Chemical Characterization, Functionality, and Baking Quality of Intermediate Wheatgrass (*Thinopyrum intermedium*)

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Dedication

This thesis is dedicated to my parents, Prijo Handoko Rahardjo and Tjoesoesanti Tunggono, who always give and sacrifice their all for their children. And to my husband, Lie Philip Santoso, for his full support and love throughout my master's journey.

Abstract

Environmental problems have exacerbated the challenges of food production. Soil erosion, run-off from irrigation, and greenhouse gas emissions have significantly impacted the ecosystem through their cumulative effects over decades. As the population increases, these aforementioned problems will grow in scale and scope. Thus, it is important to address these concerns by investigating sustainable solutions and conducting research on new approaches to food supply. Specifically, the development of perennial crops for food use would have environmental benefits such as reduced soil and water erosion. Further, the usage of perennial crops for food applications would provide alternatives to the current and ever diminishing food supply, provide incentives to farmers for planting these crops, and address consumers' growing interest in sustainable food systems.

One of several perennial crops available for potential food use is intermediate wheatgrass (IWG) (*Thinopyrum intermedium*). Little is known about the chemical and functional characteristics of IWG. Therefore, the overall objective of this work was to characterize the chemical and functional properties of IWG grains from multiple breeding lines for food applications, namely bread baking.

Sixteen IWG experimental lines along with one bulk IWG sample and two wheat controls (Arapahoe and commercial hard red wheat) were analyzed for proximate composition following standard methodologies. Dietary fiber, total starch content, and percent damaged starch were determined using Megazyme kits. Amylose/amylopectin

ratio and their molecular weight distribution were determined using size exclusion chromatography. Gluten forming proteins profile and molecular distribution were determined using gel electrophoresis and size exclusion chromatography. Dough rheology was assessed using a farinograph and a texture analyzer equipped with a Kieffer rig, while starch pasting properties were monitored using a rapid visco analyzer. Bread baking tests were performed following the AACCI 10-10.03 method.

Compared to wheat controls, IWG samples had higher protein, dietary fiber, and ash contents, yet were lower in starch content and deficient in high molecular weight glutenins (HMWG), important protein components responsible for dough strength and elasticity. Specifically, wheat controls had more high molecular weight polymeric proteins (HMWPP), while IWG samples had more albumins and globulins. The ratios of amylose to amylopectin among the IWG samples and the wheat controls were similar. However, percent damaged starch was higher in the wheat controls than in IWG samples. On the other hand, the soluble to insoluble dietary fiber ratio was higher in wheat controls than in IWG samples.

Dough rheology data showed that IWG dough was weaker than that of the controls. Farinograph and Kieffer data demonstrated that doughs made from wheat controls were more stable, more resistant to extension, and more extensible than doughs made from IWG samples. In terms of starch pasting properties, wheat controls had higher peak, hold, and final viscosities than the IWG samples, indicating the superiority of wheat controls over IWG samples as viscosity builders. The starch pasting properties data

illustrated the samples' behavior upon heating and cooling treatments, which are important characteristics to consider when evaluating IWG for commercial applications.

Even though IWG had similar specific volumes to one of the wheat controls (hard red wheat), both wheat controls had a higher rising capability due to the wheat's gluten network forming ability. Deficiency in HMWG and high fiber content of IWG samples, contributed to the poor gluten network and consequently inferior baking quality.

Overall, results of this work suggest that IWG has a superior nutritional profile as compared to wheat, but poses challenges for baked products that require dough rising properties. Further studies on IWG, such as investigating the effect of conditioners on enhancing protein functionality, determining the effect of fiber on dough development, effect of blending with wheat, and exploring other food applications would enhance its potential utilization as a food crop. This research and future efforts will support breeders in their current screening and future breeding efforts for the development of IWG lines suitable for food applications.

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1. Literature Review

1.1 Introduction and Objectives

Environmental problems such as soil erosion, run-off from irrigation, and greenhouse gas emissions have significantly impacted the ecosystem through their cumulative effects over decades. Soil erosion has caused 10 million ha of cropland loss per year, a rate of 10 to 40 times higher than the soil formation rate (Pimentel & Burgess, 2013). This is a concerning issue given that 99.7% of global food is produced on land (Pimentel & Burgess, 2013). The regular tillage practices used to plant annual crops have also increased soil erosion rates by 4-10 times (Pimentel et al., 2012). Additionally, the increased demand on water supply and global water shortages have also negatively impacted agriculture and food production practices. In 2004, more than one billion people were lacking access to drinking water (Pimentel, Berger, Filiberto, et al., 2004). During the same year, an average rate of water consumption in the US including that for irrigation, was 5,500 L per person (Pimentel, Berger, Filiberto, et al., 2004). This high demand for water accentuates the importance of water conservation. Agriculture practices, specifically, have used 70% of fresh water resources due to the high amount of water needed for grain production ("Securing the Food Supply.," 2001; Pimentel, Berger, Filiberto, et al., 2004). For example, rice, wheat, and corn need 1,600 L, 900 L, and 650 L of water, respectively, per 1 kg of cereal grain produced (Pimentel, Berger, Filiberto, et al., 2004; Pimentel, Berger, Newton, et al., 2004). On the other hand, the rate of global greenhouse gas emissions has increased very rapidly in the last 40 years, contributing to the growth of the global warming phenomenon (*Climate Change 2014 Synthesis Report*,

2014). The huge amount of energy, pesticides, and fertilizers used to grow annual crops is also concerning, both for the environment and the cost associated with them.

The aforementioned environmental problems will continue to exacerbate the challenges of food production as the population continues to grow. Thus, it is important to address these concerns with sustainable solutions and food supply. Development of perennial crops, with substantial environmental benefits, for food use, will positively help the ecology as a whole. Developing perennial crops for food applications will not only provide environmental benefits, but also will provide alternatives to the current diminishing food supply, provide incentives to farmers for planting these crops, and address consumers' growing interest in sustainable food systems. One of several perennial crops available for potential food use is intermediate wheatgrass (IWG) [*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey]. Little is known about the chemical and functional characteristics of IWG. Thus plant breeders are seeking quality trait information to be used for developing IWG lines for food use.

For these aforementioned reasons, the overall objective of this work was to characterize the chemical and functional properties of IWG grains from multiple breeding lines for food applications, namely bread baking. Data from this work will further assist breeders in their current screening and future breeding efforts for the development of IWG lines suitable for food applications.

1.2 Perennial Crops – Their Benefits and Challenges

Perennial crops have unique characteristics that can bring benefits from agricultural perspectives. Perennial crops are crops that can survive without replanting for several years, which means they can be harvested several times before replanting is needed. Perennial crops also have extensive root systems. Being perennial with extensive root systems, these crops can contribute to several environmental and agricultural benefits when compared to annual crops. Because of their root systems, perennial crops can reduce soil erosion (Pimentel et al., 1987), fix nitrogen (Dinnes, Karlen, Jaynes, Kaspar, & Hatfield, 2002), hold more carbon dioxide in the soil (Freibauer, Rounsevell, Smith, & Verhagen, 2004), and capture more nutrients and water. Perennial crops, which produce high amount of biomass, are also drought and frost resistant, and more resistant to diseases commonly present in annuals, thus requiring less energy and pesticides (Culman, Snapp, Ollenburger, Basso, & DeHaan, 2013; Glover et al., 2010; Robertson, Paul, & Harwood, 2000; Wagoner & Schauer, 1990). Annual crops are 50 times more prone to soil erosion than perennial crops (Gantzer, Anderson, Thompson, & Brown, 1990). Specifically, annual grain crops can lose five and 35 times as much water and nitrate respectively, as perennial crops (Randall et al., 1997; Zhang et al., 2011).

However, despite the benefits they can offer, perennial crops have encountered some challenges from the breeding and management perspectives. Some perennial crops' seed size, for example IWG, is small and the yield is low compared to annual crops like wheat (DeHaan, 2015; Lubofsky, 2016). Thus, increasing its endosperm to bran ratio can take years of development. In addition, perennial crops are prone to falling over due to

their tall and weak stems, a phenomenon known as lodging. Lodging not only makes harvest more challenging, but can also inhibit seed heads from growing and producing seeds (Lubofsky, 2016). Seed shattering is another challenge of perennial crops (Lawrence, 1983). Over time, as the crops grow and fields get denser, lower grain yields are obtained. From the management side, challenges such as determining the proper dosage of nitrogen and spacing issues also need to be determined. Applying enough nitrogen, but not too much to cause extensive growth and lodging, is crucial to maximize grain yields. Row spacing, as mentioned, is also important to ensure that the fields are not over crowded to maintain the grain yields (Lubofsky, 2016). IWG, as one of the perennial crops that are associated with the benefits and challenges described above, will be further examined and discussed throughout this thesis.

1.3 Intermediate Wheatgrass (IWG) as a Perennial Grain Crop

IWG is classified in the kingdom of Plantae (plants), subkingdom of Tracheobionta (vascular plants), superdivision of Spermatophyta (seed plants), division of Magnoliophyta (flowering plants), class of Liliopsida (monocotyledons), subclass of Commelinidae, order of Cyperales, family of Poaceae (grass), genus of *Thinopyrum* (wheatgrass), and species of *Thinopyrum intermedium* (intermediate wheatgrass) ("Classification | USDA PLANTS," n.d.). *Thinopyrum intermedium* or IWG is formerly known as *Agropyron intermedium* (Host) Beauv., *Elytrigia intermedia* (Host) Nevski, *Elymus hispidus* (Opiz) Melderis, and *Agropyron glaucum* (Desf.) Roemer & Schultes (Nowick, 2015).

IWG originated from Europe, western Asia, and southern Africa, and some species have been established globally, including in the US. IWG has been used for hay and pasture seedlings for many years. Currently, it is mainly used as forage (Lawrence, 1983; Culman et al., 2013)

Like other perennial crops, IWG has a deep root system (**Figure 1**). According to Culman et al. (2013), this extensive root system can reduce nitrogen leaching and increase carbon sequestration by 86% and 13% respectively, when compared to annual wheat plants. IWG plant grows 3-4 feet tall with 4-8 mm wide green leaves. Around 2-6 florets per spikelet are usually found in this crop (Lawrence, 1983; Ogle, 2003). IWG is adapted to an area with 12-14 inches of yearly rainfall and an elevation of 3,500-9,000 feet (Ogle, 2003). On average, IWG offers yields of 250-350 lbs/acre under dryland conditions and 450-550 lbs/acre under irrigated conditions. After four harvest years, the production yield typically drops significantly (Lawrence, 1983; Ogle, 2003).

The history of IWG development as a perennial grain crop was pioneered by the Rodale Research Center (RRC) in 1983. The RRC analyzed almost 100 different species of perennial grasses for their potential cultivation as perennial grain crops. Based on several preferable traits including acceptable flavor, ease of threshing and harvest, seed size, shattering and lodging rate, and perenniality, IWG was selected for further development. Thus, breeding efforts continue to be conducted to maximize IWG capabilities as a perennial grain crop.

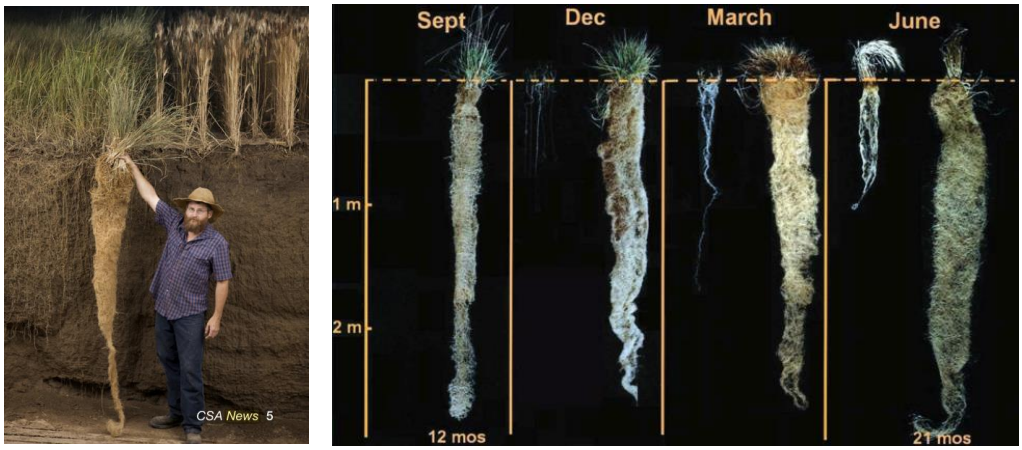


Figure 1. (a) Root systems comparison between perennial intermediate wheatgrass (IWG) (*Thinopyrum intermedium*) and annual winter wheat. *Photo taken by Jim Richardson courtesy of Patagonia Provisions* (Lubofsky, 2016). (b) Root systems comparison over a growing cycle between annual wheat and perennial IWG (Cox et al., 2006). Used with permission.

1.3.1 IWG Breeding Efforts

There are two types of breeding approaches that are used to develop perennial grains, domestication and wide hybridization. Direct domestication involves cycles of selection, which is a method of selecting the better grain producing plant through crossing, evaluation, and selection process of the seed produced as shown in **Figure 2** (DeHaan et al., 2013). Direct domestication practices of IWG may require a great amount of time before achieving preferable traits in their offspring. Favorable flavor, seed size, shatter resistance, and good yield are some of many preferable traits the breeders consider in the selection process (Wagoner & Schauer, 1990).

Wide hybridization, on the other hand, involves crossing two different parents, each of which carry favorable traits (DeHaan et al., 2013). However, the stability (same

number of chromosomes for all of the offspring at every selection cycle) and fertility of the progeny is sometimes a challenge (DeHaan et al., 2013). IWG breeding efforts use direct domestication techniques as will be discussed in **section 1.3.1.1**, while perennial wheat development uses wide hybridization as will be discussed in **section 1.3.1.2**. Based on the breeding efforts via direct domestication and/or wide hybridization, perennial grain crops are estimated to become commercially available in the next 20 years (Glover et al., 2010).

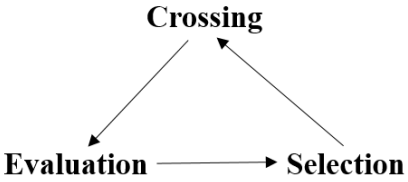


Figure 2. One cycle of selection involving crossing, evaluation of offspring, and plant selection based on preferable traits.

1.3.1.1 IWG Lines Selection

In 1988, the RRC began the development of IWG as a perennial grain crop in Kutztown, PA to improve its fertility and seed size (DeHaan et al., 2013). The RRC conducted two cycles of selection, in which the progenies were then used by the Land Institute in Kansas to continue the study in 2003. After two cycles of selection by the Land Institute, the IWG yield and seed mass improved by 77% and 23%, respectively, when grown in a solid plot (planted in rows that are very close together, about 7 inches or less) (DeHaan et al., 2013). In 2011, the University of Minnesota (UMN) began its involvement in the project, using the third cycle selection from the Land Institute, and

focused on increasing the yield and size of IWG (Zhang et al., 2016). The history of IWG breeding efforts is illustrated in **Figure 3.**

A group of scientists from the University of Minnesota closely studied the characteristics, namely gene transcription of gluten forming proteins, of various IWG lines. Zhang et al. (2014) isolated five high molecular weight glutenin (HMWG) genes from IWG plants. IWG deduced proteins were smaller in size but similar in structure to the HMWG present in wheat. Compared to HMWG in wheat, the authors reported that the HMWG in IWG had a larger range of molecular weight (45-90 kDa). Futher studies on the molecular interactions of the smaller sized glutenins in IWG and gluten forming proteins in wheat are necessary to broaden the understanding of their effect on dough rheology/functionality.

Zhang et al. (2016) studied the relationship between the genotypes and phenotypes of IWG using molecular markers for genetic analysis. Through their study, a formulation that relates the genotypes to the phenotypic outcome of IWG was determined. These results allow breeders the use of genotyping to enhance the efficiency of the selection process and further the development of IWG with desirable phenotypes.

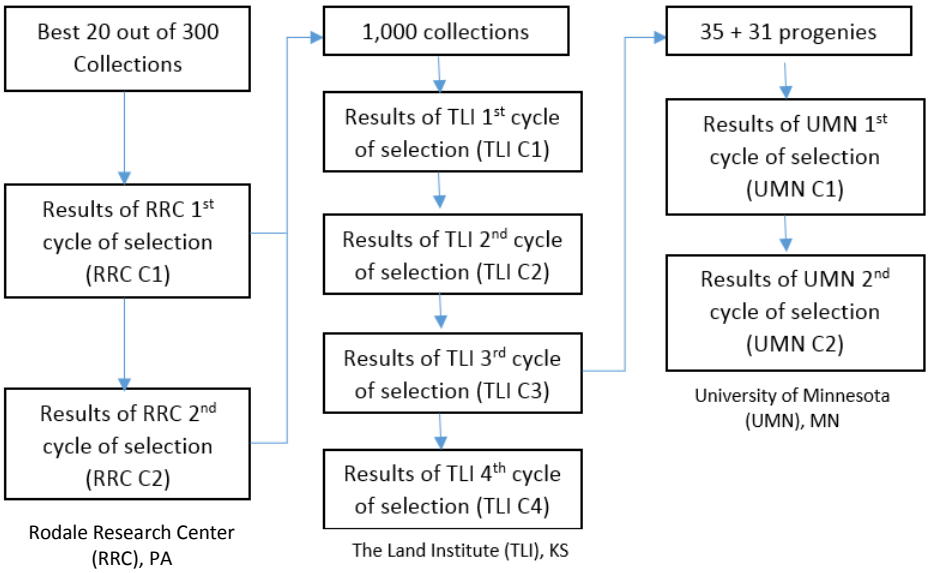


Figure 3. History of IWG breeding efforts to maximize its potential as a perennial grain crop, adapted and simplified from Zhang et al. (2016).

1.3.1.2 Wheat Crossing with IWG

Another effort to develop perennial grain crop was also conducted by crossing wheat and IWG. The progeny is called perennial wheat. Past efforts by the Soviet Union to develop perennial wheat were abandoned in the 1960s due to sterility, inconsistent perenniality, and other undesirable agronomic characteristics (Cox, Glover, Van Tassel, Cox, & DeHaan, 2006). In 2011, The Land Institute again took on the challenge of developing perennial wheat, with the goal of creating good grain yield and perennial offspring. As of now, there are a few stable lines of perennial wheat. However, these progenies still lack the perenniality trait. Thus, breeders still continue to seek a functional strain of perennial wheat hybrid.

