	7.7	2.3
1st Middlings	5.4	4.7	67.2	194.7	6.5	1.5
2nd Middlings	5.5	4.7	68.7	188.0	6.1	1.4
3rd Middlings	5.1	4.9	68.6	185.9	6.2	1.7
4th Middlings	3.9	4.6	68.0	205.8	7.0	1.9
6th Middlings	4.1	4.7	69.7	163.8	5.3	1.0
Tail Flour	3.9	4.5	70.5	150.8	4.8	0.1
Tail Cyclone Flour	4.3	4.7	68.4	208.1	6.9	1.9
LSD* ( $P < 0.05$ )	0.6	0.2	1.0	13.9	0.5	0.3

\* LSD – Least Significant Difference

Similarly, early reduction millstreams had longer MPT, while 4<sup>th</sup> and 6<sup>th</sup> reduction millstreams had significantly ( $P < 0.05$ ) lower MPT. Machet (2005) also reported that dough mixing results for the last two middling flours (M5 and M6) gave very flat mixograms, showing

little dough development, despite having high protein content. This low MPT in later reduction streams could be due to the high arabinoxylan (%) reported in these millstreams (Chapter 5). There was a negative association between AX and MPT ( $P < 0.05$ ,  $r = -0.24$ ). High AX content in later reduction millstreams could explain the shorter dough development time observed in these millstreams. Wang et al. (2006) also reported that M4-M6 contained substantially higher pentosan content, which resulted in shorter dough development time compared to M1-M3 flours. Therefore, the break flours on average produced the strongest dough, in which protein content was higher for these streams. However, the relationship between dough strength and protein content was less clear for the reduction flours. The low MPT for M4 and M6 in current study could be due to the AX content and protein composition, despite the high protein content observed in these millstreams.

On the contrary, there was no significant ( $P > 0.05$ ) difference in the baking mix time between break and reduction flours. On average, baking mix time for break flours was 4.6 min, while reduction millstreams had mixing time of 4.7 min (Table 6.10). There was a very small variation in the baking mix time, as it ranged from 4.5-4.9 min. In contrast, there was a significant ( $P < 0.05$ ) difference in the baking water absorption between break and reduction flours. Baking water absorption was higher (68.4%) for reduction flours, while break flours had baking water absorption of 66.5% (Table 6.10). This was expected as these millstreams varied greatly in their particle size distribution and flour starch damage (Chapter 5). Break flours had starch damage of 4.84%, while starch damage in reduction flours was 8.94%. High flour starch damage and finer particle size obtained in reduction millstreams could explain the high water absorption in these reduction flours.

Flour absorption during baking is related to the flour particle size as well as damaged starch from mechanical means (Posner and Hibbs, 2005). 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 breadmaking. Kweon et al. (2011) reported that native wheat starch can hold 0.3-0.45g of water per gram of dry starch whereas damaged starch (from mechanical stress during milling) can hold 1.5-2g of water per dry starch. Therefore, high starch damage and finer particle size obtained for reduction millstream flour could explain the higher baking water absorption in these millstreams. These results are also in agreement with Wang and Flores (2000), who also reported that starch damage had negative association with flour particle size. There was a high and positive correlations for starch damage and arabinoxylan content with baking water absorption ( $P < 0.001$ ,  $r = 0.80$  and  $r = 0.75$ , respectively). High correlation between these parameters with baking absorption is expected, as damaged starch can hold twice 0.6-0.0 g of water per gram of dry starch while arabinoxylan can hold 10 g of water per gram of dry starch (Kweon et al., 2011).

The ANOVA (Table 6.6) showed that millstream had significant ( $P < 0.001$ ) effect on the bread loaf volume. The large was variation in bread loaf volume for different millstreams was due to the differences observed between millstreams, which were indicated by the high F-value. Break millstream flours produced larger bread loaves. On average, break flours had bread loaf volume of 234cc, while reduction millstreams produced much lower bread loaf volume of 188cc. In addition, 5<sup>th</sup> break (B5) stream flour resulted in the greatest loaf volume of 247cc, while 6<sup>th</sup> middling (M6) produced the smallest bread loaf volume of 164cc (Table 6.10). Flour protein

content may be responsible for these differences in the bread loaf volume. There was a positive but low correlation between flour protein content and bread loaf volume ( $P < 0.05$ ,  $r = 0.56$ ).

Table 6.11. The Correlation Coefficients Between Flour and Baking Quality Parameters for Millstream

Quality Parameters		Baking Mix Time	Baking Abs.	Loaf Volume	Specific Volume	Oven Spring	Crumb Color
Flour Ash		-0.33ns	0.45ns	-0.04ns	-0.09ns	-0.39ns	-0.75***
Flour Protein		-0.18ns	-0.06ns	0.56*	0.51*	0.23ns	-0.30ns
Color	L*	0.37ns	-0.23ns	-0.04ns	-0.01ns	0.23ns	0.74**
	a	-0.35ns	0.48ns	-0.21ns	-0.24ns	-0.47ns	-0.87***
	b	-0.31ns	-0.26ns	0.37ns	0.36ns	0.16ns	-0.29ns
Particle Size	100 um	0.30ns	0.33ns	-0.17ns	-0.20ns	-0.33ns	-0.61*
	50 um	-0.01ns	0.09ns	0.07ns	0.05ns	-0.01ns	-0.02ns
	<50 um	0.37ns	-0.46ns	0.19ns	0.23ns	0.43ns	0.79***
Starch Damage		0.28ns	0.80***	-0.72**	-0.73**	-0.64**	-0.25ns
Arabinoxylan		-0.28ns	0.75***	-0.60*	-0.62*	-0.78***	-0.96***
Mixograph Midline	Left Time	0.21ns	-0.71**	0.75***	0.73**	0.66**	0.53*
	Left Width	-0.08ns	-0.55*	0.79***	0.77***	0.66**	0.33ns
	Peak Time	0.21ns	-0.71**	0.75***	0.73**	0.66**	0.53*
	Peak Width	-0.17ns	-0.42ns	0.78***	0.74**	0.50*	0.24ns
	Right Time	0.21ns	-0.71**	0.75***	0.73**	0.66**	0.53*
	Right Width	-0.09ns	-0.56*	0.83***	0.80***	0.58*	0.37ns
	Tail Time	0.04ns	-0.22ns	0.66**	0.61*	0.36ns	0.01ns
	Tail Width	0.14ns	-0.59*	0.67**	0.64**	0.54*	0.47ns

\*, \*\*, and \*\*\* represent significance at 0.05, 0.01, and 0.001, respectively. ns – not significant

However, this relationship was found to be more apparent for break millstream flours. The flour protein content was significantly ( $P < 0.05$ ) higher for break flour thus leading to greater loaf volume (Chapter 5). For example, 5<sup>th</sup> break (B5) flour had the highest protein content of 20.3% which yielded bread loaf volume of 247cc compared to other break streams. In contrast, 6<sup>th</sup> middling (M6) stream had the highest protein content (except M4) of 13.3% but yielded the smallest bread loaf among reduction flours. Therefore, the relationship between flour protein content and bread loaf volume was not as apparent in reduction flours. In other words, when flour protein is above 15% (as seen for break flours), the relationship between protein content and bread loaf volume appears to be strong. However, this relationship appears to be weaker when flour protein content is less than 15%, as was the case for reduction millstream flours. Although there was a positive correlation between protein content and bread loaf volume for millstreams, flour protein content may not be the only parameter explaining the bread loaf differences observed among millstreams.

In the production of bread, high flour water absorption, good gluten strength, and relatively high damaged starch and arabinoxylan are required (Kweon et al., 2011). In addition to flour protein content, both starch damage and total arabinoxylan content were negatively associated with bread loaf volume, specific volume, and oven spring (Table 6.12). Damaged starch acts like a sponge resulting in increased water absorption thus bread yield (Tipples, 1969). However, excessive damaged starch leads to sticky dough that is difficult to handle during breadmaking. Baasandorj et al. (2016) determined the optimum starch damage to be 6.6-8.5% for HRS wheat. It was found that M2, M3, and M6 streams had much higher starch damage (above 9%) (Chapter 5) in which bread loaf volume was significantly lower compared to other

millstreams (Table 6.10). These results confirm that excessive starch damage is inferior on dough quality ultimately leading to smaller bread loaves.

In addition to starch damage, total arabinoxylan content also had negative correlation with bread loaf volume ( $r = -0.62$ ), which indicates that high arabinoxylan content negatively affects bread loaf volume. In other words, high arabinoxylan content could lead to lower bread loaves. It was observed that M5 and M6 streams had significantly ( $P < 0.05$ ) higher total arabinoxylan content (6.1% and 2.5%). It is important to note that M5 was excluded from the straight grade-flour due to very high ash content hence M5 stream was not used in the breadmaking. However, M6 stream had the lowest bread loaf volume, which is partly explained by the very high starch damage and total arabinoxylan content. Courtin and Delcour (2002) reported that water-unextractable arabinoxylans, which are present in discrete cell wall fragments, can form physical barriers for the gluten network during dough development thus lowering the stability of the dough structure. On the other hand, water-extractable arabinoxylans interfere gluten network by competing for water thus changing conditions for gluten development (Wang et al., 2002). Therefore, high total arabinoxylan content in later reduction flours could explain the low bread loaves obtained for these millstreams. It was also found that mixograph parameters were positively correlated with bread loaf volume, specific volume, and oven spring parameters. These results indicate that mixograph parameters (especially mid-line parameters) may explain the large variation in baking quality characteristics especially when evaluating very diverse millstream flours.

Protein molecular weight distribution (MWD) was also determined for millstreams. Although flour quality and mixing parameters showed associations with baking quality parameters, protein MWD was performed to investigate the relationship between protein MWD

and breadmaking quality. Protein MWD has been extensively studied and reported to have strong associations with end-use quality of HRS wheat flour (Baasandorj et al., 2015ab; Baasandorj et al., 2016; Hamed et al., 2016; Ohm et al., 2009; Ohm et al., 2010, Tsilo et al., 2010). It has been reported that flour protein content primarily varied with SDS-extractable protein and gliadin levels, and SDS-unextractable polymeric proteins are known to have higher associations with bread loaf volume. Table 6.12 shows the protein molecular weight distribution for different millstreams.

Table 6.12. Protein Molecular Distribution (% flour) for Millstreams

Millstream	SDS-Extractable				SDS-Unextractable			
	F1	F2	F3	F4	F1	F2	F3	F4
1st Break	0.21	0.49	1.30	6.45	0.32	0.77	1.5	1.3
2nd Break I	0.22	0.49	1.38	7.01	0.37	0.91	1.7	1.4
Break Dust	0.20	0.39	1.14	5.83	0.34	0.81	1.5	1.5
Sizing I	0.18	0.35	1.01	5.20	0.32	0.72	1.2	1.0
2nd Break II	0.20	0.45	1.33	6.91	0.40	0.98	1.7	1.4
3rd Break	0.20	0.44	1.33	7.00	0.45	1.08	1.8	1.5
Sizing II	0.18	0.31	0.96	5.12	0.33	0.70	1.3	1.1
5th Break	0.22	0.49	1.59	8.16	0.51	1.29	2.1	1.6
4th Break	0.20	0.39	1.29	6.91	0.44	1.05	1.7	1.4
1st Middlings	0.15	0.29	0.91	4.90	0.32	0.71	1.3	1.0
2nd Middlings	0.15	0.31	0.97	5.11	0.34	0.75	1.3	1.0
3rd Middlings	0.16	0.32	1.01	5.23	0.33	0.77	1.4	1.1
4th Middlings	0.17	0.36	1.12	5.70	0.37	0.82	1.4	1.1
6th Middlings	0.17	0.32	1.04	5.14	0.34	0.71	1.3	1.0
Tail Flour	0.20	0.36	1.30	6.29	0.42	0.85	1.5	1.3
Tail Cyclone	0.16	0.30	1.14	6.12	0.48	0.97	1.5	1.6
Flour								
LSD* (P<0.05)	0.01	0.03	0.05	0.20	0.04	0.08	0.1	0.1

\* Least Significant Difference

Flour protein primarily varies with SDS-extractable protein and gliadin levels (Baasandorj et al., 2015a,b). The F3 protein fraction is primarily composed of gliadins. The ANOVA showed that millstream had significant ( $P<0.0001$ ) effect on the SDS-extractable F3 fraction. This indicates that gliadin content varied among different millstreams. Break millstream

flours found to have higher gliadin compared to reduction flours. On average, break flours had significantly ( $P < 0.05$ ) higher gliadin of 1.37%, while reduction millstreams had 1% of gliadin (Table 6.12). Therefore, the high gliadin observed for break flours could explain the larger bread loaves obtained from break millstreams. Among break flours gliadin content was greatest for B5 stream. The significant ( $P < 0.05$ ) gliadin for B5 stream could be due to the very high protein content (20.3%), as there was a very high and strong correlation between SDS-extractable F3 fraction and flour protein content. Among reduction millstreams M1 and M2 had significantly ( $P < 0.05$ ) lower gliadin compared to the later reduction flours. Machet (2005) also reported that the most highly refined reduction streams (M1 and M2) had poorer protein quality, on a total flour protein basis. In addition, SDS-unextractable high molecular weight (HMW) polymeric proteins have been reported to have high associations with end-use quality of HRS wheat. Similar to gliadins, the SDS-unextractable HMW polymer proteins (F1 and F2) were higher for break flours compared to reduction flours. For examples, SDS-unextractable F1 fraction (primarily composed of HMW glutenin) was found to be higher for break flours of 0.41%, while reduction millstreams contained 0.34% on total flour protein basis. Overall, break streams possessed high protein content and good quality (high percentages of gliadins and HMW-glutenins) thus leading to larger bread loaves for break millstreams. In addition, both SDS-extractable and –unextractable protein fractions were positively correlated with bread loaf volume and specific volume (Table 6.13).