Wheat crossing with IWG had also been studied to transfer the protein quality and disease resistance characteristics of IWG to wheat. Li et al. (2013) found that a specific chromosome of IWG's HMWG, 1St#2, has the potential to improve the quality of wheat end-products. The authors showed that the addition of this specific chromosome increased wheat's protein content and dough's strength when compared to control wheat. Additionally, Li & Wang (2009) evaluated IWG's capability to be used as a disease resistant source for the many diseases wheat is susceptible to genes transfer from wheatgrass to wheat was shown to improve wheat resistance to diseases such as leaf rust, stem rust, powdery mildew, barley yellow dwarf virus, wheat streak mosaic virus, and wheat curl mite (Turner, De Haan, Jin, & Anderson, 2013). Resistance to different diseases is typically associated with different IWG's chromosomes. However, a single IWG's chromosome can carry genes for multiple diseases.

Despite the positive traits genes transfer between IWG and wheat can result in negative effects. For example, one of the genes that provides resistance to leaf and stem rust problems is associated with yellow flour pigmentation that is undesirable. Other genes are also limited in temperature range and some others bring negative effects to yield and quality parameters. Thus, the application and effectiveness of wheatgrass genes transfer to wheat are limited and only very few are used in practical breeding (Li & Wang, 2009). However, efforts are continued to improve genes transfer practicality.

1.3.2 IWG for Forage and Biofuel

While IWG has value as a potential perennial grain crop, it has several additional uses as mentioned previously (**Section 1.3**). IWG is used for hay, pasture, and forage in many regions, especially in the Great Plains region running through the US and Canada (Berdahl, Karn, & Data, 1994; Lawrence, 1983). The high amount of IWG's biomass production also indicates its potential use as a biofuel. This use is currently under evaluation (Culman et al., 2013). If all potential uses of IWG come to fruition, IWG may become the first triple-purpose perennial crop used as grain, forage, and biofuel. Of the three, however, the most attractive potential is the development of the crop for food use.

1.4 Wheat as a Staple Grain

After rice, wheat is the second largest crop harvested for human consumption globally (Stevenson, Phillips, O'Sullivan, & Walton, 2012). Wheat and IWG belong to the same family (Poaceae). Thus, a comparison between IWG and wheat is necessary to understand their similarities and differences, as well as the preferable traits for food applications, such as bread baking, that can advance IWG breeding development. In this research, wheat is used as a control grain for comparison to IWG.

Wheat is classified in the kingdom of Plantae (plants), subkingdom of Tracheobionta (vascular plants), superdivision of Spermatophyta (seed plants), division of Magnoliophyta (flowering plants), class of Liliopsida (monocotyledons), subclass of Commelinidae, order of Cyperales, family of Poaceae (grass), and genus of *Triticum* L ("Classification for Kingdom Plantae Down to Genus Triticum L.," n.d.). There are 19

known species of wheat, some of which are frequently used by consumers for food applications, such as durum wheat (*Triticum durum* Desf.) and common wheat (*Triticum aestivum* L.) ("Classification for Kingdom Plantae Down to Genus Triticum L.," n.d.). Common wheat or *Triticum aestivum*, contributes roughly to around 90-95% of world wheat production (Peña, 2002).

Similar to IWG, wheat also belongs to the Poaceae (grass) family. However, unlike IWG, wheat is an annual plant that has been cultivated for over 10,000 years. Based on genetic analysis, it is highly probable that wheat originated in what is now southeast Turkey (Shewry, 2009). Currently, wheat is planted in almost all countries, with more than 855 million metric tons of production in 2014 (Atwell, 2001; *Wheat Crop Production Data*, 2014).

Wheat is categorized based on three categories: grain hardness (hard vs. soft), color (red vs. white), and growing season (spring vs winter). Hard wheat has a firmer endosperm when compared to soft wheat and therefore, requires more milling time (Atwell, 2001). As suggested by the names, the red and white wheat differ in bran color as a result of red pigments present in wheat. These pigments are polyphenol compounds, namely phlobaphene or proanthocyanidin (Atwell, 2001; Himi & Noda, 2005). Spring wheat is planted in the spring and harvested in late summer or fall, while winter wheat is planted in the fall and harvested in the summer due to its requirement of cold weather for the grain to flower (Atwell, 2001). Thus, three letters are generally used to describe any common wheat. For example, "HRW" is used when referring to hard red winter wheat.

Not all types of wheat are suitable for all wheat products. HRW, HWW, HWS, and HRS wheat varieties are usually used for bread or other baked products that require rising, due to their capacity to hold gas. On the other hand, because they lack elasticity, SRW, SWW, and SWS are the types of wheat typically used for cakes, crackers, and cookies (Atwell, 2001). A summary table of wheat types, characteristics, and their general uses can be seen in **Table 1.**

Table 1. Wheat Types, Characteristics, and General Uses (Atwell, 2001). Used with permission.

Class	General Characteristics	General Uses
Hard red winter (HRW)	High protein, strong gluten, high water absorption	Bread and related products
Soft red winter (SRW)	Low protein, weak gluten, low water absorption	Cakes, cookies, pastries, pie crusts, crackers, biscuits
Hard red spring (HRS)	Very high protein, strong gluten, high water absorption	Bread, bagels, pretzels, and related products
Hard white	High protein, strong gluten, high water absorption, bran lacks pigments	Bread and related products
Soft white	Low protein, weak gluten, low water absorption, bran lacks pigments	Noodles, crackers, wafers, and other products in which specks are undersirable
Durum	High protein, strong gluten, high water absorption	Pasta

1.4.1 Wheat Kernel Composition

Typically, a wheat kernel measures 5-9 mm long and weighs 35-50 mg (Šramková, Gregová, & Šturdík, 2009). A wheat kernel has three major components: the

outer layer (bran), the middle layer (endosperm), and the embryo (germ) (**Figure 4)** (Atwell, 2001; Šramková et al., 2009). The bran represents 13-17% of the kernel's weight and is high in fiber and ash (minerals) contents. The germ represents 2-3% of the kernel's weight and contains most of the wheat lipids (Atwell, 2001; Šramková et al., 2009). The endosperm represents 80-85% of the kernel's weight and consists of two different layers: the outer and inner endosperm (Atwell, 2001; Šramková et al., 2009). The outer layer of the endosperm is located between the bran and the inner endosperm. This layer is called the aleurone and contains proteins and enzymes crucial for germination (Atwell, 2001; Šramková et al., 2009). The inner endosperm is often referred to as starchy or storage endosperm due to its role in energy storage in the form of starch. This inner endosperm is rich in starch and protein. It is also important to note that even though the aleurone layer is part of the endosperm, most people and millers consider it part of the bran (Atwell, 2001; Šramková et al., 2009).

Several ingredients can be produced from wheat grain, but the most common are whole-wheat flour and refined flour. While whole-wheat flour is made from the whole-wheat kernels, refined flour is made from kernels that have been processed to remove the bran, the germ, and the aleurone layer, to isolate the inner/starchy endosperm.

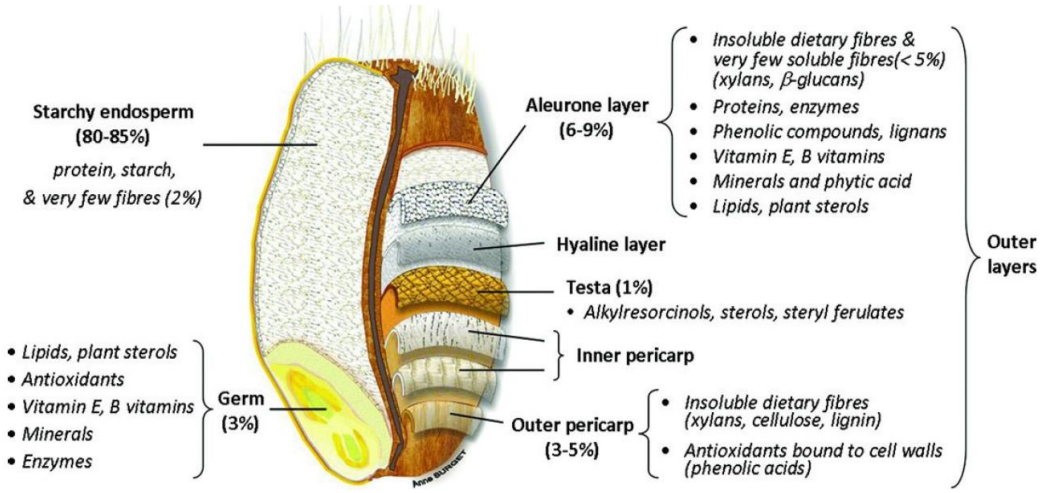


Figure 4. Wheat kernel diagram, adapted from Surget & Barron (2005) and Brouns, Hemery, Price, & Anson (2012). Used with permission.

1.4.2 Wheat Chemical Composition and The Effect on Functionality

Wheat is considered as a good source of protein, minerals, B vitamins and dietary fiber. Generally, wheat grain is composed of 10-18% protein, 2-3% lipid, and 60-75% carbohydrate (Haard, Odunfa, Quintero-Ramírez, Lorence-Quiñones, & Wacher-Radarte, 1999; Šramková et al., 2009). Wheat has around 2% ash (Becker, Wagoner, Hanners, & Saunders, 1991) and around 13% dietary fiber (Dhingra, Michael, & Rajput, 2012). Wheat also contains around 20-35 mg/kg zinc, 29-57 mg/kg iron, and 0.02-0.60 mg/kg selenium (Alfthan & Neve, 1996; Cakmak I, 2004; Šramková et al., 2009). Like other major cereals, wheat is abundant in essential amino acids, excluding lysine, which is the limiting amino acid in wheat (Shewry, 2009). In the following sections, the major

constituents of wheat, protein, starch, and dietary fiber, and their impact on functionality and bread baking quality will be further discussed.

1.4.2.1 Gluten Forming Proteins

Total protein content in wheat is an important factor in determining wheat price and end product usage. Historically, wheat that has a high protein content garners a premium price, commonly referred to as the"protein premium" (Bale & Ryan, 1977). The price of the protein premium varies depending on the protein content, wheat type, and the supply and demand of wheat protein (Bale & Ryan, 1977). For example, according to the Grain Farmers of Ontario (2017), the wheat protein premium price is charged when the protein content is ≥11% for hard red winter wheat and ≥ 12% for hard red spring wheat.

Different wheat types typically have different protein contents. Whole durum wheat protein content ranges from 9-18% (Feillet, 1988; Delcour et al., 2012), while whole bread wheat (hard wheat) protein content ranges from 10-14%, and soft wheat protein content ranges from 8-11% (Huebner, Bietz, Nelsen, Bains, & Finney, 1999; Delcour et al., 2012). This differing protein contents result in each variety of wheat being suitable for different applications (**Table 1)**.

In addition to the importance of the total protein content, the individual protein components also have a distinct impact on wheat functionality for various applications, specifically bread baking. There are four types of wheat proteins that are classified by Osborne (1924) based on their extractability and solubility in various solvents. The four types are albumins, globulins, prolamins, and glutelins. Albumins are soluble in water;

globulins are soluble in salt solutions; prolamins (gliadins) are soluble in 70% ethyl alcohol; and glutelins (glutenins) are soluble in dilute acid or alkali solutions (Šramková et al., 2009).

Albumins and globulins are cytoplasmic or membrane proteins located mainly in the germ and bran and are important for germination. (Belderok, Mesdag, & Donner, 2000; Merlino, Leroy, Chambon, & Branlard, 2009; Žilić, 2013). They are mainly monomeric proteins and represent 20-25% of the total wheat protein (Kasarda, Autran, Lew, Nimmo, & Shewry, 1983; Bietz & Simpson, 1992;(Žilić, Barać, Pešić, Dodig, & Ignjatović-Micić, 2011). Their molecular weights (MW) are mostly below 25,000 Da, but some are between 60,000-70,000 Da (Singh, Donovan, Batey, & MacRitchie, 1990).

Gliadins (prolamin proteins) predominate in wheat. Gliadins together with glutenins (glutelin proteins) represent about 75-85% of the total wheat protein (Kasarda, et al.1983 ; Bietz & Simpson, 1992;(Žilić et al., 2011); (Žilić, 2013). Both are storage proteins located mainly in the endosperm and are used as a source of amino acids and nitrogen during germination. Composition and distribution of gliadins and glutenins directly impact, to a great extent, the rheological properties of wheat (Žilić, 2013).

Gliadins constitute α-/ β-, γ- (MW ranging from 28-35,000 Da) and ω-gliadins (MW ranging from 52-74,000 Da) (Wieser, 2007; Žilić, 2013). Gliadins constitute about 58-77% of the total gluten forming proteins (Wieser, 2007). While all gliadins are monomers, α-/ β-, and γ-gliadins can form intra-chain disulfide bonds. ω-gliadins, however, do not form these bonds because they lack cysteine residues (Žilić, 2013).

Glutenins can be categorized into two groups, low molecular weight glutenins (LMWG) and high molecular weight glutenins (HMWG). LMWG have a MW ranging between 30,000-74,000 Da, while HMWG have a MW ranging between 67,500-120,000 Da (Sivam, Sun-Waterhouse, Quek, & Perera, 2010; Žilić, 2013). LMWG represent about 19-25% of the total gluten forming proteins, while HMWG represent about 7-13% of the total gluten forming proteins (Wieser, 2007). Thus, glutenins constitute about 26-38% of total gluten forming proteins (Wieser, 2007). Glutenins are polymeric proteins constituting of polypeptides linked together through intermolecular disulfide bonds. Individual glutenin polypeptides also contain intra-molecular disulfide bonds (Žilić, 2013).

The kneading of flour with water results in gliadins and glutenins forming the protein complex known as gluten. Gluten is unique to wheat dough, contributing to the rheological properties referred to as viscoelastic properties (Hoseney, 1998; Lee & Lee, 2012). The viscoelastic properties impact the dough's gas holding capacity. Gliadins are responsible for the viscosity and extensibility, while glutenins are responsible for elasticity and dough strength. Gliadin is often referred to as the "plasticizer" for the glutenin network. The balance between these two components is important for establishing the gas holding capability necessary for baked products that require rising such as bread (Žilić, 2013).

Dhaka & Khatkar (2014) studied the gluten composition of 15 different wheat varieties and the effect of the composition on functionality for bread application. The authors confirmed that the ratio of gliadin to glutenin and HMWG to LMWG affect bread

baking quality. Results showed that higher glutenin content caused a longer mixing time, but more importantly, resulted in higher dough stability. Additionally, higher glutenin to gliadin ratio resulted in higher bread specific volume. The study demonstrated that a higher HMWG/LMWG ratio resulted in a longer dough development time and an increase in dough stability and bread specific volume. This study proved the widely held view of the importance of HMWG in producing high quality bread.

Following the recognition of its importance, HMWG's molecular structure and interactions were the subject of several studies focused on understanding gluten functionality. It was presumed that HMWG's secondary structure was related to dough formation and functionality (Mejia, Mauer, & Hamaker, 2007). To advance the understanding of HMWG's molecular structure and interactions, Shewry & Tatham (1997) had cloned and sequenced HMWG subunit genes. Their results showed that HMWG has three domains of non-repetitive N-terminus and C-terminus, with a repetitive central domain. Most of the cysteine residues that can form interchain disulfide bonds were found to be located in the non-repetitive terminus domains, which can further assist in the stabilization of HMWG polymers (Humphris et al., 2000). It was also argued that the formation of disulfide linkages are preceded by crosslinking via H-bonding resulting in β-sheet secondary structure formation (Shewry, Halford, Belton, & Tatham, 2002; Wellner et al., 2005; Mejia et al., 2007). The NMR study by Belton et al. (1995) highlighted the importance of H-bonding in dough formation to stabilize the gluten structure, which was attributed to the high level of glutamine residues (35% of the amino acids present in the gluten forming protein) in the HMWG subunits (Wellner et al., 2005;

Wrigley, Békés, & Bushuk, 2006). This further confirmed the contribution of H-bonding to gluten elasticity (Belton, 1994; Belton et al., 1995).

In general, there are multiple types of chemical bonds that can contribute to gluten network formation. Wrigley et al. (2006) discussed the importance of both the non-covalent bonds, such as hydrogen and hydrophobic interactions, as well as the covalent bonds, such as disulfide cross linkages and tyrosine crosslinking, in the development of optimum dough structure (Sivam et al., 2010; Tilley et al., 2001). Wrigley et al. (2006) stated that even though a H-bond is considered a weak bond, the large numbers of H-bonds associated with the gluten network significantly contribute to the dough structure and stability. Hydrophobic bonds may have a similar role to H-bonds (Wrigley et al., 2006), and may play a crucial role in biopolymers' interactions within the dough, assisting with dough stability (Sivam et al., 2010). In addition, disulfide linkages of the polypeptide chains may stabilize hydrogen and hydrophobic interactions (Goldstein, 1957; Wrigley et al., 2006). HPLC and NMR data observed by Tilley et al. (2001) also showed that tyrosine cross linking develops during mixing and baking. Tyrosine linkages are formed through chemical oxidation of tyrosines, as confirmed when oxidizing agent was used, leading to a linkage between two tyrosine subunits (Scheibel, 2008).

The molecular structures and interactions of proteins are very complex, but crucial to understand. Protein structure and molecular interactions dictate key functionalities. Even though protein has an important nutritional value in food products, specific types of protein have more functional properties than others. Thus, not only does protein quantity matter, but also the type of protein present is important. Further

discussion about the effect of protein interactions on dough functionality and subsequent baking quality will be explored further in following sections.

1.4.2.2 Starch

Starch is one of the major wheat components that can have a major effect on functionality in various applications. Starch properties and interactions with other constituents may result in unique functionality in different food systems. Starch constitutes around 60-75% of the total dry weight of wheat (Šramková et al., 2009). Amylose and amylopectin are the components of starch. Amylose is a mostly linear α(1-4) chain of glucose units, while amylopectin contains both linear α(1-4) and branched α(1-6) glucose chains. The MW of both amylose and amylopectin can vary greatly, but the MW of amylose is usually around 25,000 Da while the MW of amylopectin is around 10^8 Da (Šramková et al., 2009). Regular wheat starch constitutes about 20-30% amylose and 70-80% amylopectin (Konik-Rose et al., 2007).

Starch pasting properties influence starch functionality in food products. Starch pasting properties are a function of heating and cooling treatments, which affect the starch structural organization. During heating and under a sufficient level of moisture, starch granules absorb water and swell, and start to lose their crystallinity and original structure (Copeland, Blazek, Salman, & Tang, 2009; Kantar et al., 2016). Amylose has a crucial role in the initial step of starch swelling as it can form a complex with lipids that prevent leaching and reduce swelling capacity. Thus, starch swelling capacity correlates directly with the amylose/amylopectin content (Singh, Singh, & Kaur, 2003; Alcázar-

Alay & Meireles, 2015). Additional factors also contribute to swelling capacity, such as the degree of polymerization, length, degree of branching, and molecular weight (Hoover, 2001; Ratnayake, Hoover, & Warkentin, 2002; Alcázar-Alay & Meireles, 2015).