Table 6.13. The Correlation Coefficients between Protein Molecular Weight Distribution and Mass

Quality Parameters		Baking Mix Time	Baking Abs.	Loaf Volume	Specific Volume	Oven Spring	Crumb Color
SDS-Extractable (%flour)	F1	-0.33ns	-0.41ns	0.57*	0.56*	0.31ns	-0.08ns
	F2	-0.13ns	-0.46ns	0.69**	0.67**	0.45ns	0.12ns
	F3	-0.19ns	-0.14ns	0.58*	0.54*	0.25ns	-0.23ns
	F4	-0.15ns	-0.20ns	0.69**	0.65**	0.38ns	-0.13ns
SDS-Unextractable (%flour)	F1	-0.12ns	0.21ns	0.38ns	0.33ns	0.10ns	-0.43ns
	F2	-0.02ns	-0.06ns	0.65**	0.60*	0.37ns	-0.12ns
	F3	-0.07ns	-0.17ns	0.7**	0.65**	0.41ns	-0.07ns
	F4	-0.16ns	-0.17ns	0.58*	0.54*	0.35ns	-0.24ns
Molecular Weight for SDS-Extractable	F1	-0.20ns	0.72**	-0.46ns	-0.50*	-0.56*	-0.80***
	F2	-0.32ns	0.69**	-0.39ns	-0.43ns	-0.56*	-0.79***
	F3	-0.52*	0.47ns	-0.45ns	-0.47ns	-0.59*	-0.83***
	F4	-0.22ns	-0.42ns	0.15ns	0.16ns	0.18ns	0.06ns
Molecular Weight for SDS-Unextractable	F1	-0.31ns	0.44ns	-0.70**	-0.68**	-0.60*	-0.45ns
	F2	-0.32ns	0.20ns	-0.49ns	-0.46ns	-0.41ns	-0.39ns
	F3	-0.39ns	0.39ns	-0.59*	-0.58*	-0.61*	-0.67**
	F4	-0.18ns	0.79***	-0.73**	-0.75***	-0.78***	-0.70**

\*, \*\*, and \*\*\* represent significance at 0.05, 0.01, and 0.001, respectively. ns – not significant

Protein molecular weights in these fractions (F1 to F4) for both SDS-extractable and SDS-unextractable fractions were also determined using the high performance size exclusion chromatography (HPSEC) with multi angle light scattering (MALS). Table 6.14 shows the molecular weights for different millstreams for both SDS-extractable and –unextractable fraction

Table 6.14. Protein Molecular Weight (Da) for Different MIAG Millstreams

Millstream	SDS-Extractable				SDS-Unextractable			
	F1	F2	F3	F4	F1	F2	F3	F4
1st Break	1.0x10 <sup>7</sup>	2.3x10 <sup>6</sup>	1.2x10 <sup>6</sup>	1.6x10 <sup>6</sup>	2.5x10 <sup>6</sup>	7.2x10 <sup>5</sup>	5.2x10 <sup>5</sup>	6.2x10 <sup>5</sup>
2nd Break I	1.3x10 <sup>7</sup>	2.5x10 <sup>6</sup>	1.0x10 <sup>6</sup>	7.3x10 <sup>5</sup>	2.0x10 <sup>6</sup>	7.0x10 <sup>5</sup>	4.8x10 <sup>5</sup>	5.5x10 <sup>5</sup>
Break Dust	1.3x10 <sup>7</sup>	2.6x10 <sup>6</sup>	1.1x10 <sup>6</sup>	5.2x10 <sup>5</sup>	2.1x10 <sup>6</sup>	6.0x10 <sup>5</sup>	4.6x10 <sup>5</sup>	4.6x10 <sup>5</sup>
Sizing I	1.1x10 <sup>7</sup>	2.3x10 <sup>6</sup>	8.8x10 <sup>5</sup>	3.3x10 <sup>5</sup>	2.1x10 <sup>6</sup>	6.3x10 <sup>5</sup>	5.0x10 <sup>5</sup>	6.0x10 <sup>5</sup>
2nd Break II	1.2x10 <sup>7</sup>	2.6x10 <sup>6</sup>	8.0x10 <sup>5</sup>	2.6x10 <sup>5</sup>	1.9x10 <sup>6</sup>	5.3x10 <sup>5</sup>	4.4x10 <sup>5</sup>	5.6x10 <sup>5</sup>
3rd Break	1.5x10 <sup>7</sup>	3.2x10 <sup>6</sup>	9.0x10 <sup>5</sup>	2.5x10 <sup>5</sup>	1.7x10 <sup>6</sup>	5.4x10 <sup>5</sup>	4.5x10 <sup>5</sup>	5.7x10 <sup>5</sup>
Sizing II	1.6x10 <sup>7</sup>	3.4x10 <sup>6</sup>	1.2x10 <sup>6</sup>	3.3x10 <sup>5</sup>	2.3x10 <sup>6</sup>	6.6x10 <sup>5</sup>	4.9x10 <sup>5</sup>	6.7x10 <sup>5</sup>
5th Break	1.6x10 <sup>7</sup>	3.8x10 <sup>6</sup>	1.1x10 <sup>6</sup>	2.5x10 <sup>5</sup>	1.6x10 <sup>6</sup>	5.0x10 <sup>5</sup>	4.1x10 <sup>5</sup>	5.9x10 <sup>5</sup>
4th Break	2.1x10 <sup>7</sup>	4.0x10 <sup>6</sup>	1.0x10 <sup>6</sup>	2.8x10 <sup>5</sup>	1.8x10 <sup>6</sup>	5.6x10 <sup>5</sup>	4.7x10 <sup>5</sup>	6.3x10 <sup>5</sup>
1st Middlings	1.6x10 <sup>7</sup>	3.3x10 <sup>6</sup>	1.0x10 <sup>6</sup>	2.6x10 <sup>5</sup>	2.0x10 <sup>6</sup>	5.8x10 <sup>5</sup>	4.7x10 <sup>5</sup>	6.3x10 <sup>5</sup>
2nd Middlings	1.4x10 <sup>7</sup>	2.7x10 <sup>6</sup>	7.9x10 <sup>5</sup>	2.4x10 <sup>5</sup>	2.0x10 <sup>6</sup>	5.7x10 <sup>5</sup>	4.8x10 <sup>5</sup>	6.4x10 <sup>5</sup>
3rd Middlings	1.3x10 <sup>7</sup>	2.3x10 <sup>6</sup>	8.3x10 <sup>5</sup>	2.3x10 <sup>5</sup>	2.0x10 <sup>6</sup>	5.6x10 <sup>5</sup>	4.4x10 <sup>5</sup>	6.5x10 <sup>5</sup>
4th Middlings	1.6x10 <sup>7</sup>	3.2x10 <sup>6</sup>	1.0x10 <sup>6</sup>	2.5x10 <sup>5</sup>	2.6x10 <sup>6</sup>	7.0x10 <sup>5</sup>	5.2x10 <sup>5</sup>	6.4x10 <sup>5</sup>
6th Middlings	2.3x10 <sup>7</sup>	4.4x10 <sup>6</sup>	1.3x10 <sup>6</sup>	3.2x10 <sup>5</sup>	3.1x10 <sup>6</sup>	7.2x10 <sup>5</sup>	4.8x10 <sup>5</sup>	7.2x10 <sup>5</sup>
Tail Flour	2.6x10 <sup>7</sup>	5.0x10 <sup>6</sup>	1.6x10 <sup>6</sup>	3.6x10 <sup>5</sup>	2.7x10 <sup>6</sup>	7.2x10 <sup>5</sup>	6.1x10 <sup>5</sup>	7.9x10 <sup>5</sup>
Tail Cyclone	2.6x10 <sup>7</sup>	4.4x10 <sup>6</sup>	1.4x10 <sup>6</sup>	3.2x10 <sup>5</sup>	2.2x10 <sup>6</sup>	6.5x10 <sup>5</sup>	5.4x10 <sup>5</sup>	7.0x10 <sup>5</sup>
LSD* (P<0.05)	4.3x10 <sup>6</sup>	1.1x10 <sup>6</sup>	2.6x10 <sup>5</sup>	9.5x10 <sup>4</sup>	7.3x10 <sup>5</sup>	1.6x10 <sup>5</sup>	9.0x10 <sup>4</sup>	1.5x10 <sup>5</sup>

\*Least Significant Difference

The ANOVA showed that millstream had significant effect on both SDS-extractable ( $P < 0.001$ ) and SDS-unextractable ( $P < 0.05$ ). These results indicated that molecular weights varied among different millstreams. It was also observed that molecular weight for SDS-extractable fractions were much higher than the SDS-unextractable fractions. For example, high molecular weight glutenin (F1) in SDS-extractable ranged from  $1 \times 10^7$  to  $3 \times 10^7$  daltons, while F1 fraction in SDS-unextractable ranged from  $2 \times 10^6$  to  $3 \times 10^6$  daltons. Similar trend was observed other fractions (F2-F4), where molecular weights for these fractions were much higher in SDS-extractable than SDS-unextractable. The low molecular weight observed for all SDS-unextractable fraction could be due to the use of sonication used in the extraction. In the extraction, the unextractable protein in the residue was solubilized by sonication. 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. Therefore, the degradation of the glutenin as well as other protein fractions may have been degraded by the sonication procedure. Sodium dodecyl sulfate (SDS) are the most efficient solvents extracting wheat flour proteins (Bietz, 1984; Danno and Hosoney, 1982; Moonen et al., 1982). Because of the low solubility of glutenin in aqueous media, vigorous dissolution methods are required to bring it into solution. It has been reported that increased solubility of flour proteins (especially glutenin) by sonication method has been previously explained by a reduction in size of glutenin molecules (Moonen et al., 1982). These authors concluded that sonication reduces glutenin polymers by breaking covalent bonds. Singh et al. (1990) also suggested that breakage of peptide bonds after prolonged sonication. However, since disulphide bonds are weaker than peptide bonds (MacRitchie, 1975), sonication for as short as 15s may result predominantly in cleavage of disulphide bonds (Weegels et al, 1994).

However, Arfvidsson et al. (2004) also reported that the molecular weight range of the high molecular weight material in the sonicated sample was lower ( $10^5$ - $10^7$  Da) than the gently stirred sample ( $10^6$  to  $10^8$  Da). The results in the current study are in agreement with Arfvidsson et al. (2004). The SDS-extractable protein fractions are not sonicated, and the molecular weight was in the range of  $2 \times 10^6$  to  $3 \times 10^7$  Da, while the molecular weight of the SDS-unextractable protein fractions, which were sonicated, was in the range of  $5 \times 10^5$  to  $3 \times 10^6$  Da (Table 6.14). These results indicate that high and molecular weight glutenin, gliadins, and albumins (F1-F4) were all degraded into much lower molecular weight species in the case of SDS-unextractable protein fractions. Therefore, using sonication to dissolve glutenin resulted in degradation of the protein.

Reduction millstreams had higher molecular weight for both high molecular weight (HMW) and low molecular weight (LMW) glutenin for both SDS-extractable and SDS-unextractable, although SDS-unextractable fractions were much lower possibly due to a sonication procedure. On average, reduction millstreams had molecular weights of  $1.62 \times 10^7$  Da and  $2.32 \times 10^6$  Da for HMW-glutenin in the SDS-extractable and –unextractable protein, respectively (Table 6.14). In contrast, break millstreams had lower molecular weights of  $1.44 \times 10^7$  Da and  $1.9 \times 10^6$  Da for SDS extractable and –unextractable proteins, respectively. Similar trend was observed for low molecular weight (LMW) glutenin subunits for break and reduction millstreams. These results indicated that very high molecular weights in both HMW and LMW-glutenins had negative effect on the bread loaf volume. It was found that F1 fraction (HMW-glutenin) in SDS-unextractable had a negatively correlated with bread loaf volume ( $P < 0.01$ ,  $r = -0.70$ ). This indicated that very high HMW-glutenin has inferior effect on the bread loaf volume. These results explain the differences observed in the bread loaf volume between

break and reduction millstreams. Break flours produced larger loaves owing to lower molecular weights for both HMW and LMW-glutenins, while reduction flours produced smaller loaves owing to a higher molecular weights for these glutenin subunits. In addition, the molecular weights of these fractions in both SDS-extractable and –unextractable proteins had negative correlation with bread specific volume and oven spring (Table 6.13). These results indicate that as the molecular size increased for these protein fractions more dense bread was obtained. In other words, bread did not rise thus resulting in more dense bread with smaller loaf volume as the molecular weights in the four fractions increased.

## 6.6. Conclusion

The current research was carried out to investigate various MIAG Multomat millstreams for their baking quality characteristics when evaluating five HRS wheat cultivars from two regions. Main effects wheat cultivar, millstreams, and interactions between the main effects showed significant differences on the breadmaking quality characteristics. These results indicate that there was a varietal difference for quality parameters as well as growing regions. When comparing flour millstreams, reduction millstream flours had higher baking absorption owing to higher starch damage and arabinoxylan content. Break flours produced larger bread loaves, while reduction flours resulted in smaller bread loaves. Mixograph mixing parameters were positively correlated with bread loaf volume indicating that strong dough mixing parameters for break flours were associated with larger loaves. In contrast, there was starch damage and total arabinoxylan content were negatively correlated with bread loaf volume. This indicated that high starch damage and arabinoxylan content in reduction flours had negative effect on the bread loaf volume. In addition to breadmaking, protein molecular weight distribution (MWD) as well as molecular weights were determined for protein fractions. SDS-extractable gliadin SDS-

unextractable polymeric proteins (HMW and LMW-glutenins) were found to be higher in break flours explaining the larger bread loaves obtained for break millstreams. It was found that reduction flours had very high molecular weights for HMW and LMW-glutenin subunits, and HMW-glutenin in SDS-unextractable had a negatively correlated with bread loaf volume ( $P < 0.01$ ,  $r = -0.70$ ). This indicated that very high HMW-glutenin has inferior effect on the bread loaf volume. These results explain the differences observed in the bread loaf volume between break and reduction millstreams. Break flours produced larger loaves due to lower molecular weights for both HMW and LMW-glutenins, while reduction flours produced smaller loaves due to a higher molecular weights for these glutenin subunits. The knowledge of wheat kernel distribution in different millstreams as well as the flour protein composition on end-use quality in these millstreams can provide millers with very important information to optimize the functionality of flour blends.