Structural organization further degrades with continued water absorption and heat application. As the starch granule swells, the H-bonds responsible for starch granule structure begin to break, leading to partial starch solubilization, loss of crystallinity and molecular order of the granules, and an increase in viscosity (Hoover, 2001; Alcázar-Alay & Meireles, 2015). This irreversible phenomenon is called gelatinization (Copeland et al., 2009; Kantar et al., 2016). Gelatinization transforms starch from a semi-crytalline to an amorphous form (Tester & Debon, 2000; Alcázar-Alay & Meireles, 2015). The progress of gelatinization depends on water availability and other applied parameters such as the temperature, time, and stirring (Alcázar-Alay & Meireles, 2015; Schirmer, Jekle, & Becker, 2015). The gelatinization/pasting temperature for refined wheat flour is around $61.0 \pm 0.2°C$ (Marti, Qiu, Schoenfuss, & Seetharaman, 2015). For the same amount of sample, the gelatinization/pasting temperature for whole wheat flour is lower simply because it contains less starch. Sun, Wu, Bu, & Xiong (2015) further justified this expectation when they found that higher fiber substitution resulted in lower starch pasting temperature.

As the heating treatment is continued, the viscosity will decrease due to the rupture of granules (Copeland et al., 2009). Upon cooling, viscosity will increase again as the starch molecules reform into different structures and retrogradate (Copeland et al.,

2009). Retrogradation involves intermolecular interactions between amylose and amylopectin molecules (Tang & Copeland, 2007). During retrogradation, amylose molecules reassociate forming double helices, while amylopectin molecules recrystallize (Singh et al., 2003; Alcázar-Alay & Meireles, 2015). Retrogradation is a process that takes an extended period of time to complete. While amylose can complete its retrogradation process in hours, amylopectin may take days. Higher amylose content will result in products with firmer gels upon retrogradation (Thomas & Atwell, 1999; Tang & Copeland, 2007).

An example of a common pasting profile is shown in **Figure 5**. The peak viscosity of the pasting profiles can be used as an indication of starch water holding capacity (Gupta, Bawa, & Semwal, 2009). The breakdown value is related to the solubility of the starch, with a higher breakdown value indicating a higher starch solubility (Hoseney, 1998). The final viscosity depends on the reassociation of starch molecules, predominantly the amylose, resulting in gel formation and therefore, increasing the viscosity (Saunders, Izydorczyk, & Levin, 2011).

Retrogradation affects the application of starch in different food products. For example, high amylose content in starch is desirable for products requiring fast retrogradation such as pasta (Dexter & Matsuo, 1979; Vignaux et al., 2005). On the other hand, amylopectin retrogradation is an important factor in determining baked products' staling period (Copeland et al., 2009).

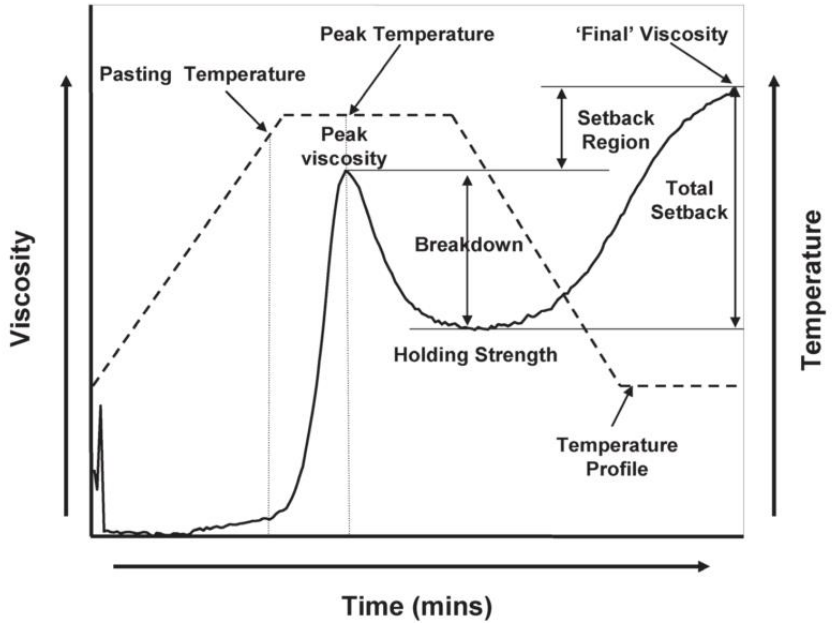


Figure 5. A common starch pasting profile obtained using a rapid visco analyzer (RVA) (Saunders et al., 2011)**.** Used with permission.

Staling in bread is defined by an increase in the bread's firmness, moisture migration from the center to the crust, and loss of flavor (BeMiller, 2007). Ghiasi, Hoseney, Zeleznak, & Rogers (1984) compared two different breads with different amylose/amylopectin ratios. They observed that one day after baking, the bread with the higher amylopectin content was softer. However, at days three and five, the firmness of the two different breads was the same. This finding confirmed that the amylose/amylopectin ratio is an important factor contributing to bread staling.

Another factor that affects starch functionality is the amount of damaged starch present in wheat flour. The amount of damaged starch is found to be proportionally related to kernel hardness - the harder the kernel the higher the amount of starch damage

after milling (Mok & Dick, 1991; Bass, 1998). As grain milling time increases, starch is more likely to get damaged. Research has shown that high damaged starch content may result in lower bread specific volume (Barrera, Pérez, Ribotta, & León, 2007). There are two explanations for this phenomenon in the literature. First, as the amount of damaged starch increases, the water absorption capacity increases. Therefore, the competition of starch with the fiber and protein for water will prevent the optimization of the gluten network formation during mixing. Another possible reason is starch degradation during the fermentation process. Damaged starch degrades more readily than intact starch leading to a decrease in the consistency of the dough, and thus a reduction in gas retention capacity (Barrera et al., 2007).

The role of starch pasting properties in bread baking is widely recognized. Many studies have demonstrated the effects of starch composition on bread quality parameters (Hung, Maeda, & Morita, 2007; Morita et al., 2002). For example, starch gelatinization within the dough's gluten matrix is essential for porous and elastic crumb development due to the semi-rigid form of the gluten network that is maintained by the uptake of water by the starch during gelatinization (Rotsch, 1954; Sandstedt, 1961; Medcalf, 1968; Dennett & Sterling, 1979). Thus, limited gelatinization of amylose is considered insufficient for bread baking purposes (Montgomery & Senti, 1958; Kim & Deruiter, 1968; Dennett & Sterling, 1979). Moreover, amylose gelatinization and amylopectin crystallization are two important factors that determine starch retrogradation (Morita et al., 2002; Hung et al., 2007). The linear structure of amylose reassociates and form a gel

upon cooling, while the branched structure of amylopectin crystallizes upon cooling (Wang, Li, Copeland, Niu, & Wang, 2015).

Hung et al. (2007) showed the effect of increasing amylopectin content on both functionality and bread baking quality. Whole waxy flour (WWF) is mainly composed of amylopectin and is significantly higher in protein and fiber content as compared to common wheat flour (CWF). Hung et al. (2007) showed that the pasting temperature and viscosities of WWF were lower than that of CWF, and that during mixing, the WWF dough had higher water absorption and lower stability than that of CWF. Because protein, fiber, and starch all compete for water, the higher protein and fiber content of WWF as compared to CWF increased the water absorption and reduced the strength of the gluten network (Hung et al., 2007). The bread made with WWF had a lower specific volume, but also a lower bread firmness during storage, which delayed bread staling (Hung et al., 2007). Higher retention of water in bread made with higher amylopectin content had also been observed in other studies (Morita et al., 2002; Hayakawa et al., 2004; Hung et al., 2007).

Overall, the chemical properties of starch are crucial in grain based products. Starch pasting properties demonstrate the impact of the heating and cooling treatments that a particular grain product will undergo, and highlights the effect of amylose to amylopectin ratio. These properties, specifically, have an important impact on bread baking, which will be further discussed in **Section 1.5**.

1.4.2.3 Dietary Fiber

Many studies have demonstrated the health benefits of dietary fiber (DF) consumption in relation to obesity, constipation, hypertension, and the normalization of blood lipids, as well as its reduction of the risk of some chronic diseases such as heart disease, cancers, and type 2 diabetes (Fung et al., 2002; Koh-Banerjee et al., 2004; Sahyoun, Jacques, Zhang, Juan, & Mckeown, 2006; Seal, 2006; BeMiller, 2007; Lam et al., 2007; Mellen, Walsh, & Herrington, 2008). Dietary fiber is defined as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, [and] associated plant substances" (*The definition of dietary fiber*, 2001). Resistant starch is also considered a DF as it is indigestible in the small intestine but subject to fermentation in the large intestine (Slavin, 2013).

Whole wheat contains around 13% DF, located mostly in the bran (~45% DF) and some in the germ (~18% DF) (Fardet, 2010). Due to being mostly associated with the bran and the germ components, the fiber content in whole wheat is higher compared to the fiber content of refined flour (bran and germ mostly removed). This has raised consumer awareness around the consumption of whole grains (Šramková et al., 2009). Wheat DF content and composition not only impacts health, but also the functionality of wheat flour.

DF is categorized as soluble fiber (SDF) and insoluble fiber (IDF) based on water solubility (Šramková et al., 2009). Whole wheat contains around 9-12% IDF and 1-3%

SDF (Fardet, 2010). Whole grains are typically good sources of IDF (Šramková et al., 2009). Whole wheat DF is made up of arabinoxylans (AX), cellulose, β-glucans, arabinogalactan peptide, resistant oligosaccharides, and lignin (Loosveld, Grobet, & Delcour, 1997; Zorb, Betsche, Langenkamper, Zapp, & Seifert, 2007; Gebruers et al., 2008). AX is one of the major components of wheat endosperm cell walls (Šramková et al., 2009). Even though AX can be categorized as either SDF or IDF depending on the MW and substitution pattern, it acts more like SDF in the GI tract, rapidly subjected to bacterial fermentation in the colon (Lattimer & Haub, 2010).

AX's structure varies among different grains, but in general, AX has a xylan backbone and branches with arabinose residues (Nandini & Salimath, 2001). Based on solubility, AX can be divided to two different categories, water extractable (WEAX) and water unextratable (WUAX). In wheat, WEAX constitutes around 25-30% of the total AX (Izydorczyk & Biliaderi, 1995; Koehler & Wieser, 2013). Typically, around 25% of water added to wheat dough binds to WEAX (Atwell, 1998; Koehler & Wieser, 2013). Through oxidation, ester linkages can form between ferulic acids and arabinose (Vinkx, Van Nieuwenhove, & Delcour, 1991; Figueroa-Espinoza & Rouau, 1998; Adams, Kroon, Williamson, & Morris, 2003; Koehler & Wieser, 2013). On the other hand, WUAX is not soluble in water due to its covalent cross linking with the cell wall structure (Courtin, Roelants, & Delcour, 1999; Courtin & Gelders, 2001; Koehler & Wieser, 2013).

AX can absorb water up to ~20 times its weight (Gan, Ellis, & Schofield, 1995; Koehler & Wieser, 2013). Conflicting results had been reported on the AX high water absorption capacity and its effects on bread baking (Izydorczyk & Biliaderi, 1995).

Because of this unique property of AX, AX can create a highly viscous solution, which stabilizes gas bubbles and increases the gas holding capacity of the dough (Gan, Ellis, & Schofield, 1995; Koehler & Wieser, 2013). However, despite its high water holding capacity, a high concentration of WUAX could negatively affect bread volume due to the build up of dough viscosity, which disturbs gluten network formation (Izydorczyk & Biliaderi, 1995). The optimum amount of AX for the bread baking process varies according to the flour type and AX's molecular weight (Izydorczyk & Biliaderi, 1995). Hydrolyzing WUAX using endoxylanases makes it soluble, resulting in functionality enhancement similar to that of WEAX (Courtin, Roelants, & Delcour, 1999; Courtin & Gelders, 2001; Koehler & Wieser, 2013).

The understanding of the molecular interactions of the fiber components is, therefore, important for the understanding of their effects on dough functionality and bread baking quality. Though dietary fiber may bring many health benefits, its presence in formulations is not always beneficial to the production process. Fiber can both positively and negatively impact dough functionality and baking quality. Thus, depending on the final application, the level of fiber should be adjusted accordingly.

1.5 Bread Baking

The importance of each ingredient and the baking steps will be described in this section to further understand the chemistry of baking as a whole. In the bread baking process, the basic ingredients needed are flour, yeast, sugar, salt, shortening, and water.

Ingredients are usually combined and mixed together to make a dough. The dough is then divided, proofed (fermented) and kneaded several times, and finally shaped and baked.

1.5.1 Bread Ingredients

Each ingredient (flour, yeast, sugar, salt, shortening/oil, and water) in bread contributes to the overall quality. However, DiMuzio (2009) argued that flour is the most important ingredient in bread baking. Different types of flour are used for different baked products as shown in **Table 1**. Wheat flour with high gluten forming proteins is typically used to make breads that require rising.

In addition to flour, yeast is used to ferment both endogenose (produced upon hydrolysis of starch by native α-amylose) and added sugars present in the formulation. Yeast produces the enzyme zymase, which catalizes the sugar's fermentation into carbon dioxide and alcohol (DiMuzio, 2009). This fermentation process is essential for bread leavening (DiMuzio, 2009). Sugar is usually added for flavor and serves as a source for yeast fermentation. However, due to its hygroscopic nature (binding and absorbing water), at levels above 12%, sugar can actually decrease the rate of fermentation (DiMuzio, 2009). Thus, sugar in bread is usually added at ~6% of flour weight (*AACCI Approved Methods*, 2010).

Salt also enhances flavor while strengthening the gluten network by shielding the charges of the gluten proteins. The presence of salt reduces electrostatic repulsions and promotes hydrophobic interactions (Butow, Gras, Haraszi, & Bekes, 2002; DiMuzio, 2009). A stronger gluten network will increase the dough's capability to hold the gas

produced by fermentation, creating bread with a desirable loaf volume. On the other hand, the hygroscopic characteristic of salt reduces the amount of available water, decreasing the rate of fermentation. Thus, the level of salt added is typically ~2% of flour weight (DiMuzio, 2009).

Shortening/oil serves as a tenderizing agent for bread dough. Further, the addition of lipid crystals distributed throughout the gluten network leads to the formation of expandable gluten, which aids in air bubble stabilization (Ghotra, Dyal, & Narine, 2002; Watanabe, Larsson, & Eliasson, 2002; Watanabe, Yokomizo, & Eliasson, 2003; Pareyt, Finnie, Putseys, & Delcour, 2011). On the other hand, shortening/oil shortens gluten strands during mixing, a phenomenon called the shortening effect (DiMuzio, 2009). The shortening effect weakens gluten network development. Thus, the amount of shortening added is typically ~3% of flour weight (*AACCI Approved Methods*, 2010).

Finally, water plays a significant role in bread baking. Water aids in the fermentation process and supports the formation of the gluten network. Additionally, water is needed for the gelatinization of starch (DiMuzio, 2009).

1.5.2 Bread Baking Process

The steps involved in baking good quality bread are mixing, fermentation/proofing, kneading, shaping, and baking. The first step in the bread baking process is mixing the liquid and solid ingredients to ensure homogenous distribution of ingredients and form a solid mass. In this step, starch, fiber, and proteins hydrate and begin to interact with each other in an unorganized way. Through the mixing process and

under sufficient hydration, both glutenin and gliadin also begin to interact and form gluten tangled chains. With continued mixing and turning over, the dough will start to get smoother as the gluten network forms (DiMuzio, 2009). Bakers usually check the optimum dough development using the gluten window test (**Figure 6**). In the gluten window test, the dough is slowly stretched. If the dough does not break, then optimum dough condition has been reached. If the dough breaks, then more kneading is needed to further develop the gluten network (DiMuzio, 2009; Hitz, 2011). As a technical alternative to the gluten window test, the farinograph can be used to determine the optimum dough water absorption and mixing time (*Wheat and Flour Testing Methods*, 2004). For wheat, a measurement of 500 BU is the optimum dough consistency (El-Dash, 1978). It is important not to overmix the dough because overmixing can break the gluten network, resulting in un-balanced dough extensibility and elasticity (DiMuzio, 2009).

Figure 6. In a favorable gluten window test, the dough is stretched without breaking (Hitz, 2011). Used with permission.

Fermentation, which follows mixing, can be defined as the breakdown of organic matter (carbon containing substances) by yeast, bacteria, and/or molds (DiMuzio, 2009).

In bread baking, yeast is the fermention agent. The fermentation step allows the yeast to convert the sugar to carbon dioxide and alcohol, producing gas that supports the bread's rising (DiMuzio, 2009). The period during which the fermentation process occurs is called proofing. Fermentation can be categorized into three different periods: bulk fermentation, intermediate proofing, and final proofing. Bulk fermentation refers to the time period just after the dough is mixed and before the dough is divided. Intermediate proofing refers to the time period between dividing the dough and shaping the loaves. Final proofing refers to the time period after the dough is shaped, placed into the baking pan, and allowed to rest before baking. The time periods of each fermentation step vary depending on the bread types (DiMuzio, 2009).

Kneading the dough results in protein denaturations, unfolding, and structural rearrangement that facilitate gluten network formation through several molecular interactions discussed in the previous sections. During this step, gluten proteins are also aligned through the stretching of the bonds forming parallel β-sheets through H-bonding in addition to the formation of hydrophobic interactions, disulfide linkages, and tyrosine linkages, thus increasing air trapping efficiency. Kneading also helps with the gluten hydration process, trapping water in between gluten proteins. The trapped water will convert to steam during baking, and thus contribute further to the rising of the bread (Claire, 2014; Corriher, 1997).

During baking, bread undergoes a browning reaction caused by caramelization and maillard reactions. Caramelization and maillard reactions are considered non-enzymatic reactions and require the presence of heat. Caramelization happens when

direct heating is applied to sugars, while maillard reactions require the presence of heat, protein, reducing sugars, and water (Purlis & Salvadori, 2007).

Additionally, during the baking process as the heat penetrates the dough and increases its temperature, the bread undergoes a phenomenon called “oven spring”. Dough fermentation is accelerated during the oven spring, resulting in the production of a large amount of carbon dioxide. The bread loaf then rises rapidly. However, when the bread loaf is placed into the oven, the intense heat will cause a formation of skin on the bread’s surface, limiting loaf expansion. Thus, the use of steam in the oven before baking is crucial to slow down the formation of the skin. Too much steam, however, can also limit the caramelization rate and therefore, it is only used right before baking but not during the baking process. Starch gelatinization and protein coagulation also occur during baking. Evaporation of water helps with the final rise of the dough. Water evaporation continues until the bread fully cools. Thus, complete steam loss and sufficient cooling are essential steps prior to cutting or packing (DiMuzio, 2009).

While the process of bread baking can be straightforward, several factors in addition to those mentioned are involved in creating a good quality bread, such as the use of dough conditioners, dough mixing time, and baking temperature and time. In this thesis, breads made with wheat controls and IWG samples were made and compared. The next section will explore the chemical composition and functionality of IWG to understand their effect on bread baking quality.

1.6 IWG Chemical Composition and Functionality

While wheat chemical composition and functionality have been well studied, research on IWG has been limited. Becker et al. (1991) examined the compositional, nutritional, and functional properties of IWG. The seed samples were obtained from crops grown in Penssylvania in 1985 through 1988 and in Colorado in 1987. In this study, the group found that IWG was higher in protein (20.8%), fat (3.21%), and ash (2.64%) than wheat. Similar to wheat, IWG also had lysine as the limiting amino acid, but was higher in all of the essential amino acids compared to wheat. The IWG starch gelatinization/pasting temperature was found to be higher than that of wheat with midpoints of 65.5°C and 61°C, respectively.

Preliminary work by our research group on IWG compositional, functional, and nutritional properties used IWG seeds planted in 2002, harvested in 2007, and de-hulled in 2008. Similar to the results of Becker et al. (1991), it was found that IWG was higher in protein (17%) and in most of the essential amino acids, except for histidine. However, unlike the results shown by Becker et al., some of the IWG lines tested by our group were lower in lysine content when compared to wheat. IWG was also found to have lower starch content (~44%) than wheat (60-70%) and higher DF content (~16% in IWG vs. ~13% in wheat) (Shewry, 2009; Dhingra et al., 2012). Results also indicated that IWG was deficient in HMWG. Additionally, IWG had noticeable differences in flavor development in bread, compared to wheat.

Based on the preliminary data collected, breeders are continuing efforts to develop IWG suitable for food applications. Continued team effort between breeders and

food scientists is crucial for the development of IWG suitable for food applications. The present research focused on characterizing 16 different IWG experimental lines grown in St. Paul, MN, USA. The samples were derived from the first cycle of selection performed by the IWG breeding group at University of Minnesota. Through this research, a broader understanding of IWG grown in Minnesota was attained. The research sought the identification of superior IWG lines via correlation between IWG chemical composition, functionality, and bread baking.

1.7 IWG Potential for Food Use

Currently, IWG is sold under the name of Kernza®, a trademark by The Land Institute (TLI) in Salina, KS. IWG was also sold under the name Wild Triga by the Rodale Institute in Kutztown, PA (Marti, Bock, Pagani, Ismail, & Seetharaman, 2016). Kernza® is used in several restaurants such as The Perennial in San Francisco, CA to make breads; Birchwood Café in Minneapolis, MN to make tortillas; and Patagonia Provisions in Sausalito, CA to make beer. All of these places provide food service and are committed to using sustainable ingredients for their products. Kernza® is mostly mixed with wheat (20-50%) to make breads or can be 100% used for baked products that do not require rising property such as muffins and pancakes (DeHaan & Christians, 2016).

Despite its current use in food service industries, IWG also faces many challenges and limitations. IWG usage in food production is still very low when compared to wheat. Therefore, our initiative seeks to increase the use of IWG in product formulations and

diversify the range of products in which IWG can be used. This research is important for the development of IWG for food use through breeding efforts. Expansion of food applications is one of the altimate goals both breeders and food scientists share in order to establish a good market for IWG as a sustainable food option.

2. Materials and Methods

2.1 Materials

Sixteen experimental lines of intermediate wheatgrass (IWG) from different female parents grown in St. Paul, MN, USA along with a pure line of hard red winter wheat control (Arapahoe) grown in Lamberton, MN, USA, were harvested in 2014 and provided by Professor James Anderson from the Agronomy and Plant Genomics Department at the University of Minnesota. Each of the sixteen IWG experimental lines, which originated from UMN-C1 (**Figure 3),** came from individual plant. Another hard red winter wheat control was obtained from a commercial source and will be referred to as hard red wheat (HRW). A bulk IWG sample, grains of which are a combination from several plants (from several lines/populations), was provided by the Land Institute (Salina, KS, USA). Glycine standard (99%, 502-211) was purchased from Leco, Corp. (St. Joseph, MI, USA). TRIS - HCl 4-15% polyacrylamide gel (345-0028), laemmli sample buffer (161-0737), broad range molecular weight standard (345-0024), and concentrated tris-tricine-sodium dodecyl sulfate running buffer (161-0744) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Coomassie brilliant blue R250 (786-498) was purchased from G-Biosciences (St. Louis, MO, USA). HPLC-grade water (WX0004-1) was purchased from EMD milipore (Billerica, MA, USA). Enzymes and assay kits for total starch (K-TSTA-50A/K-TSTA-100A 08/16) and total dietary fiber (K-TDFR-100A/K-TDFR-200A 12/15) were purchased from Megazyme (Megazyme International, Ireland). Diatomaceous earth (DE) (ANKOM XTC, Celite 545 AW, C8656, Sigma Chemical Co. or equivalent), TRIS-TROMETHAMINE 2-Amiono-2-

Hydroxymethyl (>99/9%, 77-86-1), MES Anhydrous Buffer 2-(N-Morpholino) ethanesulfonic acid (100%, 4432-31-9), dietary fiber IDF bag (DF-I), and dietary fiber SDF bag (DF-S) were purchased from ANKOM Technology (Macedon, NY, USA). All of the other reagent grade chemicals were purchased from Fisher Scientific (Waltham, MA, USA), Sigma Aldrich (St. Louis, MO, USA), or VWR International (Radnor, PA, USA). Bread-making ingredients were purchased locally.

2.2 Milling of the Grains

All grain samples were milled using a ball mill (Fritsch Pulverisette, Idar-Oberstein, Germany). Fifteen grams of samples were ground at a time, at 600 rpm for 30 min with breaks every 10 min to prevent over-heating. The resulting flour samples were stored in closed containers at -20°C until further analyses were conducted.

2.3 Experimental Plan

Chemical characteristics, functional properties, and baking quality of all 19 flour samples were analyzed in either duplicate or triplicate. Chemical characterization included the analysis of proximate composition, dietary fiber, starch content, amylose/amylopectin ratio, starch damage, amylose/amylopectin molecular weight, gluten forming proteins profile, as well as molecular weight distribution. Functional properties analyzed included determination of dough rheology following farinograph analysis, dough extensibility using Kieffer, and starch pasting properties using rapid

visco analyzer (RVA). Finally, bread baking tests were performed following the AACCI method 10-10.03 (*AACCI Approved Methods*, 2010). Bread dimensions (height, width, and length) were measured using a caliper (Neiko 01407A, Neiko, Taiwan, China). Bread volume was determined by the rapeseed displacement method (AACCI method 10-05.01, *AACCI Approved Methods*, 2010). Bread crumb moisture was determined using Ohaus Moisture Analyzer (MB45, Parsipanny, NJ, USA). Bread firmness was determined following AACCI method 74-09.01 (*AACCI Approved Methods*, 2010), and FIJI software was used to obtain cell counts and sizes.

2.4 Chemical Characterization

2.4.1 Proximate Analysis

Proximate analysis was performed following standard methods of analyses. Protein content of the samples was determined following the Dumas nitrogen combustion method (AOAC 990.03, *Official Methods of Analysis of AOAC International*, 2016) using a Nitrogen Analyzer (LECO® TruSpecNTM, St. Joseph, MI, USA). A nitrogen conversion factor of 5.70 was used. Fat content was determined following the Mojonnier method (AOAC 922.06, *Official Methods of Analysis of AOAC International*, 2016). Moisture content was determined following the vacuum oven method (AACCI 44-40.01, *AACCI Approved Methods*, 2010). Ash content was measured following the dry ashing method (AOAC 923.03, *Official Methods of Analysis of AOAC International*, 2016). Finally, total carbohydrate content was determined by difference.

2.4.2 Total Dietary Fiber Quantification

To determine the dietary fiber content of the samples, an ANKOM automated Dietary Fiber Analyzer (ANKOM Technology, New York, USA) and Megazyme kit were used to perform the AOAC enzymatic assay (AOAC 991.43, *Official Methods of Analysis of AOAC International*, 2016). Duplicate samples were digested sequentially with heat stable α-amylase (95-100°C, 30 min), protease (60°C, 30 min), and amyloglucosidase (60°C, 30 min). For insoluble dietary fiber (IDF), the digested sample was filtered; and the residue was washed with warm water, 95% ethanol, and acetone; dried; and weighed. For soluble dietary fiber (SDF), the combined filtrate and water wash was precipitated with 95% ethanol; filtered; and the residue was washed with 78% ethanol, 95% ethanol, and with acetone respectively; dried; and weighed. Dietary fiber results were corrected for protein and ash. Filtrations were performed using ANKOM filter bags specifically designed to trap fiber particles. ANKOM SDF bag is designed to trap the SDF particles, while ANKOM IDF bag is designed to trap the IDF particles. Diatomaceous earth (DE) as a filter aid was used only for the SDF bag.

2.4.3 Total Starch Quantification

Total starch content was determined following the AOAC enzymatic assay using a Megazyme kit (AOAC 996.11, *Official Methods of Analysis of AOAC International*, 2016). The method used was the one specified for samples containing D-glucose and/or maltodextrin. Post enzymatic hydrolysis glucose concentration was determined

spectrophotometrically using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Glucose concentration was then used to back calculate total starch content.

2.4.4 Amylose/Amylopectin Ratio and Molecular Weight

Potassium hydroxide (KOH, 1M), 6 M urea, and 1 M hydrochloric acid (HCl) were filtered through 0.2 μm nylon syringe filter (285145-487, VWR, Radnor, PA, USA). While gently vortexing, 4.5 mL of 1M KOH and 0.5 mL of 6 M urea were added to 30-40 mg flour sample in a 15 mL tube. The sample then was flushed with nitrogen for 5 min and heated at 100°C in a heating block for 90 min. The tube was vortexed for several times while heating. Room temperature aliquots (1.0 mL) were transferred to 5 mL test tubes and the pH was adjusted to 7.0 using 1 M HCl. The amount of 1 M HCl added was recorded to calculate the sample concentration in the final volume. The neutralized samples were then filtered through 0.45 μm syringe filter into HPLC vials. To determine the amylose/amylopectin ratio and molecular weight in the prepared samples, size exclusion high performance liquid chromatography (SE-HPLC) linked to refractive index detector (Agilent 1200 HPLC, Agilent Technologies, Inc. Santa Clara, CA, USA) (Simsek, Whitney, & Ohm, 2013) and coupled with multi angle light scattering (MALS) detector (Dawn Helios-II, Wyatt Technologies, Santa Barbara, CA, USA) (Whitney, 2013; You & Lim, 2000) was performed. A waters ultra-hydrogel guard column (WAT011565), ultra-hydrogel 1000 column (WAT011535), in line with linear ultra-hydrogel column (WAT011545) (Waters Co., Milford, MA, USA) were used. The temperature of the column and RI detector was set to 50°C and the solvent, HPLC-grade

water, was pumped at 0.4 mL/min flow rate. The volume of injection was 60 µL and the total run time was 45 min (Grant, Ostenson, & Rayas-Duarte, 2002; Simsek et al., 2013). The area under the curve (IR detector) was used to measure amylose and amylopectin contents using Chem Station version B.04.03 (Agilent Technologies, Santa Clara, CA, US), which were then used to obtain ratio value. An example chromatogram can be found in **Appendix A** (**Figure 17**). The dn/dc value (change in refractive index with concentration) of 0.146 (for MALS detector) (Fiedorowicz, Tomasik, Cracow, You, & Lim, 1999) and the Debye model (Radosta, Haberer, & Vorwerg, 2001) with a fit degree of 3 were used for calculation of the molecular weight (MW) of the amylopectin and amylose in the samples using Astra version 6 (Wyatt Technology, Santa Barbara, CA, USA).

2.4.5 Starch Damage Quantification

Starch damage content was determined following the AACC enzymatic assay using a Megazyme kit (AACCI 76-31.01, *AACCI Approved Methods*, 2010). Similar to total starch assay, glucose concentration was determined spectrophomotrically. However, procedural steps varied to allow only the determination of damaged vs. total starch.

2.4.6 Gluten Forming Proteins Profiling

To determine the profile of gluten forming proteins, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed, following the

procedure developed by Tatham, Gilbert, Fido, & Shewry (2000). Two grams of each sample, weighed in 50 mL centrifuge tube was defatted using 20 mL of water saturated butanol solution. The mixture was placed on a shaker for 1 hour at room temperature, then centrifuged (Beckman J2-MC, Brea, CA, USA) at 9,000 g for 10 min at 20°C. The supernatant was discarded and the pellet was washed with water saturated butanol for two more times. To remove the salt soluble proteins (albumins), 20 mL of 0.5 M sodium chloride (NaCl) was used to disperse the pellet. The dispersion was placed on the shaker for 1 hour at room temperature, followed by centrigugation at 9,000 g for 10 min at 20°C. The supernatant was discarded and the pellet was washed with salt solution two more times. To remove the residual salt, 20 mL of double distilled water (DDW) was added and the sample was placed on the shaker for 30 min at room temperature, followed by centrifugation at 9,000 g for 10 min at 20°C. The supernatant was discarded and DDW wash of the pellet was repeated two more times.

Glutenins and gliadins were then extracted from the pellet using 20 mL of aqueous1-propanol (50% v/v), beta-mercaptoethanol (2% v/v), and acetic acid (1% v/v) while each sample were placed on a shaker for 1 hour at room temperature, followed by centrifugation at 9,000 g for 10 min at 20°C. The supernatant, which contain gluten forming proteins (glutenins and gliadins), was then collected into a 250 mL round bottom flask. The pellet was washed two more times with the same solvent and the supernatants were combined. A rotary evaporator was used to evaporate the solvent at 60°C. Extracted gluten forming proteins were then lyophilized and stored at -20°C for later analysis.

The lypholized protein extracts were analyzed for their protein content following the Dumas method as outlined in section 2.3.1. Protein profile of the extracts were monitored by SDS-PAGE under reducing conditions. The amount of sample needed to obtain 10 μg protein was weighed and mixed with 190 μL SDS Laemmli buffer and 10 μL beta-mercaptoethanol, stirred for 30 sec, boiled at 100°C for 5 min, and cooled down to room temperature. The sample was centrifuged at 9,279 g (Eppendorf 5415D, Hamburg, Germany) for 5 min. An aliquot (5 μL) of each sample was loaded into the wells of a 4-15% Criterion Tris-HCl gel. The gel was electrophoresed at 200 V for approximately 45 min, followed by staining using a coomassie blue staining solution (45% v/v methanol, 10% v/v glacial acetic acid, 45% v/v DDW, and 3g/L coomassie brilliant blue R250), and destaining using a destaining solution (10% v/v glacial acetic acid, 5% v/v methanol, and 85% v/v DDW). Gels were scanned using Molecular Image Gel Doc XR system (BioRad, Hercules, CA).

2.4.7 Protein Molecular Weight Distribution

To determine the molecular weight distribution of the IWG proteins, SE-HPLC was performed following the procedure developed by Gupta, Khan, & Macritchie (1993) and modified by Ohm, Ross, Ong, & Peterson (2006). Ten mg of flour (14% moisture content) was weighed into a 2 mL tube, and 1 mL of extraction buffer was added. The extraction buffer consisted of 0.5% SDS in 0.05 M sodium phosphate buffer (pH 6.9). The tube was vortexed at 2,500 rpm for 5 min using a pulsing vortex mixer (Fisher Scientific, Waltham, MA, USA) to solubilize the SDS extractable protein (SEP). The

mixture was centrifuged at 17,000 g (Eppendorf Centrifuge 5424, Hamburg, Germany) for 15 min. The supernatant was filtered through a 0.45 μm filter and heated at 80°C for 2 min. The heating step was done to suppress endogenous protease activity.

For the SDS-unextractable protein (UEP), 1 mL of the extraction buffer was added to the tube containing the residue/precipitate, sonicated at 10 W for 30 sec (Sonic dismembrator 100, Fisher Scientific, Waltham, MA, USA), and vortexed at 2,500 rpm for 5 min using a pulsing vortex mixer. The solution was centrifuged at 17,000 g for 15 min. The supernatant was filtered through a 0.45 μm filter, and the filtered solution was heated at 80°C for 2 min.

SE-HPLC was performed using an Agilent system (1100 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with BIOSEP SEC-S4000 500 Å (300*4.5 mm) size exclusion column (Phenomenex, Torrance, CA, USA) and a guard cartridge (BIOSEP SEC S4000). Ten μL of the protein extract (SEP or UEP) was injected onto the column. Aqueous acetonitrile (50% in water) with 0.1% trifluroacetic acid was used to elute the proteins at a flow rate of 0.5 mL/min. Temperature of the column was set at 35°C. The total run time per sample was 10 min. Proteins were detected at 214 nm using a photodiode array detector (1200, Agilent Technologies, Santa Clara, CA, USA) (Ohm et al., 2010).

MATHLAB 2008 software (The MathWorks, Natick, MA) was used to analyze the absorbance data. The absorbance values were interpolated to an interval of 0.002 min to calculate the absorbance area (AA). Data analysis was conducted between 3.5 and 8.0 min of runtime. There were five distinct fractions (F1-F5) observed in the generated

chromatogram. F1 extended over 3.5-5.5 min, and corresponded to the high molecular weight polymeric proteins (HMWPP). F2 extended over 5.5-5.9 min, and corresponded to the low molecular weight polymeric proteins (LMWPP). F3 extended over 5.9-6.2 min, and corresponded to ω-gliadin. F4 extened over 6.2-6.9 min, and corresponded to α-, β-, and γ-gliadins. Finally, F5 extended over 6.9-8.0 min, and corresponded to the albumins and globulins. See **Appendix B** (**Figure 18** and **Figure 19**) for sample chromatograms. Area under the curve for each fractions was converted to a relative percent value based on total areas and total protein content (Ohm et al., 2010).

2.5 Functionality Testing

2.5.1 Dough Rheology/Mixing Properties

Dough behavior during mixing was determined using a Farinograph – AT (C.W. Brabender, Duisburg, Germany) following the AACCI method 54-21.02 (*AACCI Approved Methods*, 2010). A 10 g farinograph mixing bowl set at 30 ± 0.2°C was used to determine the optimal water absorption and stability time for each flour sample. An optimum consistency was set at 500 BU. Water absorption data was then used to prepare the dough with optimum consistency for the Kieffer extensibility experiment, as outlined in section 2.4.2.