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## 7. OVERALL CONCLUSION

The current research was carried out to determine whether the overall ranking of Hard Red Spring Wheat cultivars for quality evaluation was affected by mill type, and breadmaking methods and loaf sizes. The overall quality scoring system was developed in order to assist in comparing and ranking HRS wheat objectively. Although there were differences between various roller mill types used in the quality evaluation, the ranking of the HRS wheat cultivars did not change for mill types. In other words, based on the overall scoring system, the ranking of the wheat cultivars was consistent across different roller mills. Therefore, the overall scoring system was effective in objectively ranking these HRS wheat cultivars on their end-use quality. From the results obtained in this study we can conclude that the roller mill type does not affect the overall ranking of HRS wheat cultivars for quality evaluation when using a developed scoring system. When comparing different breadmaking methods, the results in the current research study indicate that although there are differences in the breadmaking methods on the end-use quality evaluation, the ranking of HRS wheat flours is not affected by the baking methods and processing conditions. In other words, cultivars considered to have “fair” quality tend to have low breadmaking quality, while “excellent” breadmaking cultivars will have superior end-use quality regardless of the baking method and processing conditions.

The current research was also carried out to determine the effect of roller mill type on solvent retention capacity (SRC) test for Hard Red Spring wheat. The mill type had significant ( $P < 0.001$ ) effect on the SRC results. There were differences in the SRC results between roller mills. The results from this research study indicated that SRC values for different solvents were significantly different across various roller mills. This indicated that SRC results are much dependent on the roller mill that is being used for quality evaluation. This is very important for

the milling industry and wheat quality labs, as there can be different rollers mills used in the quality evaluation for HRS wheat. Therefore, the selection of roller mill to produce flour can have a significant impact on the SRC results due to the significant differences observed in this study. Therefore, selecting a certain mill type for SRC test for quality evaluation is crucial knowing that the differences exist between various roller mills.

Lastly, the current research was carried out to investigate various MIAG Multomat millstreams for their physicochemical and breadmaking quality characteristics when evaluating 5 HRS wheat cultivars from 2 regions. It was found that main effects wheat cultivar, millstreams, and interactions between the main effects showed significant differences on the flour and end-use quality of these millstreams. These results indicate that there was a varietal difference for quality parameters as well as growing regions.

When comparing flour millstreams, reduction millstreams accounted for 47.6% of the total flour yield, while break and sizing millstreams combined 20.2% of the total flour yield. Break millstreams had average ash content of 0.77%, while reduction streams (excluding the 5<sup>th</sup> midds.) was 0.48%, which significantly lower than the break millstreams. It was also found that the most refined (reduction) streams have the brightest color, while high ash (break) streams had the darkest color. The break flours along with tail cyclone flour had very high content with average of 17.0%, while reduction millstreams had much lower protein content average of 13.5%. Break millstreams had significantly lower starch damage, while reduction millstreams had greater flour starch damage. Reduction millstreams were slightly higher AX content compared to the break millstreams. This indicates that streams containing higher ash content yield higher AX%. Reduction millstream flours had higher baking absorption due to higher starch damage and arabinoxylan content. It was found that break flours produced larger bread

loaves, while reduction flours resulted in smaller bread loaves. Mixograph mixing parameters were positively correlated with bread loaf volume indicating that strong dough mixing parameters for break flours were associated with larger loaves. In contrast, there was starch damage and total arabinoxylan content were negatively correlated with bread loaf volume. This indicated that high starch damage and arabinoxylan content in reduction flours had negative effect on the bread loaf volume.

In addition to breadmaking, protein molecular weight distribution (MWD) as well as molecular weights were determined for protein fractions. SDS-extractable gliadin SDS-unextractable polymeric proteins (HMW and LMW-glutenins) were found to be higher in break flours explaining the larger bread loaves obtained for break millstreams. It was found that reduction flours had very high molecular weights for HMW and LMW-glutenin subunits, and HMW-glutenin in SDS-unextractable had a negatively correlated with bread loaf volume ( $P < 0.01$ ,  $r = -0.70$ ). This indicated that very high HMW-glutenin has inferior effect on the bread loaf volume. These results explain the differences observed in the bread loaf volume between break and reduction millstreams. Break flours produced larger loaves owing to lower molecular weights for both HMW and LMW-glutenins, while reduction flours produced smaller loaves owing to a higher molecular weights for these glutenin subunits. The knowledge of wheat kernel distribution in different millstreams as well as the flour protein composition on end-use quality in these millstreams can provide millers with very important information to optimize the functionality of flour blends. The knowledge of wheat kernel distribution in different millstreams as well as the flour composition in these millstreams can provide millers with very important information to optimize the functionality of flour blends.

## **8. FUTURE RESEARCH AND COMPLICATIONS**

To complement the study of evaluating Hard Red Spring (HRS) wheat quality using different roller mill types, a commercial flour milling company could have been partnered in this study to show variation in flour, dough, and breadmaking quality. This would be very interesting to see whether “lab scale” and “pilot scale” mills differ from a “commercial scale” mill in the flour and end-use quality evaluation. In the evaluation of HRS wheat MIAG millstreams study, it would be interesting to check for specific minerals, lipid composition as well as molecular weight (MW) of arabinoxylan (AX) in the MIAG millstreams. In addition, it would have been interesting to include more HRS wheat cultivars in the study; however, it was challenging to get a single cultivar in large quantities.

## APPENDIX

Table A.1. The ANOVA for Milling Quality Parameters

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Flour Yield (wheat)	Mill	3	808.3	490.1	<.0001
	Sample	11	7.0	4.2	0.0006
Ash Content	Mill	3	0.1	91.8	<.0001
	Sample	11	0.0	5.6	<.0001
Protein Content	Mill	3	0.2	5.0	0.0058
	Sample	11	3.8	78.8	<.0001
Protein Loss	Mill	3	0.2	5.9	0.0025
	Sample	11	0.3	8.3	<.0001
Starch Damage	Mill	3	25.6	36.1	<.0001
	Sample	11	0.9	1.3	0.2783

Table A.2. The ANOVA for Flour and Dough Quality Parameters

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Wet Gluten	Mill	3	3.5	9.7	<.0001
	Sample	11	17.3	48.2	<.0001
Flour Falling Number	Mill	3	3729.1	21.4	<.0001
	Sample	11	5470.9	31.4	<.0001
Color (L*)	Mill	3	0.7	44.8	<.0001
	Sample	11	0.2	16.8	<.0001
Color (a)	Mill	3	0.1	18.9	<.0001
	Sample	11	0.4	120.6	<.0001
Color (b)	Mill	3	3.8	102.6	<.0001
	Sample	11	5.4	145.1	<.0001
Farinograph Water Absorption	Mill	3	15.5	31.7	<.0001
	Sample	11	8.1	16.6	<.0001
Farinograph Peak Time	Mill	3	132.1	12.6	<.0001
	Sample	11	39.4	3.7	0.0016
Farinograph Stability	Mill	3	300.3	19.7	<.0001
	Sample	11	93.6	6.2	<.0001
Extensibility (45 min)	Mill	3	2.8	4.2	0.0123
	Sample	11	1.7	2.6	0.0179
Resistance (45 min)	Mill	3	85206.7	60.0	<.0001
	Sample	11	83556.3	58.8	<.0001
Area	Mill	3	3571.8	25.1	<.0001
	Sample	11	3360.3	23.6	<.0001
Extensibility (135 min)	Mill	3	3.4	4.3	0.0113
	Sample	11	3.6	4.6	0.0003
Resistance (135 min)	Mill	3	173449.8	19.2	<.0001
	Sample	11	136254.5	15.1	<.0001
Area (135 min)	Mill	3	4358.9	22.4	<.0001
	Sample	11	4066.3	20.9	<.0001

Table A.3. The ANOVA for Breadmaking Quality Parameters

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Baking Water Absorption	Mill	3	320.3	526.2	<.0001
	Sample	11	9.0	14.9	<.0001
Baking Mix Time	Mill	3	0.2	3.7	0.0221
	Sample	11	1.2	21.6	<.0001
Dough Handling	Mill	3	0.9	3.5	0.0263
	Sample	11	0.3	1.3	0.2886
Bread Loaf Volume	Mill	3	3053.6	2.8	0.054
	Sample	11	13471.7	12.5	<.0001
Bread Symmetry	Mill	3	0.9	1.7	0.1865
	Sample	11	2.1	4.1	0.0008
Bread Crust Color	Mill	3	0.1	0.5	0.6879
	Sample	11	0.7	2.5	0.0195
Bread Crumb Grain	Mill	3	0.2	0.9	0.4517
	Sample	11	0.2	0.8	0.638
Bread Crumb Color	Mill	3	0.8	13.2	<.0001
	Sample	11	0.8	13.3	<.0001
Bread Firmness	Mill	3	2758.8	24.1	<.0001
	Sample	11	1624.1	14.2	<.0001

Table A.4. The ANOVA for Milling Quality Score

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Flour Yield	Sample	3	80.5	791.7	<.0001
	Mill	11	0.2	2.4	0.0235
Flour Ash	Sample	3	32.1	51.5	<.0001
	Mill	11	1.7	2.8	0.0119
Flour Protein	Sample	3	47.8	49.5	<.0001
	Mill	11	1.2	1.3	0.2799
Protein Loss	Sample	3	42.5	24.1	<.0001
	Mill	11	16.7	9.5	<.0001
Milling Quality Score	Sample	3	4.5	18.8	<.0001
	Mill	11	0.5	2.1	0.0485

Table A.5. The ANOVA for Flour and Dough Quality Score

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Wet Gluten	Sample	3	0.9	6.4	0.0016
	Mill	11	4.6	31.8	<.0001
Falling Number	Sample	3	0.8	5.8	0.0027
	Mill	11	1.5	11.1	<.0001
Farinograph Absorption	Sample	3	4.6	23.2	<.0001
	Mill	11	2.2	11.4	<.0001
Farinograph Peak Time	Sample	3	24.7	23.5	<.0001
	Mill	11	7.8	7.4	<.0001
Farinograph Stability	Sample	3	11.8	15.9	<.0001
	Mill	11	3.7	5.0	0.0001
Extensibility (135 min)	Sample	3	1.4	5.1	0.005
	Mill	11	0.9	3.4	0.0034
Resistance (135 min)	Sample	3	24.6	16.9	<.0001
	Mill	11	20.4	14.0	<.0001
Area (135 min)	Sample	3	14.4	13.0	<.0001
	Mill	11	15.1	13.6	<.0001
Dough Quality Score	Sample	3	2.9	24.8	<.0001
	Mill	11	2.2	19.3	<.0001

Table A.6. The ANOVA for Baking and Overall Quality Score

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Baking	Sample	3	82.7	289.8	<.0001
Absorption	Mill	11	2.4	8.4	<.0001
Dough	Sample	3	0.9	3.5	0.0263
Handling	Mill	11	0.3	1.3	0.2886
Bread Loaf	Sample	3	0.7	2.3	0.0948
Volume	Sample	11	3.4	10.8	<.0001
Grain and	Mill	3	0.2	0.9	0.4517
Texture	Sample	11	0.2	0.8	0.638
Crust Color	Mill	3	0.8	13.2	<.0001
	Sample	11	0.8	13.3	<.0001
Crust Color	Mill	3	0.1	0.5	0.6879
	Sample	11	0.7	2.5	0.0195
Symmetry	Mill	3	0.9	1.7	0.1865
	Sample	11	2.1	4.1	0.0008
Baking	Mill	3	4.6	49.0	<.0001
	Sample	11	0.6	6.4	<.0001
Quality Score	Mill	3	0.5	13.9	<.0001
	Sample	11	0.7	18.7	<.0001

Table A.7. Wheat Quality Characteristics for Wheat Cultivars

Cultivar	Test Weight	Vitreous Kernel	1000KWT	SKCS			Whole-Grain Protein	Ground Wheat Protein	Whole-Wheat Ash
				Hardness	Weight	Diameter			
SD Forefront	60.6	21.0	32.6	74.1	29.8	2.3	13.8	13.7	1.6
ND Elgin	61.0	17.0	32.8	82.5	28.5	2.2	13.4	13.8	1.6
MN Bolles	60.8	31.0	34.3	85.3	31.8	2.4	14.8	14.9	1.6
ND 817	62.3	38.0	34.8	84.3	31.9	2.4	14.2	14.3	1.5
SY Ingmar	61.7	31.0	31.7	80.6	28.4	2.2	14.6	14.7	1.5
ND Glenn	63.0	83.0	32.5	92.7	30.0	2.4	14.3	14.5	1.6
ND Dapps	61.6	44.0	33.4	78.0	32.7	2.5	14.6	14.4	1.6
ND Elgin	64.2	87.0	31.0	87.3	30.1	2.3	12.5	12.5	1.6
ND Faller	64.0	30.0	40.0	70.5	39.3	2.9	13.8	13.6	1.5
SD Focus	64.9	96.0	32.7	82.0	31.9	2.4	13.5	13.4	1.7
ND Glenn	65.5	96.0	31.6	90.0	30.9	2.4	15.1	15.1	1.5
ND Prosper	64.7	45.0	39.8	72.1	39.0	2.8	11.9	11.9	1.5

Table A.8. Milling Quality Characteristics for Wheat Cultivars

Cultivar	Flour Yield (%)	Flour Ash (%)	Flour Protein (%)	Protein Loss (%)	Starch Damage (%)
G-MN Bolles	64.2	0.47	13.9	0.62	7.1
C-ND Glenn	65.9	0.51	14.1	0.79	6.9
G-ND Glenn	66.3	0.5	12.7	1.24	7.4
G-SY Ingmar	67.9	0.47	13	1.25	7.6
C-ND Dapps	66.6	0.48	13.2	1.16	8.0
C-ND Faller	67.9	0.51	12.4	1.10	7.2
G-ND Elgin	67.3	0.45	12.3	0.81	6.4
G-ND 817	67.1	0.45	12.4	1.46	7.2
C-ND Elgin	67.9	0.50	11.3	0.85	7.3
C-SD Focus	69	0.54	12.3	0.89	6.9
G-Forefront	68.5	0.44	11.9	1.57	6.4
C-ND Prosper	69.9	0.53	10.6	1.00	7.4
LSD	1.7	0.04	0.3	0.29	1.2

Table A.9. Flour and Dough Quality Characteristics for Wheat Cultivars

Cultivar	Farinograph					Extensigraph		
	Falling Number	Wet Gluten	Water Abs.	Peak Time	Stability	Extensibilit y (135min)	Resistance (135 min)	Area (135min)
	(sec.)	(%)	(%)	(min.)	(min.)			
G-MN Bolles	460	36.1	63.6	15.4	23.5	15.7	1182	241
C-ND Glenn	464	35.7	63.3	15	21	12.8	1257	208
G-ND Glenn	408	32.6	64.2	10	15.9	12.8	1208	199
G-SY Ingmar	445	32.7	61.6	12.9	21.4	15.3	1023	204
C-ND Dapps	363	35.3	62.8	9	17.2	14.7	903	168
C-ND Faller	428	33.4	64	8.7	14.6	13.9	869	157
G-ND Elgin	398	31.8	63.1	9.4	15.7	15.4	844	173
G-ND 817	385	33.9	64.6	8.6	14.5	14.7	824	161
C-ND Elgin	423	31.4	63.8	7.6	14.8	14.9	708	140
C-SD Focus	490	34.2	61.4	6.3	6.7	13.9	902	161
G-Forefront	404	30.7	59.8	9.1	15.7	14.5	924	175
C-ND Prosper	396	29.3	61.6	5.4	8.7	13.6	710	127
LSD	19	0.86	1	4.7	5.6	1.3	137	20

Table A.10. Baking Quality Characteristics for Wheat Cultivars

Cultivar	Baking	Bake Mix	Loaf	Crumb	Crumb	Firmness
	Abs.	Time	Volume	Color	Grain	
	(%)	(min.)	(cc)			(g)
G-MN Bolles	68.2	5.2	1080	7.3	7.6	78
C-ND Glenn	68.6	5.3	1003	7.7	7.6	96
G-ND Glenn	68.7	5.1	994	7.7	7.3	72
G-SY Ingmar	65.6	4.6	1050	7.1	7.9	103
C-ND Dapps	67	4.3	1018	8.1	7.6	90
C-ND Faller	67.6	4.2	961	8.3	7.9	98
G-ND Elgin	67.3	4.2	1008	7.3	7.6	89
G-ND 817	68.8	4.2	979	7.4	7.2	74
C-ND Elgin	68.7	3.9	903	7.1	7.6	113
C-SD Focus	67	3.6	928	6.9	7.7	124
G-Forefront	63.8	4.3	976	7.8	7.6	128
C-ND Prosper	66.8	3.8	897	8.1	7.6	128
LSD	1.1	0.3	47	0.4	0.6	15

Table A.11. Milling Quality Score for Mill Types

Mill Type	Flour	Flour Ash	Protein	Starch	Milling Quality
	Yield		Loss	Damage	Score
Quad. Jr	3.8d	8.6b	8.4a	4.3b	6.3b
Quad. Sr	6.9c	10.0a	3.8c	2.6c	6.4b
Buhler	9.4a	6.2d	6.4b	6.9a	7.3a
MIAG	9.0b	7.3c	5.9b	6.4a	7.4a

Table A.12. Flour and Dough Quality Scores for Mill Types

Mill Type	Wet Gluten	Falling Number	Farinograph			Extensigraph			Dough Quality Score
			Water Abs.	Peak Time	Stability	Extensibility	Resistance	Area	
Quad. Jr	4.6a	6.8a	5.3b	6.9a	4.8a	6.2a	6.8a	7.1b	5.7a
Quad. Sr	4.2b	6.6a	4.5c	5.9b	4.4a	5.9ab	7.6a	8.3a	5.3b
Buhler	4.6a	6.7a	5.7ab	3.9c	2.7b	5.4c	4.7b	5.9c	4.6c
MIAG	4.8a	6.2b	5.9a	4.2c	3.2b	5.6bc	4.9b	6.0c	4.8c

Table A.13. Baking Quality Scores for Mill Types

Mill Type	Baking Abs.	Dough Handling	Loaf Volume	Grain Texture	Crumb Color	Crust Color	Symmetry	Baking Quality Score
Quad. Jr	2.5c	9.6a	6.0ab	7.5a	7.3b	9.3a	8.2a	6.5b
Quad. Sr	3.4b	9.3ab	5.8b	7.5a	7.4b	9.3a	8.0a	6.6b
Buhler	7.5a	8.9b	6.1ab	7.6a	7.7a	9.2a	8.5a	7.5a
MIAG	7.4a	9.2ab	6.4a	7.8a	7.8a	9.4a	8.5a	7.7a