2.5.2 Extensibility using Kieffer

Dough rheological properties were also tested by a Kieffer Dough and Gluten Extensibility Rig (A/KIE) attached to a TA-XT2i texture analyzer (Texture Technologies Corp, Scarsdale, NY, USA) to characterize dough resistance to extension (mN) and extensibility (mm). The dough resistance to extension (mN) is the measure of the force needed to break the dough, measured by the force generated against the hook attached to the texture analyzer as shown in **Figure 7**. Dough resistance to extension (mN) is an indicator of dough strength. Extensibility (mm) is the distance at which the dough breaks apart, or a measure of dough deformation before it ruptures (Wang, Van Vliet, & Hamer, 2004). Five sub-duplicates were analyzed for each sample. Ten g of flour, 0.2 g of salt, and optimal amount of water based on the results of the farinograph, were mixed to make a dough that had optimum consistency at 500 BU. For the wheat controls and IWG samples, the doughs were mixed for 2.5 and 2 min, respectively. The mixing time for the controls was determined based on the gluten window test, while the mixing time for IWG samples was determined based on the dough handling capability (i.e. stickiness) determined through experimental trials. The fresh dough was then pressed and molded by the Kieffer molder (TA-105, Texture Technologies Inc., Hamilton, MA, USA) to approximately 4 mm in width and 50 mm in length. The dough strips were rested for 40 min in the molder, and each strip was then placed in the Kieffer microextension rig and stretched vertically as shown in **Figure 7**. The dough strips were pulled at a speed of 3.3 mm/s for a distance of 75 mm. The data was then automatically generated by the Texture

Exponent 32 version 6.1.4.0 software (Texture Technologies, Corp. Scarsdale, NY, USA).

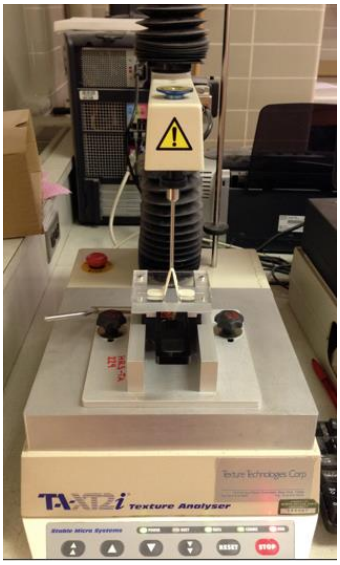


Figure 7. Dough stretched vertically using the Kieffer rig.

2.5.3 Pasting Properties

Starch pasting properties were measured using a Rapid Visco Analyzer (RVA) (Newport Scientific Inc., Jessup, MD 1998). Flour (4.0 g, 14% moisture) was mixed with 25.0 mL of double distilled water (DDW) and analyzed. The following temperature profile was applied: 50°C for 1 min, heating from 50°C to 95°C at a rate of 12°C/min (min 1:00 to 4:42), holding at 95°C for 2.5 min (min 4:42 to 7:12), cooling at a rate of 12°C/min (min 7:12 to 11:00), and holding at 50°C for 2 min. The following data were collected: pasting temperature (the temperature at which the initial swelling begins), peak viscosity (the maximum viscosity reached during heating), breakdown (extent of the decrease in viscosity during the holding period), final viscosity, and the setback value

(extent of the increase in viscosity during cooling) (Marti et al., 2015). Common pasting profile is shown in **Figure 5**.

2.6 Baking Quality

2.6.1 Bread Baking

Bread baking was done following the AACCI method 10-10.03 (*AACCI Approved Methods*, 2010), with modifications. Flour moisture was determined by OHaus MB45 (New Jersey, USA) at 130°C for 4 min. Flour (50 g), 3 g of sugar, 0.75 g of salt, 2.65 g of yeast, and 1.5 g of shortening were used to make the dough. The yeast and sugar solution was kept at 30°C and 85% relative humidity for 20 min before use.

Preparation of the IWG samples and wheat controls differed. For preparation of IWG samples, a final dough moisture of 46.5% was targeted based on a preliminary study to determine optimum moisture content. Due to sample size limitations, a food processor (Braun 4169, Kromberg im Taunus, Germany) was used to mix the dough (15 sec total mixing time). For the preparation of wheat controls, 86.3% and 87.1% added moisture were targeted for hard red wheat and Arapahoe, respectively, based on optimum moisture level previously determined by the farinograph. The dough was then mixed in a 50g farinograph bowl (C.W. Brabender, Duisburg, Germany) for 3 min, the time needed for the dough to reach optimum dough consistency at 500 BU. Each dough sample was kneaded by hand 10 times, split into two even parts, and then put in the proofer (Baxter PW2E, Orting, WA, USA) at 30°C and 85% relative humidity (RH) for 52 min.

Each dough sample was again punched by hand 10 times and proofed (30°C and 85% RH) for 25 min. Each dough sample was once more punched by hand 10 times and proofed (30°C and 85% RH) for 13 min. The dough was then sheeted to a 3/16" thickness 5 times, shaped into a ball by hand, re-sheeted to 3/16" thickness 5 times, rolled to a length of 2.1 inches, and placed in a Freshware CB-308RB trapezoidal pan (size 2.8 x 1.5 inches top, 2.1 x 1.2 inches bottom, and 1.1 inches depth) (Amazon, Seattle, WA, USA). The dough was proofed (30°C and 85% RH) for another 33 min before it was baked with steam for 10 sec followed by baking for 14 min at 425°F in a baking oven (Baxter OV500E1, Orting, WA, USA). The bread was left to cool for one hour before the analysis of bread quality parameters (**Section 2.5.2**). Bread was cut into slices of 12.5 mm thickness with a bread cutter (Oliver Products Company, Grand Rapids, MI, USA). Bread baking was conducted in duplicate for each sample.

2.6.2 Bread Baking Quality

2.6.2.1 Bread Volume and Height

Bread volume was measured using the Rapeseed Displacement method (AACCI 10-05.01, *AACCI Approved Methods*, 2010). The bread's greatest height was measured by Neiko 01407A digital caliper (Neiko, Taiwan, China).

2.6.2.2 Bread Firmness

Bread firmness was measured following the AACCI method 74-09.01 (*AACCI Approved Methods*, 2010) using TA XT-Plus Texture Analyzer (Texture Technologies Corp, NY, USA). One 12.5 mm slice of bread was used to perform the analysis. The probe used was 1.3 cm (0.5 inch) in diameter and 3.5 cm in length (1 3/8 inches). The force (in g) needed to deform the bread by the probe was recorded.

2.6.2.3 Bread Pictures

Bread pictures were taken using HP Scanjet (G4050, Palo Alto, CA, USA) at 1200 dpi.

2.7 Statistical Analysis

Analysis of variance (ANOVA) was done using R (version 3.3.2). When a factor or an effect was found to be significant ($P \leq 0.05$), differences among the means were determined using Tukey-Kramer Honest Significant Difference (HSD) test. ANOVA tables can be found in **Appendix C** (**Table 7** - **Table 17**).

3. Results and Discussions

3.1 Chemical Characterization

The bran to endosperm ratio is higher for IWG samples than that of wheat controls because IWG seed size is smaller. Among the IWG samples, bulk IWG had the smallest seed size (**Table 18, Appendix D**). Smaller seed size and consequently higher bran to endosperm ratio will have a direct bearing on the chemical constituents of the IWG grains.

3.1.1 Proximate Composition

The proximate composition of hard red wheat and Arapahoe controls, bulk IWG, and 16 IWG experimental lines is shown in **Table 2**. Protein content of the wheat controls was lower than that of the IWG samples, similar to what was observed by Becker et al. (1991). The protein component of the grain mainly constitutes albumins, globulins, gliadins, and glutenins (Brouns et al., 2012). In cereal grains, albumins and globulins are mostly found in the aleurone layer and the germ; whereas gliadins and glutenins are mostly found in the endosperm (Belderok et al., 2000; Šramková et al., 2009). Thus, the higher bran to endosperm ratio in IWG samples may result in a higher content of albumins and globulins compared to wheat. This distinction is crucial as will be discussed in **Section 3.1.7**. Even though the protein content in IWG samples was higher than that of the wheat controls, it might not necessarily constitutes a considerable amount of the functional protein needed for good baking quality.

Fat content was highest in the bulk IWG sample. Fat content among the IWG experimental lines compared to wheat controls varied, with no obvious trend. Lipids in grains are mostly found in the germ (Šramková et al., 2009). As such, the smaller seed size and higher bran to endosperm ratio of bulk IWG may explain its higher fat content as compared to the rest of the samples (**Table 18, Appendix D**).

Similarly, bulk IWG had the highest ash content followed by the IWG experimental lines and wheat controls. Since mineral content is highest in the bran component of the grain (Šramková et al., 2009), the higher bran to endosperm ratio of bulk IWG compared to IWG experimental lines and wheat controls again explains this finding.

The moisture content of the wheat controls was slightly higher than that of the IWG samples. As reported by Mutwali, Mustafa, Gorafi, & Ahmed (2016), the growing environment affects the moisture content of the grain. Therefore, the difference in the moisture content of the samples may not be a differentiation factor among the samples, but rather is a function of the growing environment, as well as the handling and storage conditions post harvest.

Lastly, the total carbohydrate content, determined by difference, was higher in the controls than in the IWG samples. Difference in carbohydrate content could also be attributed to the higher bran to endosperm ratio and the grain size of IWG because starch is mainly located in the endosperm (Šramková et al., 2009). Carbohydrate content and composition of the grain impact the functionality as will be discuseed in later sections.

The proximate composition of IWG samples may directly impact the potential functionality and application of the grain. Chemical composition results confirmed that the bran to endosperm ratio highly impact the chemical composition of the grain. In summary, the higher bran to endosperm ratio of IWG samples resulted in a higher protein, fat, and ash content, and a lower carbohydrate content compared to wheat controls. These findings, therefore, were critical to the understanding of the effect of IWG seed size on composition, functionality, and baking quality.

Table 2. Proximate composition (on wet basis) of hard red wheat and Arapahoe controls, bulk IWG, and 16 IWG experimental lines.

Samples	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Total CHO* (%) By Difference
Hard Red Wheat	$10.2^{h\wedge}$	2.85^{fg}	1.64^{e}	10.70^{a}	74.6
Arapahoe	12.0^{g}	2.46^{fg}	2.00^{d}	9.99^{ab}	73.6
Bulk IWG	18.1^{b}	4.36^{a}	2.69^{a}	9.01^{bc}	67.6
IWG L4-1	17.9^{bc}	2.36^{g}	2.40^{bc}	9.22^{bc}	68.1
IWG L4-3	19.2^{a}	3.04^{cd}	2.49^{ab}	9.31^{bc}	66.0
IWG L4-29	17.8^{bcd}	2.48^{fg}	2.42^{bc}	9.31^{bc}	67.9
IWG L4-32	17.5^{d}	2.96^{cde}	2.38^{bc}	9.08^{bc}	68.1
IWG L4-57	19.2^{a}	3.11^{bcd}	2.38^{bc}	9.28^{bc}	66.0
IWG L4-72	18.1^{b}	2.73^{def}	2.29^{bc}	8.85^{bc}	68.0
IWG L4-84	19.2^{a}	3.11^{bcd}	2.32^{bc}	9.28^{bc}	66.1
IWG L4-85	19.1^{a}	3.32^{bc}	2.32^{bc}	9.02^{bc}	66.2
IWG L4-103	16.5^{f}	3.32^{bc}	2.37^{bc}	8.87^{bc}	68.9
IWG L4-105	18.2^{b}	2.62^{efg}	2.38^{bc}	8.65^{c}	68.1
IWG L4-139	17.6^{cd}	2.56^{fg}	2.26^{bc}	8.99^{bc}	68.6
IWG L4-154	17.8^{bcd}	2.36^{fg}	2.39^{bc}	9.35^{bc}	68.0
IWG L4-157	18.0^{bc}	2.97^{cd}	2.24^{c}	9.18^{bc}	67.6
IWG L4-159	19.0^{a}	2.78^{def}	2.37^{bc}	9.11^{bc}	66.7
IWG L4-160	16.9^{e}	3.45^{b}	2.19^{cd}	9.20^{bc}	68.2
IWG L4-172	17.1^{e}	2.55^{efg}	2.19^{cd}	9.44^{bc}	68.8

* CHO stands for carbohydrate, calculated by difference.

^ Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n=3.

3.1.2 Dietary Fiber

Total dietary fiber of IWG samples was higher than that of the wheat controls. The dietary fiber content of IWG samples ranged between 15.9-18.1%, followed by Arapahoe (14.4%) and hard red wheat (13.5%) (**Figure 8**). Since most of the dietary fiber is located in the bran (Pomeranz, 1988: Brouns et al., 2012), the higher dietary fiber content in IWG samples can again be explained by the higher bran to endosperm ratio and grain size of the IWG samples. The soluble dietary fiber (SDF) represented 32.7% of the total dietary fiber for hard red wheat, 27.8% for Arapahoe, and between 23-30% for IWG samples. Daou & Zhang (2014) showed that SDF was a major determinant of samples' viscosities because SDF forms a viscous solution upon water absorption. Thus, the higher SDF content of the wheat controls may contribute to higher dough viscosity.

While it is important to consider SDF because of its viscosity builder properties, insoluble dietary fiber (IDF) represented the main component of DF in the samples and potentially may affect both the water absorption capacity and gluten network formation. IDF was between 70-76% of the total dietary fiber for IWG samples, 71.9% for Arapahoe, and 66.9% for hard red wheat. Wang, Rosell, & de Barber (2002) investigated the effect of different fiber sources on dough rheology. They found that samples with higher IDF content had a higher water absorption when compared to samples with higher SDF content. Romano, Torrieri, Masi, & Cavella (2011) also investigated the effect of SDF and IDF on the bread baking process. They found that bread made with a higher IDF content resulted in a lower volume than bread made with a higher SDF content. Thus, the

higher IDF percent coupled with high total dietary fiber content of the IWG samples might contribute to higher water absorption and lower bread volume.

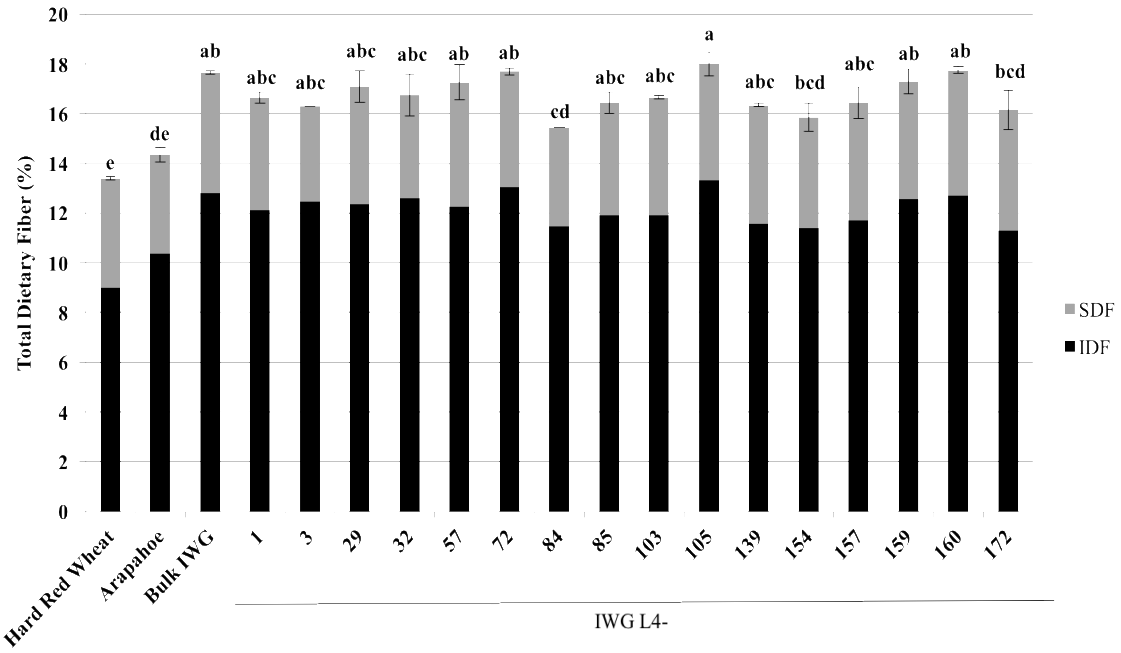


Figure 8. Total dietary fiber and soluble/insoluble dietary fiber ratio (SDF/IDF) of hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines. Error bars represent standard errors (n=2). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$).

The dietary fiber content in any sample may impact both functionality and bread baking quality. As previously mentioned, protein, fiber, and starch compete for water. Gómez, Ronda, Blanco, Caballero, & Apesteguía (2003) studied the effect of dietary fiber on bread quality and showed that higher fiber content positively correlated with water absorption, dough development time, and dough stability, but negatively correlated with bread specific volume. The hydroxyl groups in the fiber contribute to the increase in water absorption because they form hydrogen bonds with water. On the other hand, the

reduction of bread specific volume, as dietary fiber content increases, is a result of the interference of fiber with gluten network formation, causing a reduction of the gas holding capacity. In terms of the bread firmness value, adding fiber can increase firmness and preserve moisture over storage. Gómez et al. (2003) collected bread firmness data immediately after baking and showed that the bread made with 2% additional fiber had a significantly firmer texture than the bread made with no added fiber. This firmer bread texture may be due to the protein, fiber, and starch competition for water. However, the fiber's capacity to strongly bind water allows the bread to retain moisture over storage, thus slowing the bread firmness rate and increasing shelf life (Gómez et al., 2003). Therefore, dietary fiber content plays an important role in predicting the functionality and baking quality of IWG samples.

3.1.3 Total Starch and Amylose/Amylopectin Ratio

Starch, as a major component of flour, plays an essential role in baked products. In the bread baking process, starch can absorb water up to 50% of its dry weight during mixing under ideal conditions (sufficient water and at room temperature) (Goesaert et al., 2005; Onyango, 2016). Starch gelatinizes and swells during baking, and starch molecules reassociate upon cooling (Goesaert et al., 2005).

The total starch content was higher in hard red wheat and Arapahoe controls than in IWG samples (**Figure 9**). Starch is mostly found in the endosperm (Šramková et al., 2009) and therefore, the lower starch content in IWG samples is again attributed to the higher bran to endosperm ratio and smaller seed size. Starch content in bulk IWG was lower as compared to the rest of the IWG experimental lines; this is due to the smaller bulk IWG seed size as compared to the rest of IWG samples (**Table 18**, **Appendix D**). Because higher starch content leads to higher viscosity, higher viscosity results were expected for the wheat controls compared to IWG samples, as will be discussed in **Section 3.2.2**.

The amylose component of starch has a direct impact on bread firmness as it retrogradates during baking, while the amylopectin component impacts bread firmness as it retrogradates over storage (Singh et al., 2003; Alcázar-Alay & Meireles, 2015). The amylose component for wheat controls was around 25.5% of the total starch content, while it ranged between 22-25% for the IWG samples. Since the amylose to amylopectin ratio among all of the samples was relatively similar, there should be no direct impact on

bread firmness or staling rate. However, starch structure that was not analyze in this research may also have a significant impact on bread firmness or staling rate.

To the best of our knowledge, this is the first report on IWG starch content and the amylose/amylopectin ratio. This data will help explain the functionality and baking quality of IWG samples as will be discussed in **Section 3.2** and **Section 3.3**.