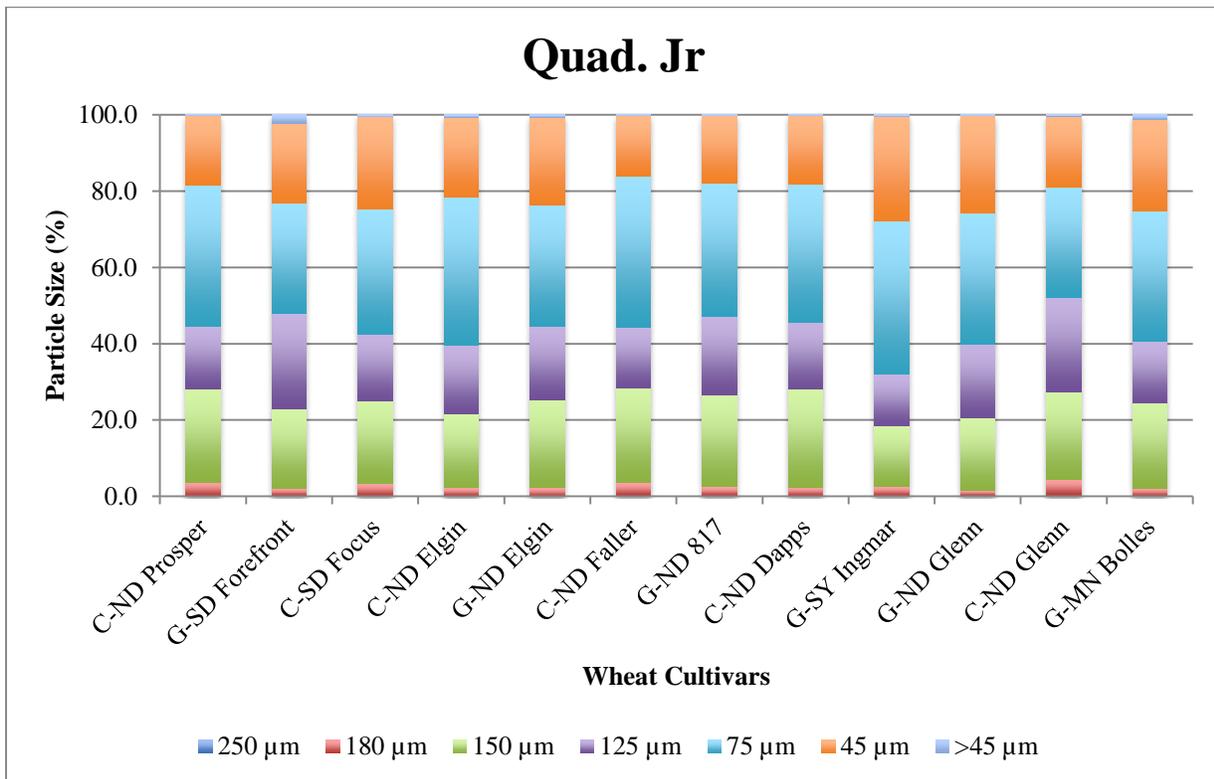


Figure A.1. Particle Size Distribution for Quadrumat Jr. Mill

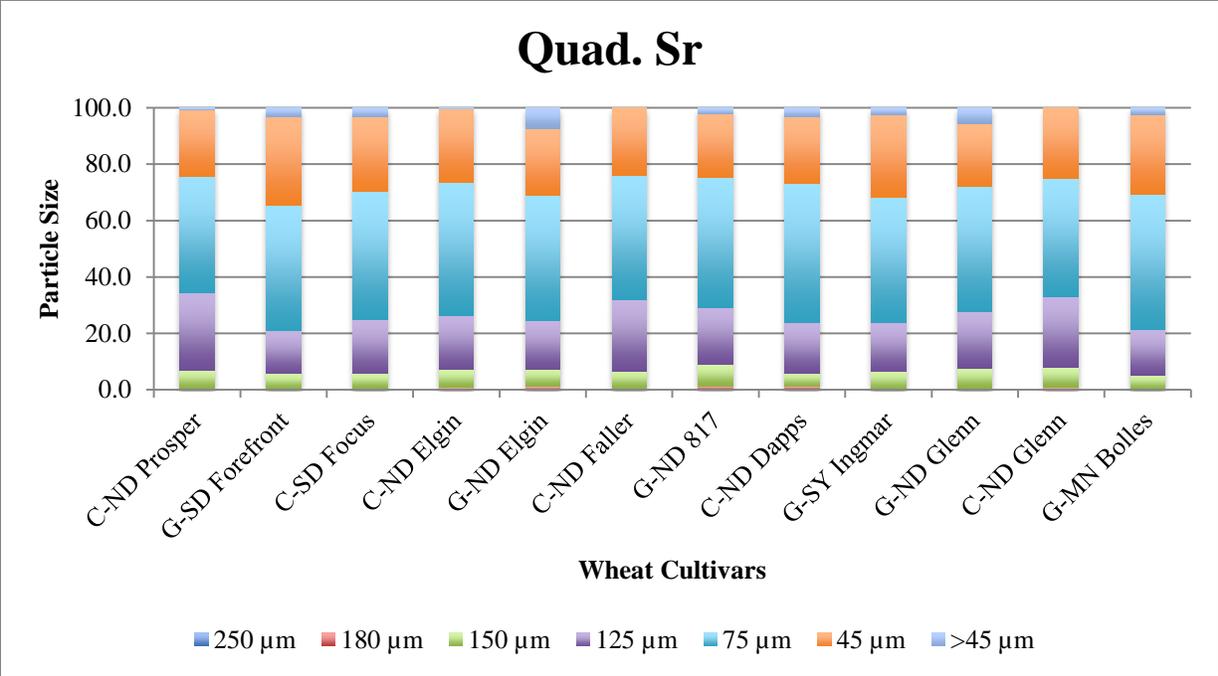


Figure A.2. Particle Size Distribution for Quadrumat Sr. Mill

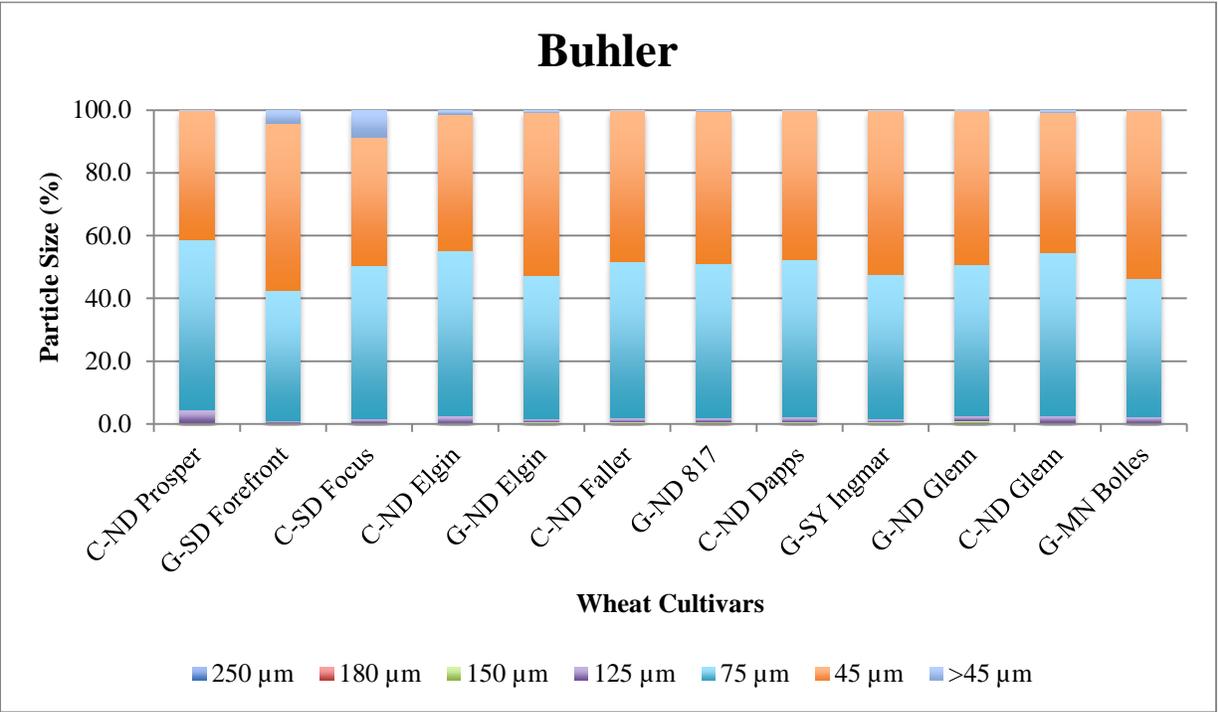


Figure A.3. Particle Size Distribution for Buhler Mill

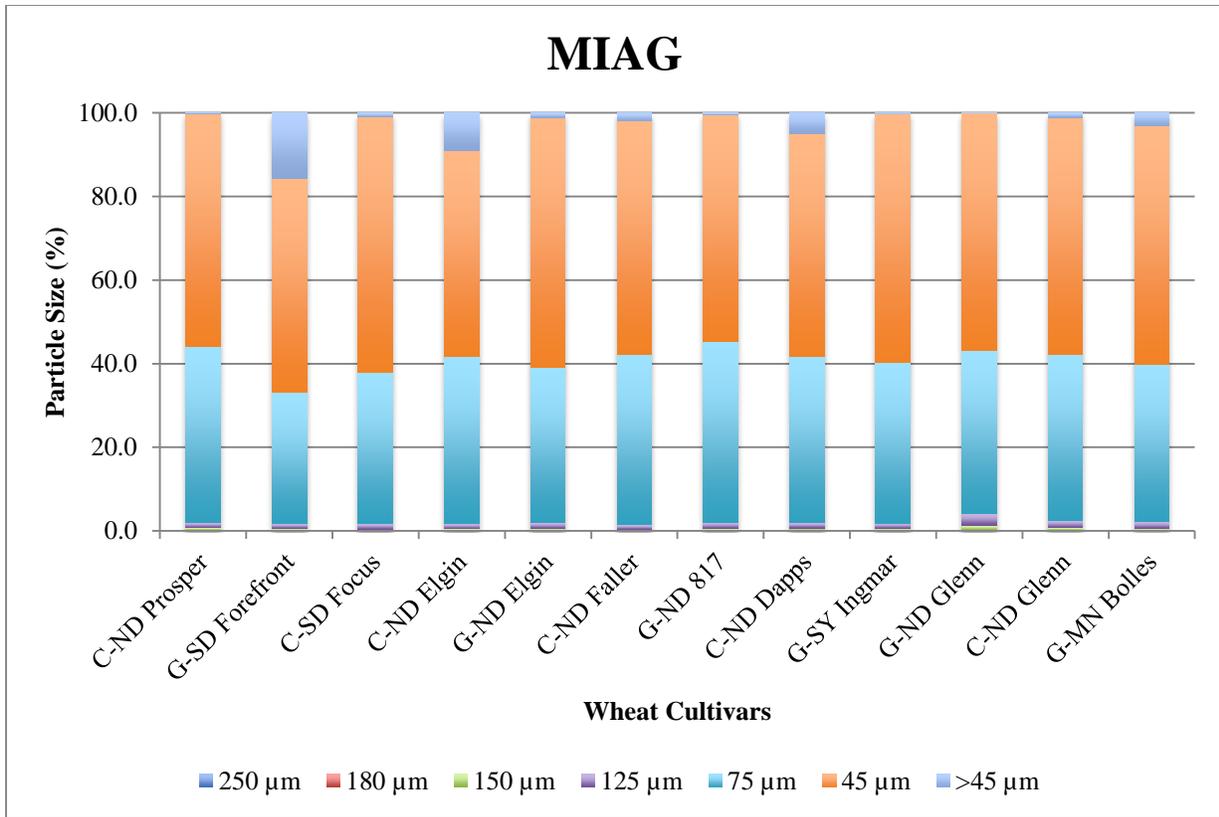


Figure A.4. Particle Size Distribution for MIAG Mill

Table A.14. The ANOVA for Baking Quality Characteristics

Dependent Variables	Source	DF	Mean Square	F Value	Pr > F
Baking Absorption	Sample	17	24.2	27.5	<.0001
	Baking Method	7	35.0	39.8	<.0001
Baking Mix Time	Sample	17	1.5	17.4	<.0001
	Baking Method	7	13.6	153.6	<.0001
Dough Handling	Sample	17	1.6	4.6	<.0001
	Baking Method	7	0.3	0.9	0.4855
Oven Spring	Sample	17	0.3	2.3	0.0049
	Baking Method	7	24.3	172.5	<.0001
Loaf Volume	Sample	17	27950.0	5.4	<.0001
	Baking Method	7	19701916.0	3820.1	<.0001
Specific Volume	Sample	17	1.1	7.2	<.0001
	Baking Method	7	13.3	86.7	<.0001
Symmetry	Sample	17	0.7	2.3	0.0056
	Baking Method	7	9.5	31.4	<.0001
Crust Color	Sample	17	0.2	1.4	0.1327
	Baking Method	7	0.2	1.6	0.1452
Crumb Grain	Sample	17	2.2	8.6	<.0001
	Baking Method	7	0.5	2.1	0.0505
Crumb Color	Sample	17	1.0	10.8	<.0001
	Baking Method	7	0.3	3.1	0.005
Firmness	Sample	17	6065.6	3.1	0.0002
	Baking Method	7	98693.0	49.9	<.0001

Table A.15. The ANOVA for Baking Quality Score

Dependent Variables	Source	DF	Mean Square	F Value	Pr > F
Baking Absorption	Sample	17	5.8	21.2	<.0001
	Baking Method	7	8.5	31.2	<.0001
Baking Mix Time	Sample	17	5.3	11.1	<.0001
	Baking Method	7	54.3	114.3	<.0001
Dough Handling	Sample	17	1.6	4.6	<.0001
	Baking Method	7	0.3	0.9	0.4855
Oven Spring	Sample	17	3.7	1.6	0.0803
	Baking Method	7	55.4	23.8	<.0001
Loaf Volume	Sample	17	6.5	5.6	<.0001
	Baking Method	7	28.9	24.9	<.0001
Specific Volume	Sample	17	4.5	6.5	<.0001
	Baking Method	7	53.7	76.7	<.0001
Symmetry	Sample	17	0.7	2.3	0.0056
	Baking Method	7	9.5	31.4	<.0001
Crumb Grain	Sample	17	2.2	8.6	<.0001
	Baking Method	7	0.5	2.1	0.0505
Crumb Color	Sample	17	1.0	10.8	<.0001
	Baking Method	7	0.3	3.1	0.005
Baking Score	Sample	17	1.8	7.6	<.0001
	Baking Method	7	6.3	27.1	<.0001

Table A.16. Baking Quality Characteristics for Wheat Cultivars

Cultivar	Baking		Dough Handling	Oven Spring	Loaf Volume	Specific Volume	Symmetry	Crust Color	Crumb Grain	Crumb Color	Firmness
	Baking Abs. (%)	Mix Time (min.)									
C-ND Elgin	69.9	3.5	8.8	2.20	1291	6.15	6.69	9.63	6.59	7.09	136.1
C-SD Focus	69.6	3.5	8.1	2.40	1314	6.32	6.72	9.56	6.09	6.69	139.3
C-ND Prosper	69.0	3.7	7.8	2.79	1324	6.08	6.88	9.75	6.69	7.94	154.4
G-Forefront	64.5	3.7	8.8	2.71	1378	6.57	7.06	9.69	6.66	7.75	190.7
G-ND 817	70.4	3.7	8.9	2.66	1390	6.48	6.75	9.56	6.25	7.50	120.2
P-ND Elgin	67.9	3.6	8.4	2.68	1427	6.74	7.25	9.81	6.41	7.41	193.9
P-WA Glee	68.6	3.5	9.0	2.65	1377	6.53	7.06	9.44	6.81	7.81	135.7
P-SY Ingmar	65.4	4.0	8.9	2.77	1418	7.14	7.19	9.94	7.19	7.38	160.1
G-SY Ingmar	66.2	3.9	8.9	2.68	1452	7.08	7.31	9.88	7.28	7.44	161.8
C-ND Dapps	68.6	3.8	8.9	2.93	1388	6.90	7.00	9.81	6.84	7.84	132.3
C-ND Faller	69.9	3.7	8.9	2.92	1407	6.64	7.00	9.75	6.75	7.97	108.2
C-ND Glenn	66.2	4.8	9.1	2.81	1373	6.56	7.38	9.75	7.81	8.03	113.2
G-ND Elgin	69.0	3.9	8.5	2.89	1445	6.92	7.13	9.94	6.31	7.38	115.0
P-MN Bolles	70.1	4.7	9.4	2.69	1445	7.03	7.44	9.81	7.19	7.50	120.4
G-ND Glenn	69.6	4.5	9.0	3.03	1458	6.96	7.28	9.56	7.44	7.94	106.9
P-ND 817	69.1	3.8	8.7	2.96	1497	7.21	7.38	9.75	6.56	7.75	99.1
G-MN Bolles	70.1	4.5	9.5	2.84	1470	7.07	7.34	9.69	7.16	7.75	134.5
P-ND Glenn	67.5	4.5	9.5	2.86	1488	7.40	7.84	9.88	8.00	8.03	113.5
LSD (0.05)	0.9	0.3	0.6	0.37	71	0.39	0.54	0.33	0.50	0.31	44.0
LSD (0.01)	1.2	0.4	0.8	0.49	94	0.51	0.72	0.43	0.67	0.40	58.2

Table A.17. The ANOVA for Solvent Retention Capacity Parameters

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Water	Sample	3	499.5	215.1	<.0001
	Mill	11	30.4	13.1	<.0001
Sodium Carbonate	Sample	3	1473.9	260.6	<.0001
	Mill	11	85.9	15.2	<.0001
Lactic Acid	Sample	3	384.3	22.6	<.0001
	Mill	11	706.1	41.4	<.0001
Sucrose	Sample	3	1668.0	293.4	<.0001
	Mill	11	118.4	20.8	<.0001
Gluten Performance Index	Sample	3	0.1	109.7	<.0001
	Mill	11	0.0	22.6	<.0001

Table A.18. The ANOVA for Rapid Visco Analyzer Parameters

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
Peak Viscosity	Sample	3	726708.3	160.2	<.0001
	Mill	11	242268.9	53.4	<.0001
Trough	Sample	3	326761.4	148.4	<.0001
	Sample	11	51211.9	23.3	<.0001
Breakdown	Mill	3	83849.0	60.8	<.0001
	Sample	11	137557.7	99.8	<.0001
Final Viscosity	Mill	3	528158.7	106.1	<.0001
	Sample	11	134818.7	27.1	<.0001
Setback	Mill	3	30156.5	31.2	<.0001
	Sample	11	31074.2	32.1	<.0001
Peak Time	Mill	3	0.0	0.5	0.6735
	Sample	11	0.0	5.5	<.0001
Pasting Temperature	Mill	3	14.4	1.7	0.1936
	Sample	11	9.2	1.1	0.4183

Table A.19. Solvent Retention Profiles for Wheat Cultivars

Cultivar	Water	Sodium Carbonate	Lactic Acid	Sucrose	Gluten Performance Index
G-Forefront	68.5	78.3	140.3	104.1	0.77
G-ND Elgin	70.6	90.6	152.0	113.4	0.75
G-MN Bolles	73.9	93.2	161.1	119.5	0.76
G-ND 817	72.5	90.8	153.0	111.5	0.76
G-SY Ingmar	69.1	86.9	157.6	110.0	0.81
G-ND Glenn	74.1	95.3	154.1	115.8	0.74
C-ND Dapps	68.5	85.3	156.1	107.9	0.82
C-ND Elgin	71.6	90.8	131.1	106.2	0.67
C-ND Faller	70.3	87.3	143.7	106.8	0.74
C-SD Focus	67.5	83.0	123.6	100.5	0.68
C-ND Glenn	71.3	89.4	146.1	111.3	0.73
C-ND Prosper	69.1	90.0	121.9	103.8	0.63
LSD	2.2	3.4	5.9	3.4	0.03

Table A.20. The RVA Profiles for Wheat Cultivars

Cultivar	Peak Viscosity	Trough	Breakdown	Final Viscosity	Setback	Peak Time	Pasting Temperature
G-Forefront	2478.63	1417.88	1060.75	2623.25	1205.38	6.18	67.8
G-ND Elgin	2573	1415	1158	2568	1153	6.18	67.4
G-MN Bolles	2834	1549	1285	2765	1216	6.23	67.8
G-ND 817	2857	1487	1371	2716	1107	6.18	66.9
G-SY Ingmar	2393	1402	991	2692	1290	6.13	67.9
G-ND Glenn	2711	1364	1347	2401	1037	6.14	67.5
C-ND Dapps	2327	1258	1069	2326	1068	6.08	72.5
C-ND Elgin	2515	1350	1165	2409	1059	6.20	67.0
C-ND Faller	2783	1631	1152	2854	1223	6.28	67.5
C-SD Focus	2376	1496	880	2716	1219	6.22	66.8
C-ND Glenn	3168	1606	1562	2909	1303	6.12	68.4
C-ND Prosper	2688	1565	1123	2751	1187	6.19	67.7
LSD	97	67	53	101	45	0.07	4.2

# PNW

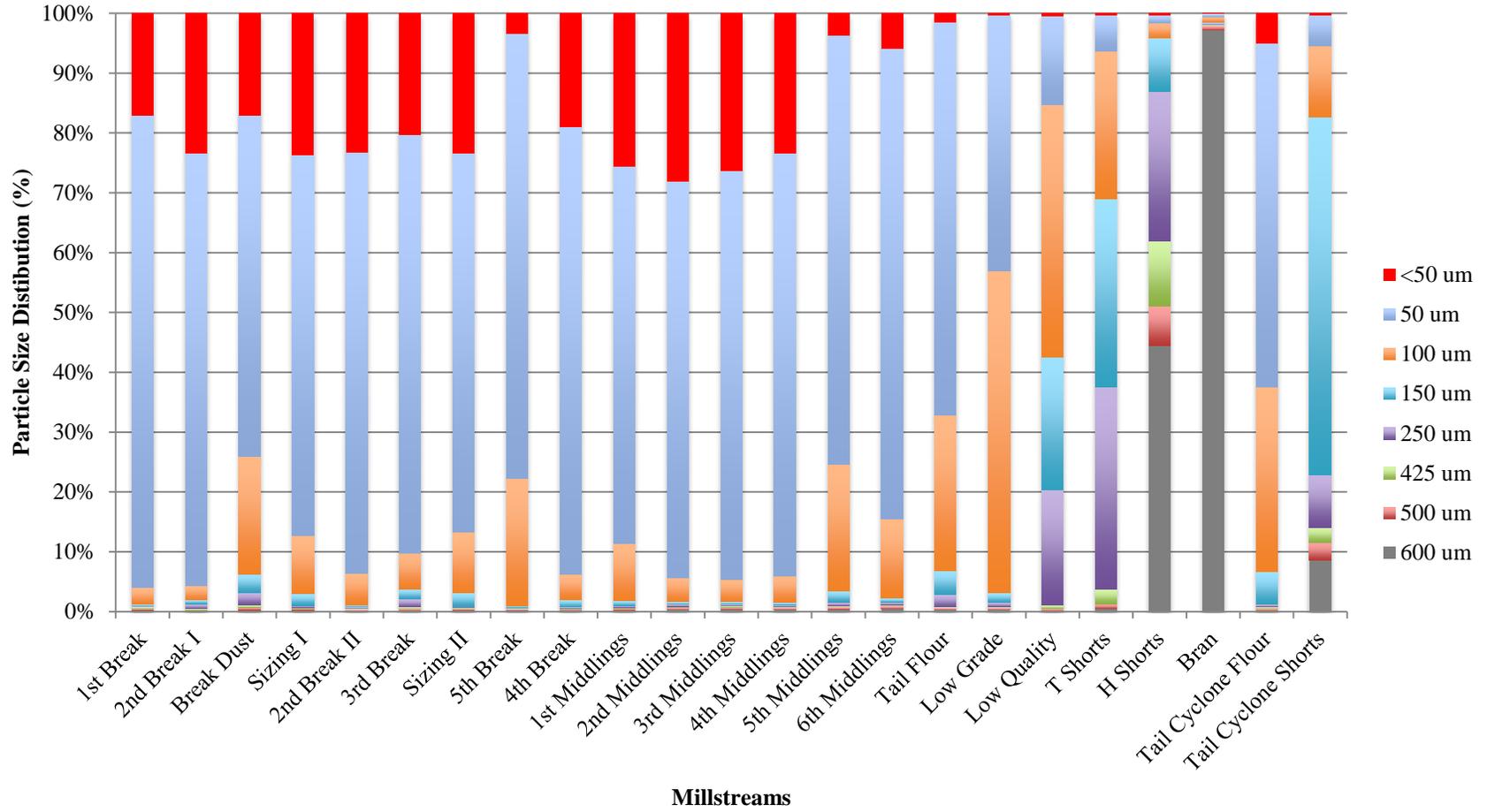


Figure A.5. Particle Size Distribution for Pacific Northwest Region

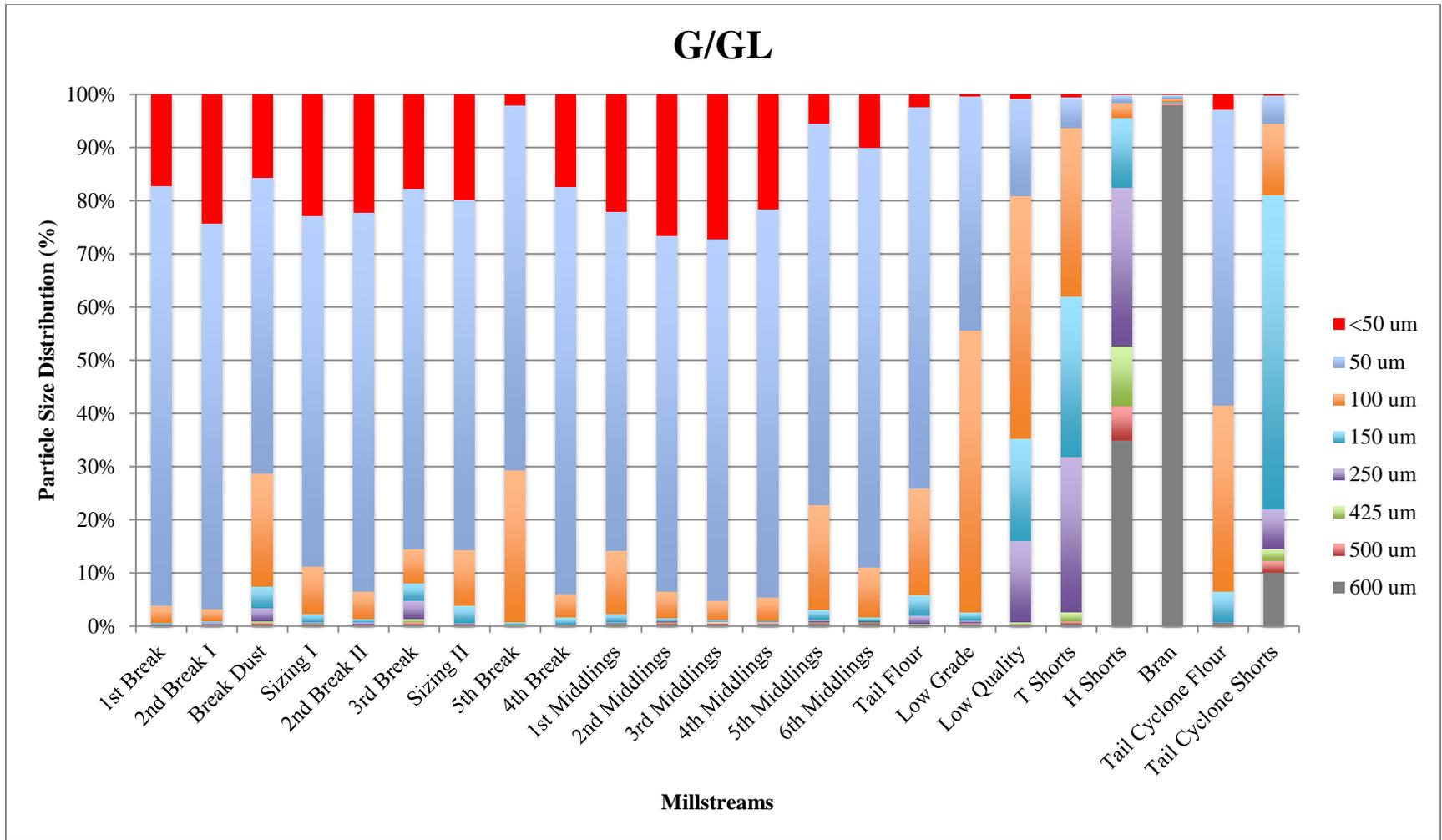


Figure A.6. Particle Size Distribution for Gulf/Great Lakes Region

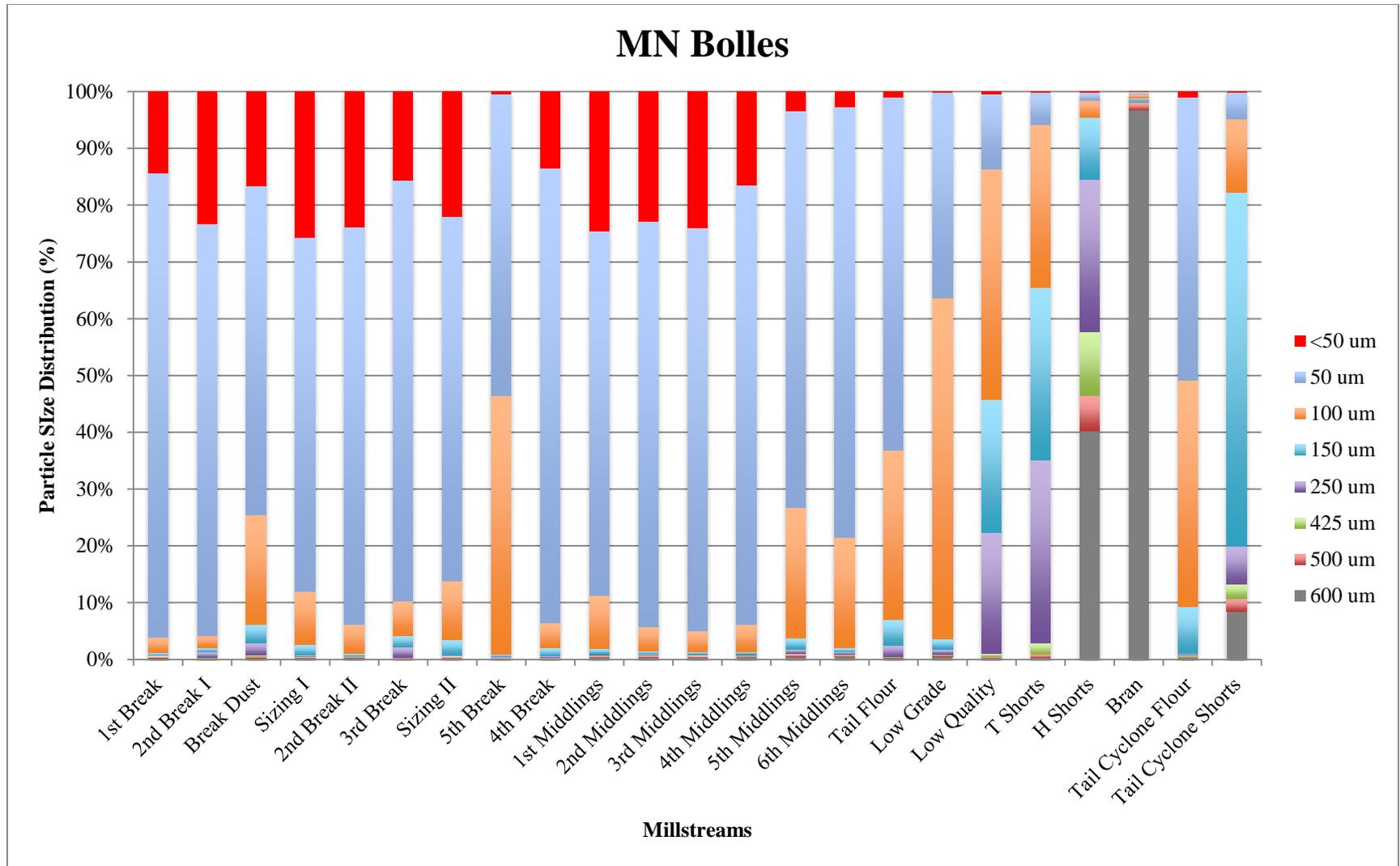


Figure A.7. Particle Size Distribution for MN-Bolles Cultivar

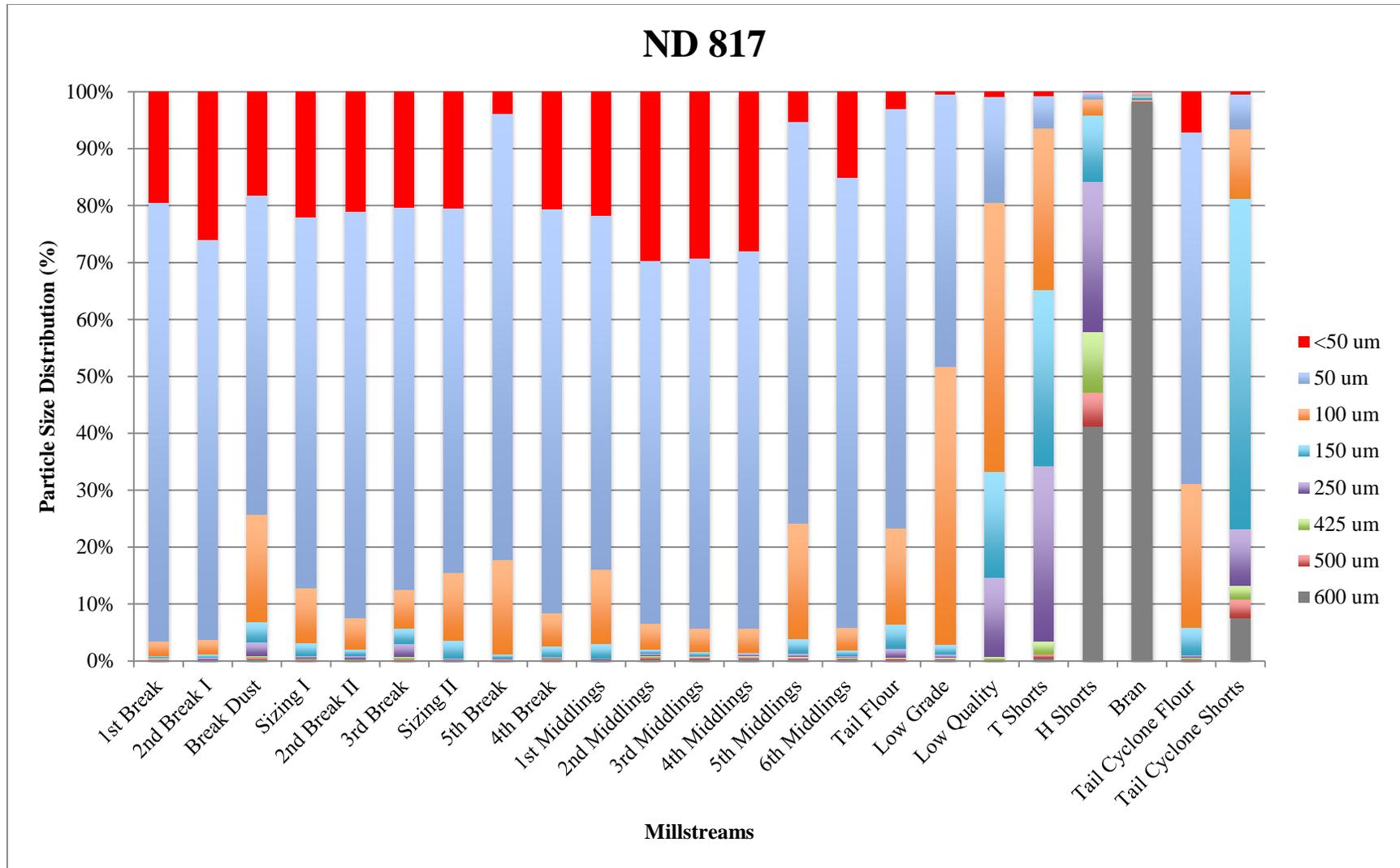