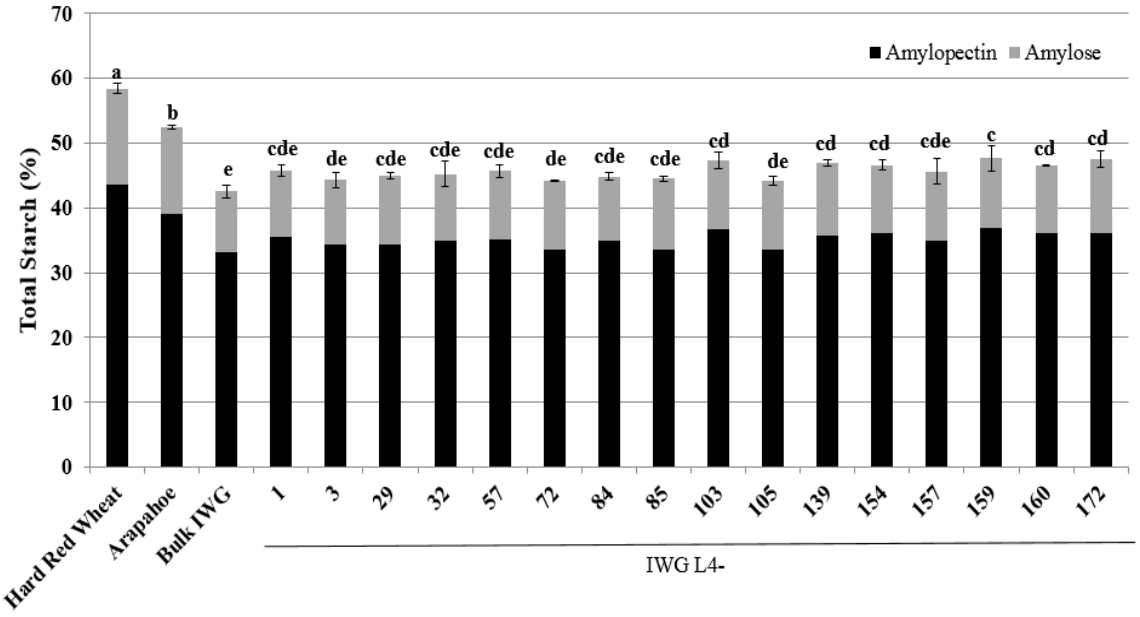


Figure 9. Total starch and amylose/amylopectin ratio of hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines. Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$).

3.1.4 Amylose and Amylopectin Molecular Weight Distribution

The molecular weight (MW) of starch molecules may affect functionality. Specifically, starch molecular size may affect its pasting properties. The MW of amylose and amylopectin were relatively similar for all of the samples with the exception of Arapahoe, which had a significantly higher amylopectin molecular weight (**Figure 10**). Reports on the effect of amylose/amylopectin MW on functionality is scarce. Kowittaya & Lumdubwong (2014) indicated that the MW of amylopectin positively affect the peak viscosity, but negatively affect the final viscosity. Thus, Arapahoe's high amylopectin molecular weight is expected to contribute to higher peak viscosity compared to other samples as will be discussed in **Section 3.2.2**.

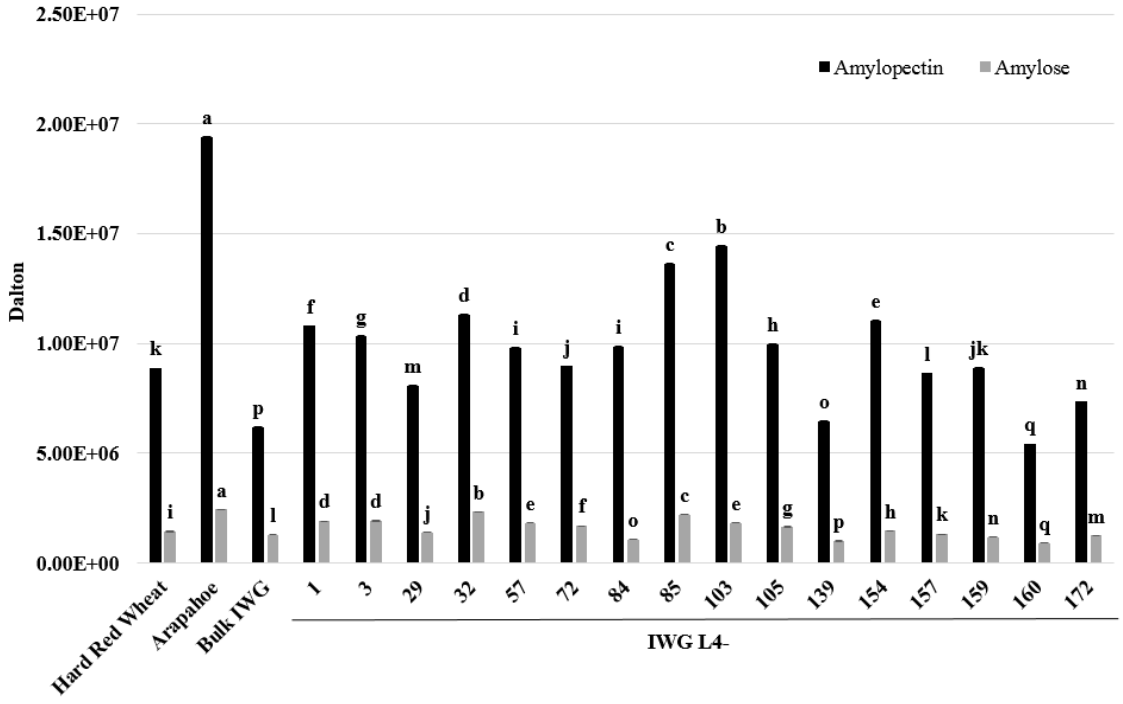


Figure 10. Molecular weight distribution of amylose and amylopectin of hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines as determined by SE-HPLC. Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$).

3.1.5 Starch Damage

Wheat controls had a higher content of damaged starch as compared to the IWG samples. Hard red wheat had the highest damaged starch content (13.8% of the total starch), followed by Arapahoe (11.5%), and IWG samples (4.7-7.5%) (**Figure 11**). As reported by Mok & Dick (1991) and Bass (1998), the amount of damaged starch is directly related to kernel hardness. Accordingly, the kernels of the wheat controls may have been harder than those of the IWG samples. The higher damaged starch content in wheat may impact wheat functionality and baking quality. With a higher damaged starch content, the water absorption increases, thus less water will be available for the gluten network (Goesaert et al., 2005; Barrera et al., 2007). High damaged starch content may also result in a lower bread specific volume (Barrera et al., 2007), as will be discussed in **Section 3.3**.

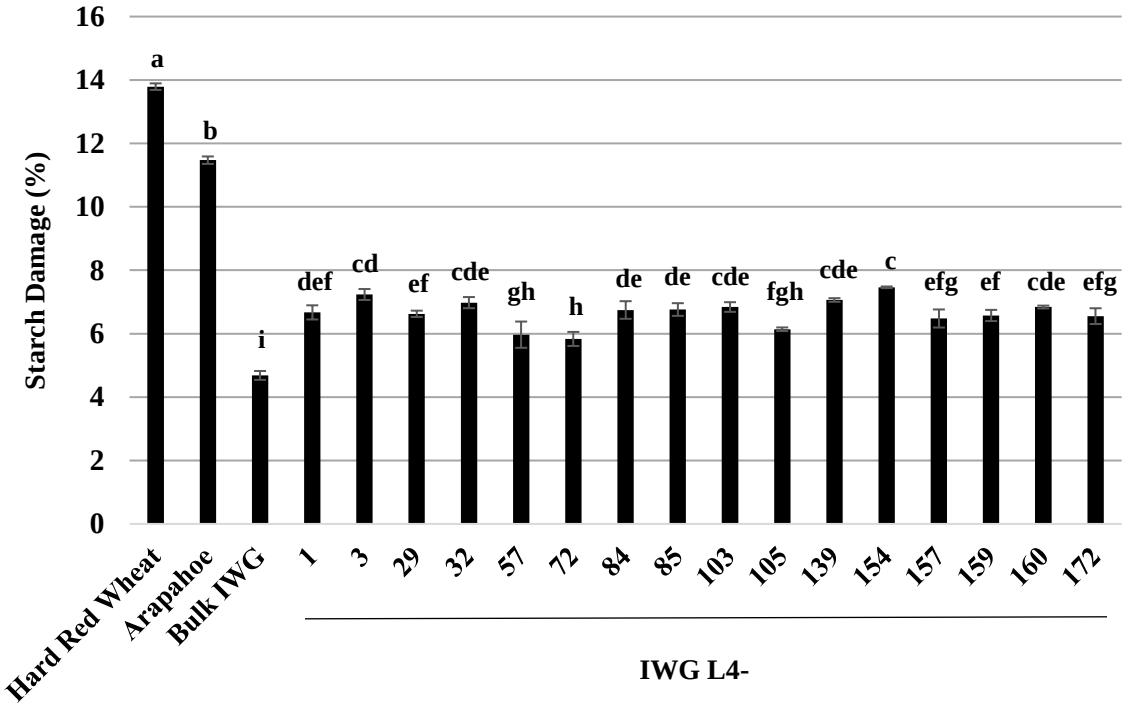


Figure 11. Percent starch damage of hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines. Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$).

3.1.6 Gluten Forming Proteins Profiling

Gluten consists of polymeric glutenins and monomeric gliadins (Žilić, 2013). Glutenins are the proteins responsible for dough elasticity and strength; while gliadins are the proteins responsible for viscosity and elasticity (Žilić, 2013). High molecular weight glutenins (HMWG) readily form large polymers and thus are important for the gluten network formation, enabling appreciable gas holding capacity and providing elastic properties (Niu et al., 2011; Žilić, 2013; Dhaka & Khatkar, 2014). Wheat is known to have a desirable balance of both glutenin and gliadin proteins for gluten network formation and stability (Žilić, 2013). Thus, baked products made from wheat have a desirable gas holding capacity, allowing the dough to sufficiently rise during fermentation and baking. Protein profiling of wheat controls and IWG samples will provide insight into the baking quality of IWG samples as compared to that of wheat.

In comparison to wheat controls, IWG samples were deficient in HMWG; but were rich in α-, β-, and γ- gliadins; and contain some low molecular weight glutenins (LMWG) (**Figure 12**). Some of the HMWG components in IWG samples were uniquely of low molecular weight (~60 kDa) compared to those of wheat controls, as pointed out with arrows in **Figure 12**. The difference in protein profiles between IWG samples and wheat controls demonstrated that the amount of protein in a sample does not confirm the presence of targeted functional proteins necessary for dough development. Thus, the deficiency of IWG samples in HMWG protein, of high molecular weight (>60 kDa), as present in the wheat controls, suggested that IWG samples may have a weak gas holding capacity and elasticity, leading to undesirable dough characteristics. Ohm et al. (2010)

showed positive correlations between the high molecular weight components of HMWG (the polymeric proteins) and strong gluten network. Larger polymers improve dough strength because the structures of these polymers may support stronger protein-protein interactions.

Zhang et al. (2014) isolated five HMWG genes from several IWG samples (third breeding cycle from The Land Institute as shown in **Figure 3**). They found that HMWG proteins in IWG samples shared similar structures but were smaller in size when compared to that of the common wheat. They also discovered that the HMWG of the IWG samples had a larger molecular weight range (45-90 kDa) than common wheat. While the impact of HMWG for wheat is understood, the impact of both smaller molecular size and larger molecular weight range of HMWG for IWG remains unknown. Thus, further study of the molecular interactions of IWG's HMWG and its impact on dough rheology and baking quality is necessary.

Differences in protein profiles were also seen among the IWG samples. For example, intensity of the protein band at ~60 kDa was stronger in IWG L4-84 (lane 11) compared to IWG L4-57 (lane 9), while the protein band intensity at ~115 kDa was stronger in IWG L4-57 (lane 9 compared to IWG L4-84 (lane 11) (**Figure 12**). Around 60 kDa, IWG L4-57 had two different protein bands, while IWG L4-84 had only one protein band at that molecular weight. Further, IWG L4-57 had one protein band around 115 kDa, while IWG L4-84 had three distinct protein bands in that region. Apparently, HMWG differed in molecular weight distribution among the different lines. These difference in HMWG distribution among the samples may have an impact on

functionality and baking quality. Estimation of each type of protein could provide additional insight into differences among the samples and the potential impact on functionality.

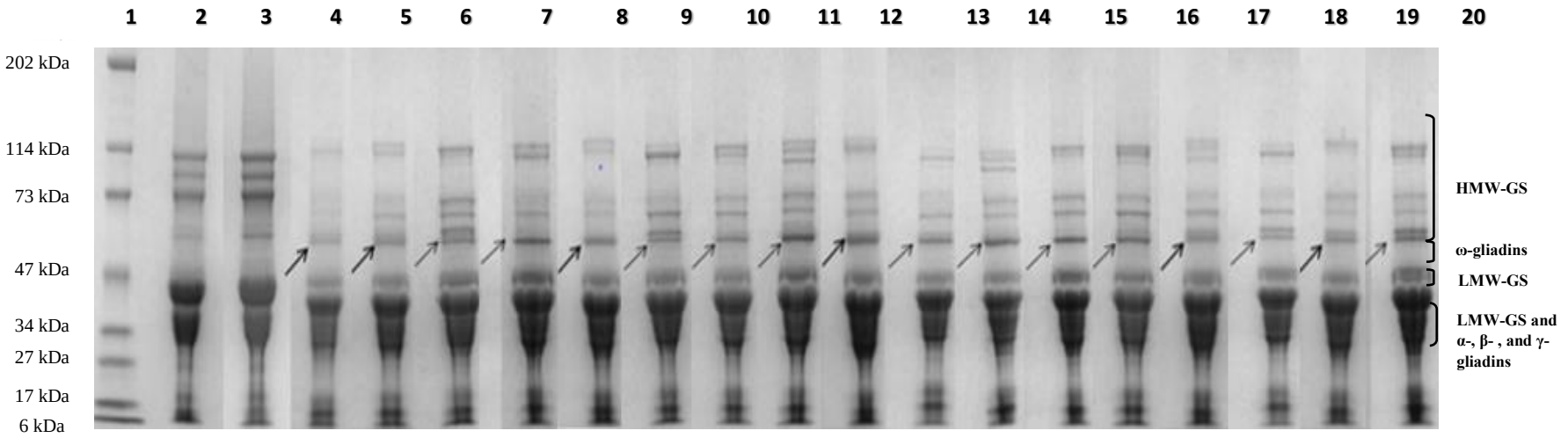


Figure 12. SDS-PAGE gel with Coomasie blue staining, visualizing the protein profiles of the samples under reducing conditions. Lane 1: molecular weight marker (MW); Lane 2: hard red wheat (HRW); Lane 3: Arapahoe; Lane 4: bulk IWG; Lane 5: IWG L4-1; Lane 6: IWG L4-3; Lane 7: IWG L4-29; Lane 8: IWG L4-32; Lane 9: IWG L4-57; Lane 10: IWG L4-72; Lane 11: IWG L4-84; Lane 12: IWG L4-85; Lane 13: IWG L4-103; Lane 14: IWG L4-105; Lane 15: IWG L4-139; Lane 16: IWG L4-154; Lane 17: IWG L4-157; Lane 18: IWG L4-159; Lane 19: IWG L4-160; Lane 20: IWG L4-172. HMWG: High molecular weight glutenins; LMWG: low molecular weight glutenins.

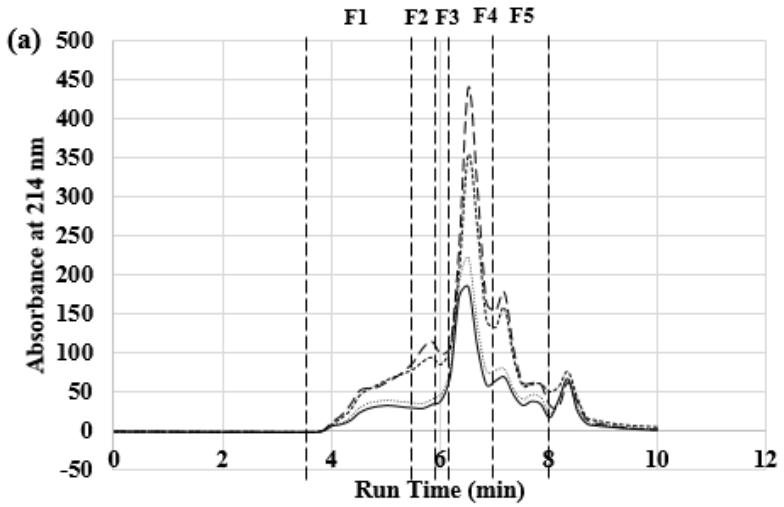
3.1.7 Protein Molecular Weight Distribution

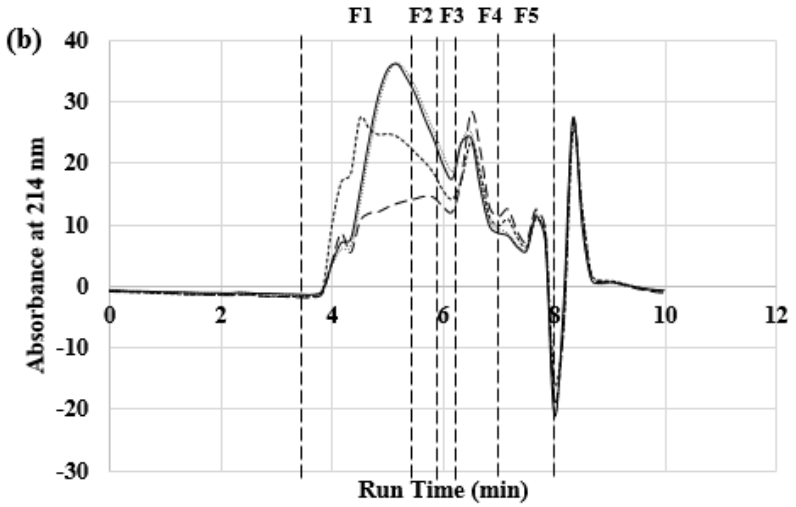
To determine the distribution and molecular weights of the IWG proteins compared to those of wheat controls, size exclusion high performance liquid chromatography (SE-HPLC) was performed. Five distinct fractions were observed (**Figure 13**, and **Figures 20** and **21** in **Appendix E**). The five fractions observed were the high molecular weight polymeric proteins (HMWPP) (F1); low molecular weight polymeric proteins (LMWPP) (F2); ω-gliadin (F3); α-, β-, γ- gliadins (F4); and albumins and globulins (F5). Fractions were identified based on previous reports (Gupta et al., 1993; Ohm et al., 2006). The area under the curve for each fraction was converted to a relative percent value (Ohm et al., 2010). The HMWPP fraction includes HMWG with some polymeric albumins and globulins aggregates. The LMWPP fraction includes LMWG with some polymeric albumins and globulins aggregates as well (Singh et al., 1990; Batey, Gupta, & MacRitchie, 1991; Gupta et al., 1993). Extractable proteins are soluble in SDS buffer solution, whereas unextractable proteins are not. Unextractable proteins contain polymers that are considerably larger than those of the extractable proteins (Singh et al., 1990; Batey, Gupta, & MacRitchie, 1991; Gupta et al., 1993). Thus, unextractable proteins underwent a sonification step to break the large polymers and solubilize the proteins to enable analysis by SE-HPLC (Singh et al., 1990).