Figure A.8. Particle Size Distribution for ND-817 Cultivar

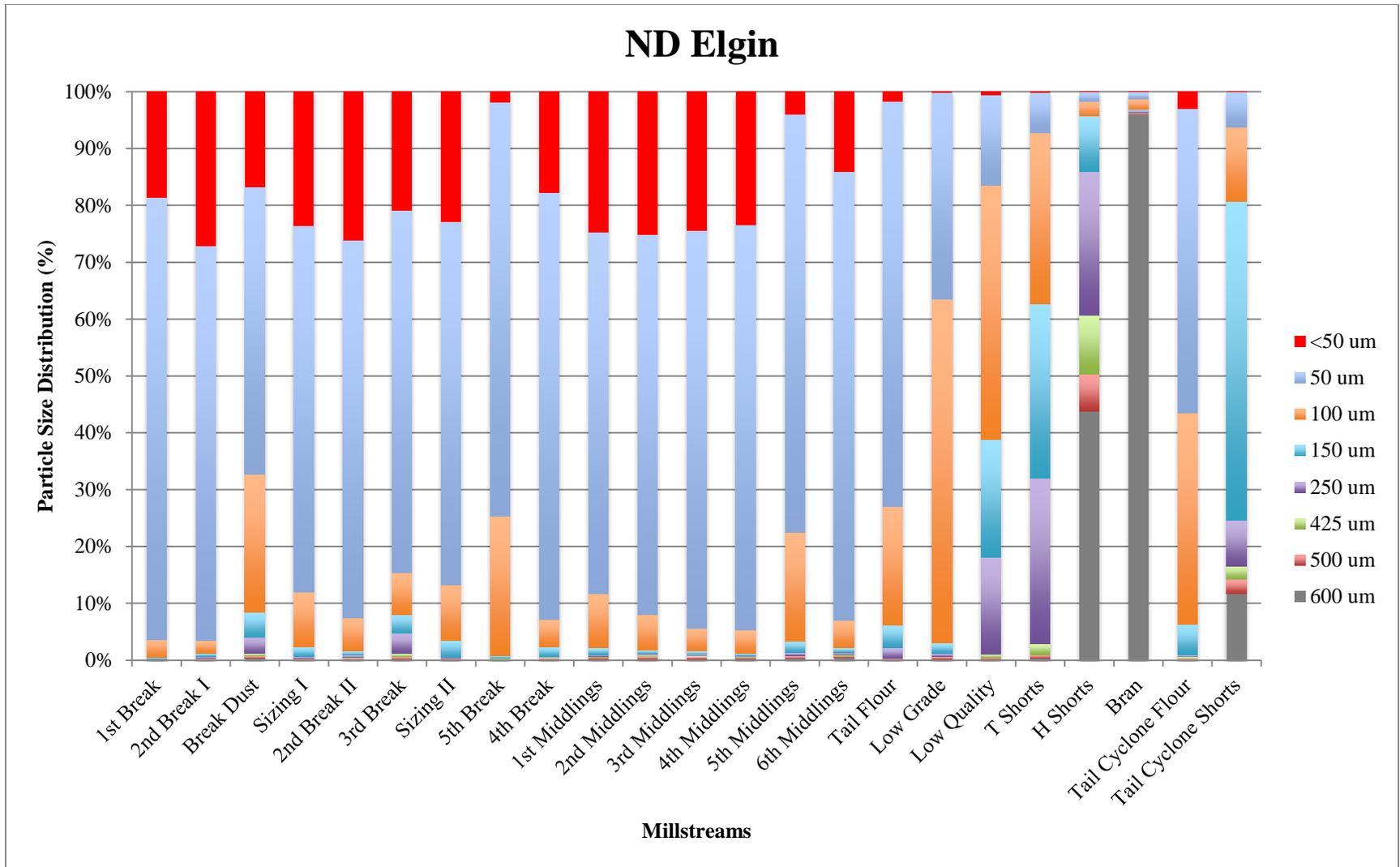


Figure A.8. Particle Size Distribution for ND-Elgin Cultivar

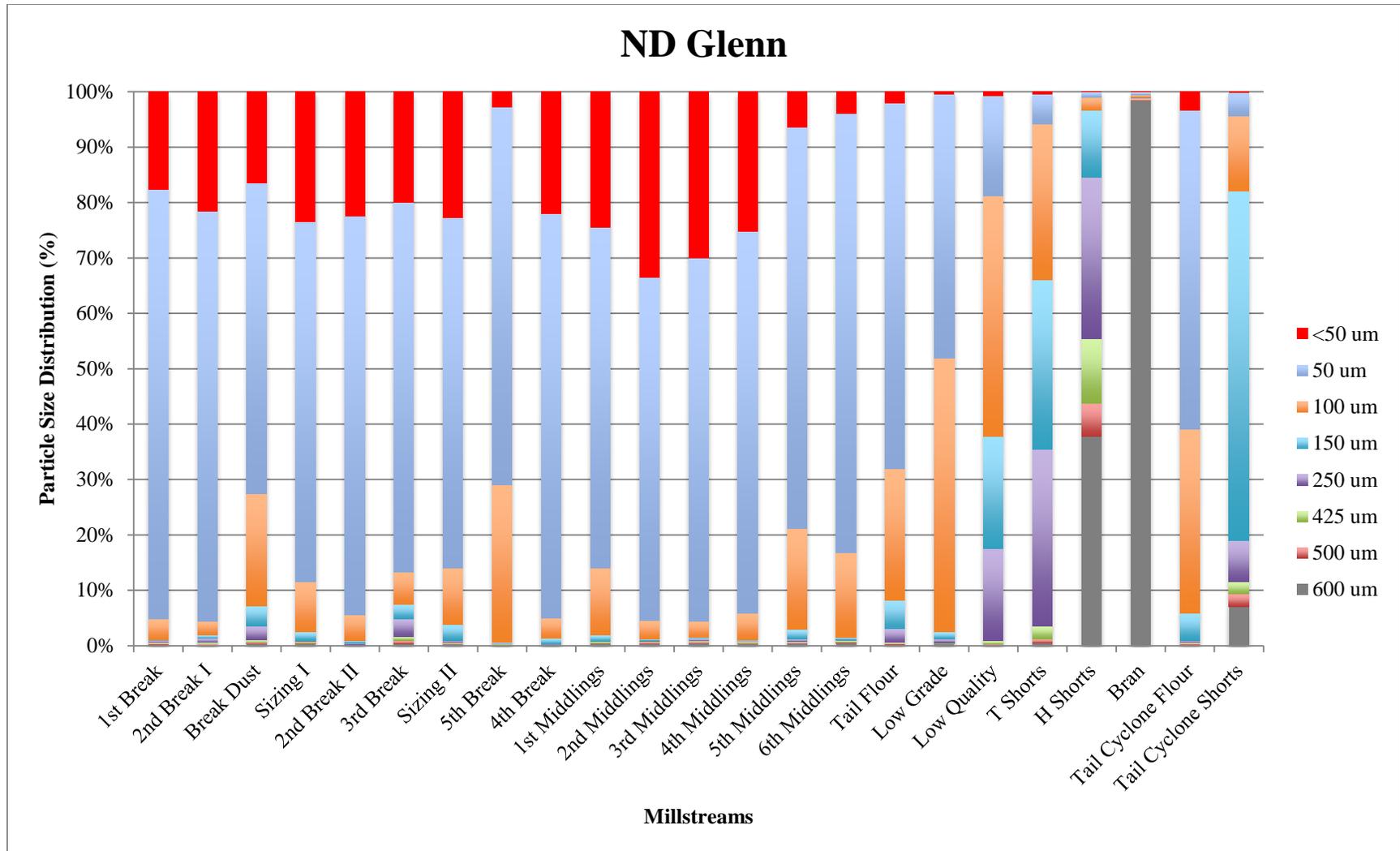


Figure A.9. Particle Size Distribution for ND-Glenn

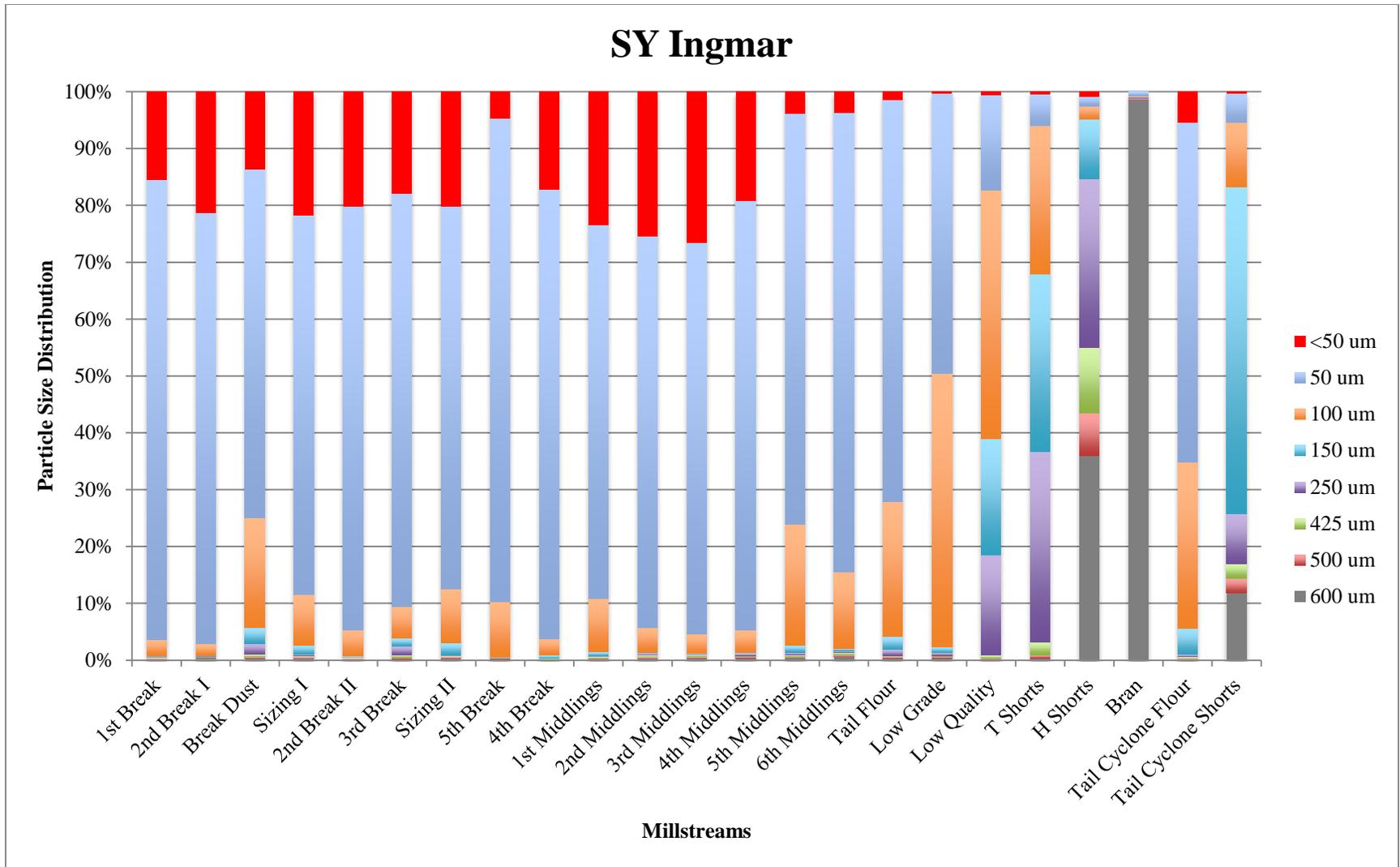


Figure A.10. Particle Size Distribution for SY-Ingmar Cultivar

Table A.21. The ANOVA for SDS-Extractable Protein Fractions (% flour)

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
F1	Location (LOC)	1	0.07	303.2	<.0001
	Cultivar (VAR)	4	0.02	107.95	<.0001
	Stream (STR)	15	0.01	24.92	<.0001
	LOC*VAR	4	0.01	64.29	<.0001
	LOC*STR	15	0.00	1.22	0.2857
	VAR*STR	60	0.00	1.31	0.1523
	F2	Location (LOC)	1	1.27	1043.11
Cultivar (VAR)		4	0.06	48.5	<.0001
Stream (STR)		15	0.05	42.37	<.0001
LOC*VAR		4	0.10	81.13	<.0001
LOC*STR		15	0.00	4.01	<.0001
VAR*STR		60	0.00	1.8	0.0125
F3		Location (LOC)	1	1.43	386.5
	Cultivar (VAR)	4	0.67	180.34	<.0001
	Stream (STR)	15	0.37	98.71	<.0001
	LOC*VAR	4	0.22	60.42	<.0001
	LOC*STR	15	0.00	0.82	0.6563
	VAR*STR	60	0.01	3.31	<.0001
	F4	Location (LOC)	1	7.15	146.92
Cultivar (VAR)		4	9.74	200.06	<.0001
Stream (STR)		15	8.95	183.88	<.0001
LOC*VAR		4	1.95	40.06	<.0001
LOC*STR		15	0.07	1.52	0.1253
VAR*STR		60	0.20	4.15	<.0001

Table A.22. The ANOVA for SDS-Unextractable Protein Fractions (% flour)

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
F1	Location (LOC)	1	0.66	276.26	<.0001
	Cultivar (VAR)	4	0.27	112.81	<.0001
	Stream (STR)	15	0.04	15.61	<.0001
	LOC*VAR	4	0.04	16.3	<.0001
	LOC*STR	15	0.00	0.72	0.7569
	VAR*STR	60	0.00	1.61	0.0332
F2	Location (LOC)	1	2.03	256.08	<.0001
	Cultivar (VAR)	4	0.19	23.97	<.0001
	Stream (STR)	15	0.28	35.74	<.0001
	LOC*VAR	4	1.25	157.99	<.0001
	LOC*STR	15	0.01	1.62	0.0943
	VAR*STR	60	0.01	1.07	0.4004
F3	Location (LOC)	1	0.45	62.58	<.0001
	Cultivar (VAR)	4	0.89	123.76	<.0001
	Stream (STR)	15	0.60	83.63	<.0001
	LOC*VAR	4	0.06	8.29	<.0001
	LOC*STR	15	0.01	1.21	0.2887
	VAR*STR	60	0.01	1.75	0.0164
F4	Location (LOC)	1	0.29	18.59	<.0001
	Cultivar (VAR)	4	0.54	35.24	<.0001
	Stream (STR)	15	0.45	29.49	<.0001
	LOC*VAR	4	0.12	7.91	<.0001
	LOC*STR	15	0.01	0.84	0.6282
	VAR*STR	60	0.02	1.04	0.4371

Table A.23. The ANOVA for Molecular Weight of SDS-Extractable Protein Fractions

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
F1	Location (LOC)	1	2.36E+15	101.05	<.0001
	Cultivar (VAR)	4	2.04E+15	87.16	<.0001
	Stream (STR)	15	2.73E+14	11.66	<.0001
	LOC*VAR	4	7.37E+14	31.53	<.0001
	LOC*STR	15	3.01E+13	1.29	0.2386
	VAR*STR	60	2.60E+13	1.11	0.341
F2	Location (LOC)	1	1.86E+14	134.05	<.0001
	Cultivar (VAR)	4	8.59E+13	61.82	<.0001
	Stream (STR)	15	7.30E+12	5.26	<.0001
	LOC*VAR	4	5.68E+13	40.89	<.0001
	LOC*STR	15	1.28E+12	0.92	0.543
	VAR*STR	60	1.36E+12	0.98	0.5304
F3	Location (LOC)	1	2.37E+12	28.24	<.0001
	Cultivar (VAR)	4	4.04E+12	48.17	<.0001
	Stream (STR)	15	4.91457E+11	5.86	<.0001
	LOC*VAR	4	1.65E+12	19.69	<.0001
	LOC*STR	15	1.08816E+11	1.3	0.2327
	VAR*STR	60	95093135366	1.13	0.3145
F4	Location (LOC)	1	8.17E+12	7.19	0.0094
	Cultivar (VAR)	4	6.38E+12	5.62	0.0007
	Stream (STR)	15	1.16E+12	1.02	0.4482
	LOC*VAR	4	5.73E+12	5.04	0.0014
	LOC*STR	15	1.11E+12	0.98	0.4866
	VAR*STR	60	1.16E+12	1.02	0.4715

Table A.24. The ANOVA for Molecular Weight of SDS-Unextractable Protein Fractions

Dependent Variable	Source	DF	Mean Square	F Value	Pr > F
F1	Location (LOC)	1	3.95E+13	58.96	<.0001
	Cultivar (VAR)	4	1.84E+13	27.4	<.0001
	Stream (STR)	15	1.52E+12	2.27	0.013
	LOC*VAR	4	1.13E+13	16.88	<.0001
	LOC*STR	15	7.71976E+11	1.15	0.334
	VAR*STR	60	7.6159E+11	1.14	0.3118
	F2	Location (LOC)	1	1.49E+12	46.71
Cultivar (VAR)		4	5.82199E+11	18.3	<.0001
Stream (STR)		15	55884412957	1.76	0.0637
LOC*VAR		4	2.27597E+11	7.15	<.0001
LOC*STR		15	26624780099	0.84	0.6343
VAR*STR		60	35943092507	1.13	0.3193
F3		Location (LOC)	1	2.14472E+11	20.75
	Cultivar (VAR)	4	1.5716E+11	15.21	<.0001
	Stream (STR)	15	22578964966	2.18	0.017
	LOC*VAR	4	35737427343	3.46	0.0131
	LOC*STR	15	10304133714	1	0.4701
	VAR*STR	60	10805551568	1.05	0.4317
	F4	Location (LOC)	1	32903703631	1.1
Cultivar (VAR)		4	453713981242	15.17	<.0001
Stream (STR)		15	58182009469	1.95	0.0359
LOC*VAR		4	378073947498	12.64	<.0001
LOC*STR		15	29485922017	0.99	0.4812
VAR*STR		60	23895434172	0.8	0.8066

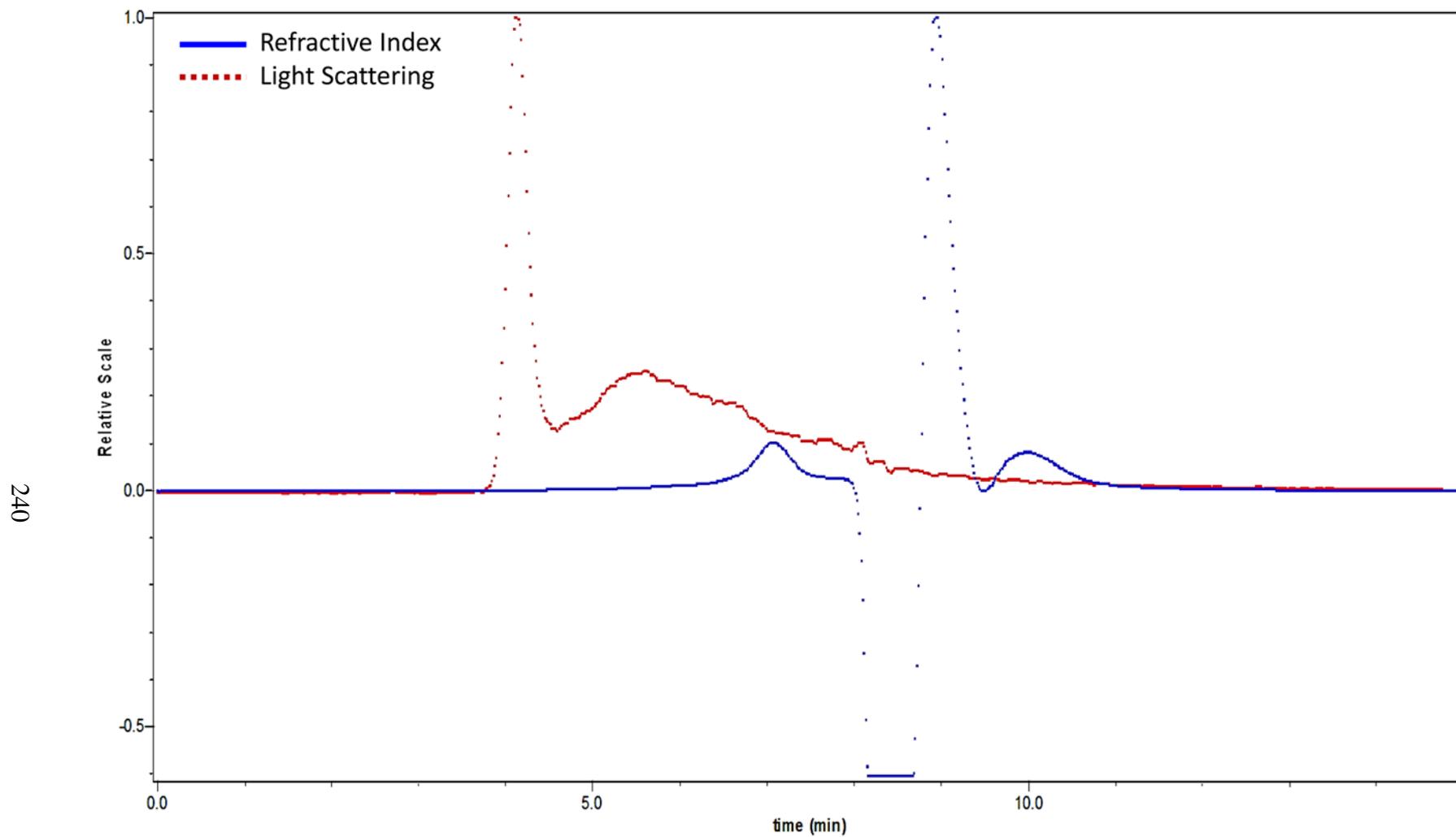


Figure A.11. A Chromatogram for SDS-Extractable Protein Sample from Tail Cyclone Flour Millstream of ND-Glenn Cultivar from Gulf/Great Lakes Region

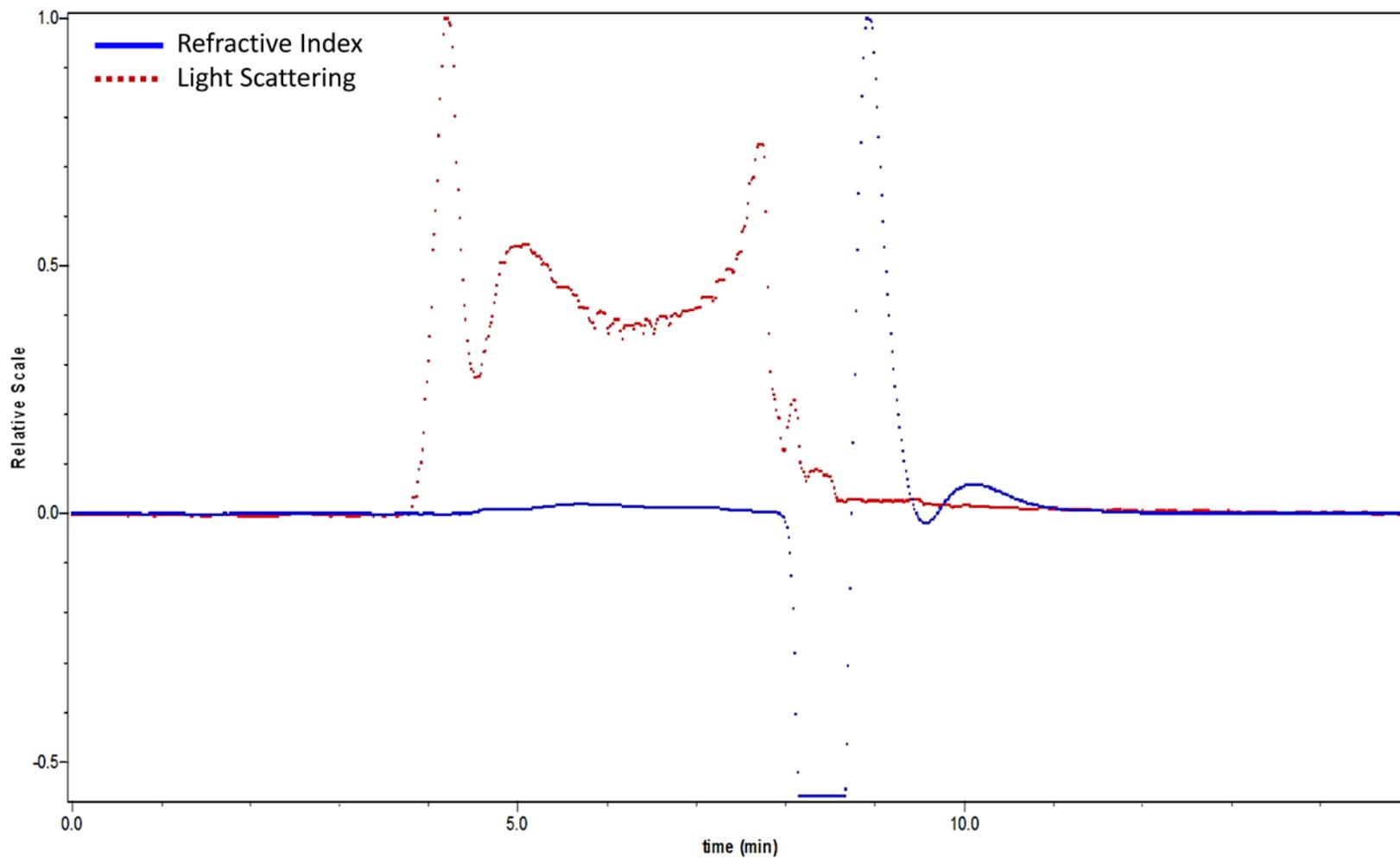


Figure A.12. A Chromatogram for SDS-Unextractable Protein Sample from Tail Cyclone Flour Millstream of ND-Glenn Cultivar from Gulf/Great Lakes Region