The amount of the extractable albumins and globulins was significantly ($P \leq 0.05$) higher in most of the IWG samples compared to wheat controls (**Table 3**). Further, the amount of unextractable HMWPP in IWG samples was significantly ($P \leq 0.05$) lower than that of the wheat controls. These results confirm that while IWG contained more

protein than wheat, it contained less of the targeted functional proteins (in this case HMWPP). Assuming HMWPP content positively correlates with HMWG content, HMWPP fraction was indicative of the presence and relative quantity of HMWG. However, the HMWPP also constitutes albumins and globulins aggregates. The presence of HMWG vs. albumins and globulins aggregates in the HMWPP fraction was not elucidated. In any case it was apparent that wheat controls had higher HMWG and thus would be superior in functionality and baking quality, followed by bulk IWG, and the rest of the IWG experimental lines.

Overall, the chemical characterization of the samples (**Section 3.1**) allowed for inferences to be made about the samples' potential functionality. In the following sections, dough rheology and starch pasting characteristics (**Section 3.2**), as well as bread baking quality of IWG (**Section 3.3**), will be evaluated. The relationhip between chemical characterization, functionality, and baking quality will be further explored.





(c)

Area	Elution Time (min)	Protein Types
F1	3.5-5.5	High Molecular Weight Polymeric Proteins
F2	5.5-5.9	Low Molecular Weight Polymeric Proteins
F3	5.9-6.2	ω-gliadins
F4	6.2-6.9	α,β,γ-gliadins
F5	6.9-8.0	albumins and globulins

Hard Red Wheat
Arapahoe
Bulk IWG
IWG L4-1

Figure 13. Protein molecular weight distribution by SE-HPLC for (a) soluble/extractable protein and (b) insoluble/unextractable protein for hard red wheat, Arapahoe, bulk IWG, and IWG experimental line L4-1 ; and (c) different fractions and elution times.

Table 3. Quantitative distribution of extractable and unextractable protein fractions based on percent of the total area of the chromatographic peaks for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

	% Total Area of Extractable Protein					% Total Area of Unextractable Protein				
Sample ID	**HMWPP***	**LMWPP^**	**ω-gliadins**	**α,β and γ-gliadins**	**Albumins and Globulins**	**HMWPP**	**LMWPP**	**ω-gliadins**	**α,β and γ-gliadins**	**Albumins and Globulins**
Hard Red Wheat	13.67$^{h\#}$	4.44^{f}	5.00^{i}	31.71^{e}	18.16^{efg}	12.83^{a}	3.90^{a}	2.13^{a}	4.85^{abcd}	3.33^{abcde}
Arapahoe	14.44^{gh}	4.67^{f}	5.10^{hi}	32.82^{de}	18.95^{efg}	10.92^{b}	3.59^{a}	2.01^{ab}	4.43^{bcd}	3.07^{abcde}
Bulk IWG	16.18^{abcde}	7.42^{bcd}	5.71^{bcdef}	33.80^{bcde}	21.17^{bc}	7.73^{c}	1.78^{bcd}	1.05^{def}	2.80^{d}	2.34^{e}
IWG L4-1	15.75^{cdefg}	8.20^{a}	5.89^{abc}	37.52^{a}	21.49^{abc}	3.77^{fg}	1.24^{de}	0.83^{ef}	2.90^{d}	2.40^{de}
IWG L4-3	14.99^{defgh}	7.79^{abc}	5.67^{bcdefg}	34.47^{abcde}	19.52^{de}	5.45^{de}	1.93^{bcd}	1.34^{cde}	5.11^{abcd}	3.72^{abc}
IWG L4-29	15.08^{defgh}	6.66^{e}	5.08^{hi}	35.46^{abcd}	18.93^{efg}	5.01^{def}	1.92^{bcd}	1.36^{cde}	6.49^{ab}	4.00^{ab}
IWG L4-32	17.45^{ab}	7.88^{ab}	5.80^{abcde}	35.60^{abc}	22.57^{a}	2.94^{g}	1.04^{e}	0.77^{f}	2.94^{d}	3.00^{bcde}
IWG L4-57	17.90^{a}	8.21^{a}	6.28^{a}	37.58^{a}	19.01^{efg}	3.36^{fg}	1.18^{de}	0.86^{ef}	3.17^{cd}	2.46^{cde}
IWG L4-72	17.15^{abc}	7.94^{ab}	5.39^{defghi}	33.41^{cde}	20.55^{cd}	5.31^{de}	1.73^{bcd}	1.13^{def}	4.13^{bcd}	3.26^{abcde}
IWG L4-84	14.57^{fgh}	6.77^{e}	5.24^{ghi}	36.34^{ab}	17.61^{g}	6.40^{cd}	2.17^{b}	1.45^{cd}	5.85^{abc}	3.60^{abc}
IWG L4-85	14.98^{efgh}	6.75^{e}	5.08^{hi}	35.95^{abc}	18.92^{efg}	5.36^{de}	1.95^{bc}	1.34^{cde}	5.86^{abc}	3.81^{ab}
IWG L4-103	16.71^{abcd}	7.87^{ab}	5.82^{abcd}	33.48^{cde}	21.91^{ab}	3.48^{fg}	1.43^{cde}	1.09^{def}	4.49^{bcd}	3.71^{abc}
IWG L4-105	16.07^{bcdef}	7.22^{cde}	5.46^{cdefgh}	36.63^{a}	21.67^{abc}	4.27^{efg}	1.36^{cde}	0.92^{ef}	3.49^{cd}	2.93^{bcde}
IWG L4-139	15.88^{cdefg}	7.53^{abcd}	5.31^{fghi}	35.55^{abc}	19.20^{ef}	4.87^{ef}	1.91^{bcd}	1.28^{cde}	5.01^{abcd}	3.46^{abcd}
IWG L4-154	15.85^{cdefg}	7.98^{ab}	5.99^{ab}	37.19^{a}	21.45^{abc}	4.33^{ef}	1.32^{cde}	0.87^{ef}	2.72^{d}	2.29^{e}
IWG L4-157	14.88^{efgh}	7.03^{de}	5.57^{bcdefg}	36.32^{ab}	21.42^{abc}	4.16^{efg}	1.54^{bcde}	1.12^{def}	4.66^{abcd}	3.30^{abcde}
IWG L4-159	15.56^{defg}	7.22^{cde}	5.42^{defghi}	33.46^{cde}	17.98^{fg}	5.22^{de}	2.20^{b}	1.62^{bc}	7.19^{a}	4.13^{a}
IWG L4-160	16.63^{abcd}	7.63^{abcd}	5.36^{efghi}	36.04^{abc}	21.77^{abc}	4.23^{efg}	1.41^{cde}	0.93^{ef}	3.2^{cd}	2.78^{bcde}
IWG L4-172	15.29^{defg}	7.95^{ab}	5.76^{bcde}	35.32^{abcd}	21.72^{abc}	4.28^{efg}	1.49^{cde}	1.07^{def}	3.77^{bcd}	3.36^{abcde}

* HMWPP stands for high molecular weight polymeric proteins

^ LMWPP stands for low molecular weight polymeric proteins

Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n=3.

3.2 Functionality Testing

3.2.1 Dough Rheology, Mixing Properties and Extensibility

The dough rheological properties of the samples were analyzed using farinograph and Kieffer. Water absorption results obtained from the farinograph show the amount of water needed by each sample to develop a 500 BU dough consistency (optimum wheat dough consistency) (El-Dash, 1978). The addition of too much or too little water will create dough outside of the optimum consistency. Based on the farinograph data, wheat controls had higher water absorption values when compared to that of the IWG samples (**Table 4**).

The farinograph also reveals dough stability time, the time between sample's arrival and depature at the 500 BU line. The amount of time a sample remains at 500 BU correlates with the strength of the dough. Hard red wheat had the highest stability time, followed by Arapahoe, bulk IWG, and the rest of the IWG experimental lines (**Table 4**, **Figure 14**; see **Appendix F**, **Figure 22** for the rest of the IWG samples). IWG lack of stability is most likely attributed to its deficiency in HMWG, the protein that is crucial for gluten network formation and stability (Niu et al., 2011; Žilić, 2013; Dhaka & Khatkar, 2014).

Table 4. Farinograph (water absorption corrected for default consistency and stability time) and Kieffer (resistance to extension and extensibility) data for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Samples	Farinograph Data		Kieffer Data	
	Water Absorption Corrected for Default Moisture Content (14%)	Stability (sec)	Resistance to Extension (mN)	Extensibility (mm)
Hard Red Wheat	74.5^{a*}	598.5^{a}	340.33^{b}	17.68^{b}
Arapahoe	74.6^{a}	247.0^{b}	425.82^{a}	21.61^{a}
Bulk IWG	68.7^{hi}	121.5^{bc}	179.14^{c}	13.51^{c}
IWG L4-1	73.2^{b}	44.0^{c}	66.69^{ijk}	6.04^{hi}
IWG L4-3	71.8^{cd}	88.0^{c}	67.47^{hijk}	7.72^{fgh}
IWG L4-29	69.0^{hi}	42.0^{c}	74.5g^{ghi}	6.69^{fgh}
IWG L4-32	67.2^{j}	42.0^{c}	65.53^{jk}	5.69^{hi}
IWG L4-57	68.5^{i}	30.0^{c}	44.99^{l}	4.41^{i}
IWG L4-72	69.6^{gh}	52.0^{c}	75.79^{fgh}	7.22^{fgh}
IWG L4-84	72.1^{c}	53.0^{c}	66.02^{ijk}	8.68^{ef}
IWG L4-85	70.3^{efg}	52.0^{c}	72.72^{ghij}	10.02^{de}
IWG L4-103	70.2^{fg}	29.0^{c}	66.86^{ijk}	6.76^{fgh}
IWG L4-105	70.8^{def}	47.5^{c}	68.73^{hijk}	6.81^{fgh}
IWG L4-139	71.2^{cde}	56.0^{c}	91.98^{e}	11.66^{cd}
IWG L4-154	71.4^{cd}	48.0^{c}	61.70^{k}	6.05^{hi}
IWG L4-157	70.3^{efg}	38.0^{c}	84.10^{ef}	8.40^{efg}
IWG L4-159	69.5^{ghi}	64.5^{c}	143.92^{d}	13.46^{c}
IWG L4-160	70.3^{efg}	42.0^{c}	71.57^{ghij}	6.34^{ghi}
IWG L4-172	68.9^{hi}	49.5^{c}	79.00^{fg}	8.42^{efg}

***** Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), where n=2 for farinograph data and n=5 for Kieffer data.

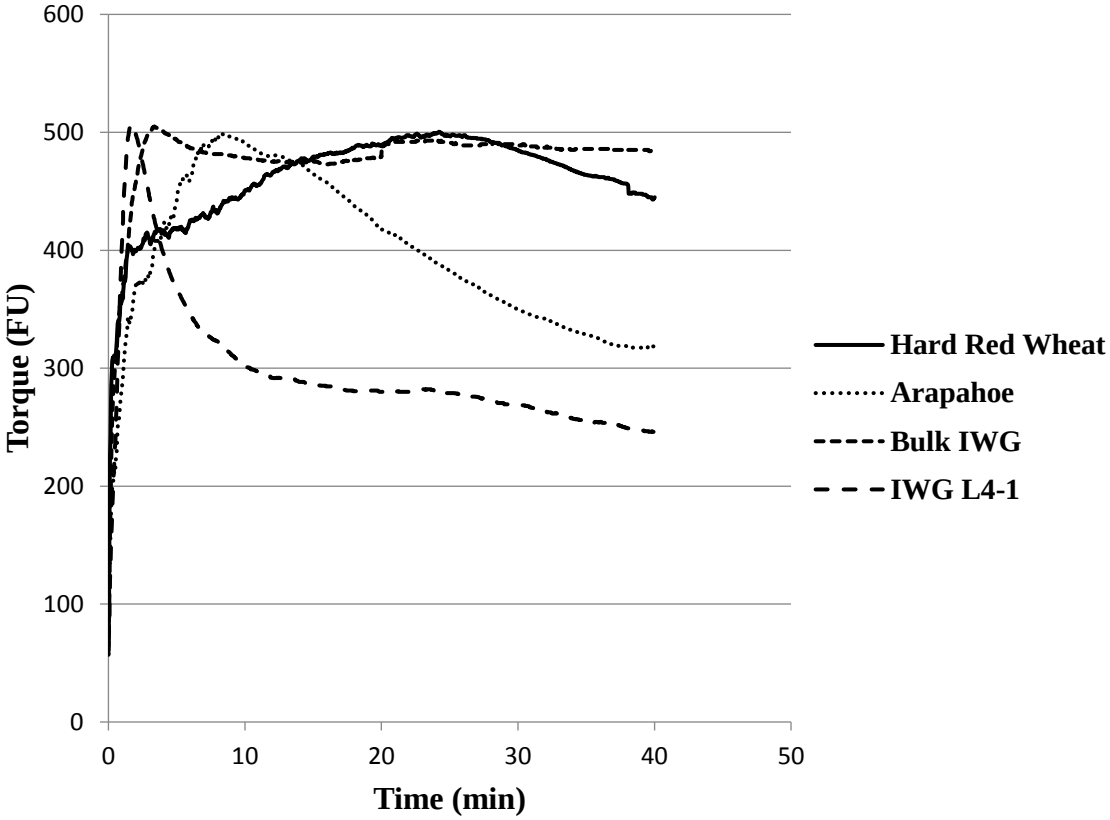


Figure 14. Farinograms of hard red wheat, Arapahoe, bulk IWG, and IWG L4-1 display that the wheat controls (hard red wheat and Arapahoe) have greater stability than bulk IWG and IWG L4-1 samples.

Kieffer dough and gluten extensibility rig was used to characterize dough extensibility (mm) and its resistance to extension (mN). The dough resistance to extension is the measure of the force needed to break the dough. Dough resistance to extension measured by the force generated against the hook attached to the texture analyzer is shown in **Figure 7**. Dough resistance to extension is an indicator of dough strength. Extensibility, also a measure of dough strength, is the distance at which the dough breaks apart - a measure of dough deformation before it ruptures (Wang et al., 2004).

Arapahoe had the highest resistance to extension and highest extensibility, followed by hard red wheat, bulk IWG, and IWG experimental lines (**Table 4**). These results were expected knowing that wheat controls have a balanced gluten forming proteins profile. The balance of both glutenin and gliadin needed for gluten network formation (Žilić, 2013) in wheat controls allowed the gluten network to form and result in a stable, high quality dough. The controls' doughs were more elastic and extensible and therefore more stable when compared to all IWG samples. The bulk IWG sample had the highest relative percent of unextractable HMWPP (**Table 3**) when compared to the rest of the IWG samples. The comparatively higher percent of HMWPP in bulk IWG may have contributed to its relatively better functionality.

Dough rheological properties are also dependent on the protein secondary structures. The importance of both β-sheets and β-turns for dough elasticity and gas holding capacity has been reported (Mejia et al., 2007; Bock, Connelly, & Damodaran, 2013; Bock & Damodaran, 2013; Quayson, Marti, Bonomi, Atwell, & Seetharaman, 2016). Whole wheat flour dough generally contain more β-sheets, while whole IWG flour dough contain more random coil (Marti et al., 2016). Further work to understand protein molecular interactions in IWG-based dough is needed. Enhancement of protein interactions in IWG dough systems using dough conditioners such as vital wheat gluten, ascorbic acid, and enzymes is currently underway.

3.2.2 Starch Pasting Properties

Starch pasting properties of the samples were determined using rapid visco analyzer (RVA). Indices such as starch pasting temperature, peak viscosity, hold viscosity, breakdown, final viscosity and setback were obtained (**Table 5**). These indices are indicative of the samples' starch functionality.

Differences in the pasting profiles were observed among the samples (**Figure 15**, and **Figure 23** in **Appendix G**). The pasting temperatures of hard red wheat and Arapahoe were significantly higher than that of IWG experimental line #1 but not higher than that of bulk IWG. Pasting temperatures ranged from 56.4°C to 60.3°C for IWG experimental lines. The pasting temperature, which depicted the temperature at which the first detectable viscosity was measured, is strongly related to the ability of starch granules to imbibe water. Thus, factors that affect the ability of starches to absorb water, such as ratio of amylose to amylopectin, concentration of starch, and the presence of lipids and other components do affect the pasting temperature and other pasting properties of starches (Jane et al., 1999; Sasaki, Yasui, & Matsuki, 2000). As discussed earlier, the protein, fat, and ash contents of the IWG samples were higher than those of hard red wheat and Arapahoe (**Table 2**). Interestingly, bulk IWG, which had the highest pasting temperature, also had the highest fat content. This observation is consistent with the fact that the presence of lipids results in the formation of starch-lipid complexes that protect the integrity of the starch granules resulting in higher pasting temperatures (Kaur & Singh, 2000).

Table 5. Starch pasting properties of hard red wheat and Arapahoe controls, bulk IWG, and 16 IWG experimental lines.

Sample	Pasting Temperature (°C)	Peak Viscosity (cP)	Hold Viscosity (cP)	Breakdown Value (Peak Viscosity-Hold Viscosity) (cP)	Final Viscosity (cP)	Total Setback (Final Viscosity - Hold Viscosity) (cP)
Hard Red Wheat	65.6^{b*}	2,299.3^{a}	1,334.3^{a}	965.0	2,804.0^{a}	1,469.7
Arapahoe	65.1^{b}	1,964.0^{b}	967.7^{b}	996.3	2,025.3^{b}	1,057.7
Bulk IWG	79.6^{a}	752.7^{de}	678.7^{c}	74.0	1,565.0^{c}	812.3
IWG L4-1	59.6^{cd}	496.7^{ij}	210.7^{l}	286.0	472.3^{j}	261.7
IWG L4-3	59.6^{cd}	236.0^{k}	132.7^{m}	103.3	181.0^{k}	48.3
IWG L4-29	59.2^{cd}	771.0^{cde}	367.7^{h}	403.3	870.0^{f}	502.3
IWG L4-32	59.8^{cd}	788.0^{cd}	435.3^{g}	352.7	1,009.7^{e}	574.3
IWG L4-57	57.4^{cd}	735.7^{def}	470.3^{fg}	265.3	1,010.0^{e}	539.7
IWG L4-72	58.7^{cd}	688.3^{f}	520.0^{e}	168.3	1,098.0^{e}	578.0
IWG L4-84	56.4^{d}	585.7^{g}	323.7^{hi}	262.0	676.0^{hi}	352.3
IWG L4-85	59.0^{cd}	709.7^{ef}	423.3^{g}	286.3	891.3^{f}	468.0
IWG L4-103	60.3^{c}	437.7^{j}	227.0^{kl}	210.7	431.0^{j}	204.0
IWG L4-105	59.8^{cd}	684.3^{f}	358.7^{h}	325.7	802.3^{fg}	443.7
IWG L4-139	60.1^{c}	576.3^{gh}	343.7^{h}	232.7	720.0^{ghi}	376.3
IWG L4-154	58.3^{cd}	549.7^{ghi}	274.3^{jk}	275.3	642.0^{i}	367.7
IWG L4-157	59.3^{cd}	522.7^{hi}	288.3^{ij}	234.3	637.3^{i}	349.0
IWG L4-159	58.4^{cd}	536.3^{ghi}	321.3^{hij}	215.0	746.0^{gh}	424.7
IWG L4-160	58.3^{cd}	820.7^{c}	623.0^{d}	197.7	1,404.0^{d}	781.0
IWG L4-172	58.1^{cd}	753.3^{de}	487.0^{ef}	266.3	1,060.0^{e}	573.0

* Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n=3.

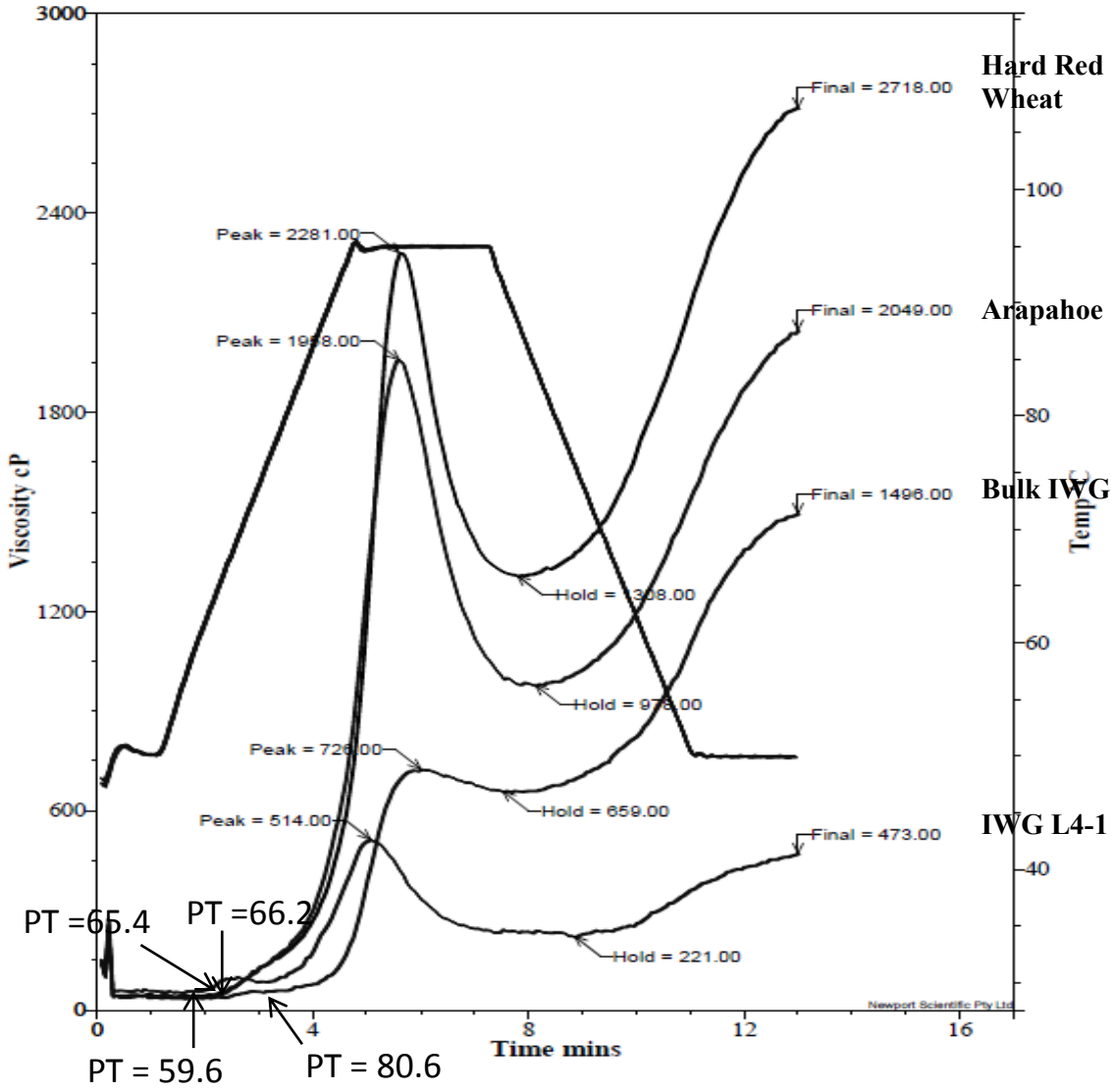


Figure 15. Starch pasting properties by rapid visco analyzer (RVA) for hard red wheat and Arapahoe controls, bulk IWG, and IWG L4-1. PT stands for pasting temperature (°C). Viscosities are expressed in centipoise (cP).

The peak, hold, and final viscosities of the wheat controls were significantly ($P \leq 0.05$) higher than those of the IWG samples (**Table 5**). Hard red wheat had the highest viscosity values, followed by Arapahoe, and IWG samples. This observation could be attributed to the presence of more protein and less starch content in the IWG samples (**Table 2**, **Figure 9**). Lim, Lee, Shin, & Lim (1999) found a similar protein effect, where protein content negatively correlated with peak viscosity but positively correlated with pasting temperature. Protein has intact disulfide bonds that can form a matrix around and/or within the starch granules, making it harder to break the starch granules. Thus, the molecular structure of protein can prevent swelling, decrease viscosity, and increase pasting temperature (Juliano, Onate, & del Mundo, 1965; Hamaker & Griffin, 1993). Moreover, it was expected that viscosity values for IWG samples would be lower than those of the wheat controls simply because IWG samples contain less starch than the wheat controls (**Figure 9**).

The breakdown value demonstrates the stability of the starch granules (Saunders et al., 2011). The breakdown value indicates the leaching of the amylose and amylopectin out of the starch granule under heat and thus, the higher the breakdown value, the less stable the starch granules are. The wheat controls' starch granules were significantly less stable than those of the IWG samples (**Table 5**). The relatively high stability of IWG starch granules compared to that of wheat, provided new information regarding IWG starch granule characteristic. Other than protein and lipid content that can affect starch pasting profiles, the amylose to amylopectin ratio also plays a crucial role in determining the final viscosity and total setback values of the sample (Jane et al., 1999). Final

viscosity and total setback values as measured by the RVA test are affected by the rate of retrogradation. Retrogradation involves intermolecular interactions between amylose and amylopectin molecules (Tang & Copeland, 2007). During retrogradation, amylose molecules reassociate forming double helices, while amylopectin molecules recrystallize (Singh et al., 2003; Alcázar-Alay & Meireles, 2015). Retrogradation is a process that takes an extended period of time to complete. While amylose can complete its retrogradation process in hours, amylopectin may take days. Higher amylose content will therefore result in products with firmer gels upon retrogradation (Thomas & Atwell, 1999; Tang & Copeland, 2007). Thus, the higher amylose content found in wheat controls (**Figure 9**) contributed to its higher final viscosity and total setback results.

Building upon previous studies, this thesis contributes to the understanding of the starch pasting properties of IWG samples and how they differ when compared to that of the wheat controls. However, starch structures and granules of samples were not analyzed, thus causation statements are restricted. Moreover, because the study was conducted using flour instead of pure starch, the understanding of starch behavior among the samples was also limited. That said, starch pasting profiles can still be used to partially explain the starch functionality of the various samples. The trends among the flour samples illustrated the samples' behavior upon heating and cooling, which are important characteristics to evaluate for commercial applications of IWG on a large scale.

3.3 Bread Baking Quality

The bread baking quality of IWG samples was compared to that of the wheat controls. Baking quality was assessed by determining bread specific volume, bread height, and bread firmness. While IWG had higher protein content than the wheat controls, IWG samples were deficient in HMWG, thus it was expected that IWG bread would be denser and have a lower specific volume than the controls.

Bread baking quality data confirmed these expectations with two exceptions (**Figure 16**, **Table 6**). The first exception was that the specific volume of hard red wheat bread was lower than expected. Hard red wheat bread had a specific volume similar to that of some of the IWG experimental lines. A factor that may have contributed to this unexpected result was the damaged starch content of the sample (**Figure 11**). Starch damage negatively impacted bread volume because it has a higher water absorption capacity, providing more competition with protein and fiber for water. The higher starch damage content also limited the development of the gluten network, again, resulting in a lower specific volume, similar to the observations reported by Barrera et al. (2007). Williams (1967) showed that starch damage content positively correlates with the hardness of the kernel. The hard red wheat starch damage content was highest among all of the samples and 2% higher than that of the other wheat control, Arapahoe (**Figure 11**), suggesting that hard red wheat had the hardest kernel among the samples.

Figure 16. Bread slices of hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Table 6. Bread quality parameters for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample	Specific Volume (mL/g)	Bread Height (cm)	Bread Firmness (g)
Hard Red Wheat	2.00^{b*}	3.81^{b}	5.71^{c}
Arapahoe	2.34^{a}	4.13^{a}	5.74^{bc}
Bulk IWG	1.58^{gh}	3.26^{d}	6.05^{a}
IWG L4-1	1.97^{bc}	3.54^{c}	6.01^{ab}
IWG L4-3	1.96^{bcd}	3.50^{cd}	6.12^{a}
IWG L4-29	1.61^{gh}	3.31^{cd}	5.88^{abc}
IWG L4-32	1.91^{bcd}	3.31^{cd}	5.98^{abc}
IWG L4-57	2.02^{b}	3.32^{cd}	5.95^{abc}
IWG L4-72	1.86^{bcde}	3.35^{cd}	5.92^{abc}
IWG L4-84	1.80^{cdef}	3.39^{cd}	5.98^{abc}
IWG L4-85	1.40^{i}	3.33^{cd}	5.95^{abc}
IWG L4-103	1.63^{fgh}	3.23^{d}	5.95^{abc}
IWG L4-105	1.72^{efgh}	3.34^{cd}	5.89^{abc}
IWG L4-139	1.87^{bcde}	3.44^{cd}	5.91^{abc}
IWG L4-154	1.67^{fgh}	3.40^{cd}	5.92^{abc}
IWG L4-157	1.55^{hi}	3.35^{cd}	5.89^{abc}
IWG L4-159	1.75^{defg}	3.45^{cd}	5.95^{abc}
IWG L4-160	1.63^{fgh}	3.31^{cd}	5.93^{abc}
IWG L4-172	1.76^{defg}	3.25^{d}	6.07^{a}

* Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n=2.

The second exception to the predicted outcomes was the specific volume of the bread baked from the bulk IWG sample. When comparing bulk IWG with the rest of the IWG experimental lines, bulk IWG had a lower bread specific volume (**Table 6**), even though it contained relatively higher percent of HMWPP (**Table 3**) and showed better rheological properties (**Table 4**). Because bulk IWG had the smallest kernel size when compared to the rest of the samples (**Table 18**, **Appendix D**), it was also one of the samples with the highest dietary fiber content (**Figure 8**). As previously mentioned, the higher dietary fiber content of bulk IWG (especially the insoluble dietary fiber), may negatively affect bread volume (Romano et al., 2011). Furthermore, bulk IWG also had the lowest amount of starch damage when compared to the rest of the samples (**Figure 11**). Even though the amount of starch damage can negatively affect bread volume, a sufficient amount is necessary to facilitate yeast fermentation during baking (Wang, Yu, Xin, Wang, & Copeland, 2017). Thus, bulk IWG seed size, fiber content, and the amount of starch damage may potentially explain the low specific volume of bulk IWG sample. However, it should be noted that the bread baking process involves many complex chemical reactions and interactions, and only some are discussed in this research project. Thus, further studies focused on the samples' molecular interactions need to be conducted to better understand their effect on bread baking quality.

Although hard red wheat had a lower than expected specific volume (similar to that of IWG samples), it had desirable rising capability unlike IWG samples. While Arapahoe had the highest bread height, it was followed by hard red wheat. As discussed previously, hard red wheat had a similar specific volume to the IWG samples. The

significantly higher height of hard red wheat bread compared to the IWG samples demonstrated its superior rising capability. Due to the gluten network of the hard red wheat dough, the bread made from hard red wheat was able to rise more than the bread made from the IWG samples. IWG samples expanded sideways rather than upwards, which may be due to the higher gliadins to glutenins ratio in IWG than in the wheat controls (**Figure 12**, **Table 3**). Since gliadins are responsible for viscosity and extensibility, while glutenins are responsible for elasticity and dough strength (Žilić, 2013), IWG dough was expected to be more viscous than elastic. This phenomenon might explain the sideways expansion of IWG bread.

In addition to specific volume and bread height, bread firmness was also measured. The bread firmness values among the samples were relatively similar, though there were a few outliers (**Table 6**). While amylose can complete its retrogradation process in hours (during baking), the retrogradation of amylopectin may take days (over storage). A higher amylose content in the sample will result in products with higher bread firmness upon baking (Thomas & Atwell, 1999; Tang & Copeland, 2007). In this research, bread firmness was only measured immediately after baking but not over storage. Since the amount of flour used to bake each sample was relatively small (25g), the effect of amylose/amylopectin content on bread firmness may not be deduced. Additionally, fiber, protein, and starch competed for water. Thus, the higher fiber content in IWG samples contributed to the firmer texture of IWG bread (**Table 6**). The influence of bread firmness was also discussed by other researchers (Abdul-Hamid & Luan, 2000; Gómez et al., 2003).

As stated previously, bread baking is affected by many factors and involves many complex mechanisms. Because of limited sample availability, bread baking in this research project was done on a small scale, using 25g of flour for each loaf. Thus, any differences in the results might have been clearer using a larger scale experiment. As supported by the data gathered from this research as well as previous research, the amylopectin and damaged starch content negatively affected bread specific volume. IWG's high gliadin and fiber content, as well as its deficiency of polymeric HMWG resulted in denser breads with low rising ability when compared to that of wheat controls. IWG's high gliadin content may explain its ability to expand but not rise. IWG's high fiber content may have also contributed to its higher bread firmness. Product applications that do not require rising properties such as pita bread, cookies, or pancakes should be explored as the next step for IWG product development.

4. Overall Conclusions, Implications, and Recommendations

Results of this work demonstrated the impact the chemical composition of IWG on its functionality and baking quality as compared to wheat. The bran to endosperm ratio had a direct impact on the grain's chemical composition. The higher bran to endosperm ratio of IWG as compared to wheat resulted in higher protein, ash, and fiber contents, but a lower starch content.

The profile of gluten forming proteins in IWG samples was markedly different than that of the wheat controls. IWG samples were deficient in HMWG as compared to the wheat controls. Wheat controls were higher in HMWPP, while the IWG samples were higher in albumins and globulins. While IWG had a higher overall protein content than wheat, the types of protein present in IWG samples did not constitute an adequate amount of the functional proteins necessary to support desirable baking qualities.

IWG samples were lower in starch content than wheat controls, but they had similar amylose to amylopectin ratios. The amylose/amylopectin molecular weight data suggested similar molecular weight distribution among the samples with one exception; Arapahoe had the highest amylopectin molecular weight. Starch content, amylose and amylopectin ratio, and starch's molecular weight distribution affected the starch pasting properties of the samples. The higher starch content in the wheat controls contributed to superior pasting properties as compared to the IWG samples. The amount of damaged starch was also higher in the wheat controls, indicating that the wheat controls had harder kernels than the IWG samples. Damaged starch absorbs more water than intact starch granules and thus had a direct impact on functionality and baking quality results.

IWG samples were higher in dietary fiber content than the wheat controls. The ratio between soluble and insoluble dietary fiber was higher for the wheat controls. The high water absorption capacity of fiber resulted in competition with protein and starch for water in IWG samples. Accordingly, higher fiber content in IWG negatively affected bread specific volume and bread height compared to wheat controls, but facilitated moisture retention over storage.

The functional properties, namely dough rheology and starch pasting properties of wheat controls were superior to those of the IWG samples. Differences in the dough rheology results were partially attributed to the wheat's glutenins and gliadins protein composition. These proteins were responsible for the wheat's desirable gluten network and thus, dough strength and viscoelasticity. Since HMWG is important for the dough's elasticity and gas holding capacity, IWG's rising capability was comparatively inferior to that of the wheat controls. The wheat dough was more stable, more resistant to breakage, and more extensible than the IWG dough. However, further research to determine protein molecular interactions and secondary structures needs to be conducted to better understand their impact on dough rheology and baking quality.

As stated above, the starch pasting properties of the wheat controls were superior to those of IWG samples. Starch pasting properties illustrated IWG's and wheat's starch behavior upon heating and cooling treatments. The amount of fat positively correlated with the starch pasting temparture due to the amylose-lipid complexes that prevent starch swelling. The data gathered suggested that the starch present in the wheat controls was superior as a viscosity builder compared to that of IWG.

Chemical composition and functional properties were used to interpret differences in the bread quality of IWG samples as compared to the wheat controls. IWG bread samples had a lower specific volume than that of Arapahoe, but a similar specific volume to that of hard red wheat. While specific volume analysis yielded mixed results, the wheat controls had a better rising capability than the IWG samples. Additionally, IWG bread samples were firmer than the wheat controls when measured immediately after baking, which is attributed to higher fiber content. Further work is needed to enhance the baking quality of IWG, possibly through the use of dough conditioners.

While the data gathered from this research provides an abundance of new information, additional studies are needed. First, the quantification of HMWG vs. albumins and globulins aggregates in the HMWPP fraction would be useful in determining the direct effects of HMWG on functionality and as such, product application. Second, analysis of starch pasting properties using isolated starch rather than flour and determination of starch granules' structures would provide a clearer picture of the samples' starch characteristics and possible applications. Third, given that the results indicated a negative effect of high fiber content on functionality and baking quality, it would be ideal to determine the effect of refinement on enhancing the functionality. Finally, given the noted characteristics of IWG, it is essential to investigate its use in other food products. Specifically, since IWG samples are deficient in HMWG, other product applications that do not require rising properties, such as pita bread, pancakes, and cookies, should be explored as potential food product applications for IWG.

Overall, this work provided valuable information regarding the chemical compostition, functionality, and baking quality of IWG. While it was clear that seed size impact composition, results showed that there are no major differences among the 16 experimental lines of IWG, and no superior line was identified. However, IWG experimental lines had different HMWG bands, suggesting that it may be possible to improve dough strength and loaf volume by breeding. The information gathered highlighted the potential of IWG for use as a food source. Finally, the data collected will assist breeders in their current screening and future breeding efforts for the development of IWG lines suitable for food applications.

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Appendices

Appendix A. Amylose/Amylopectin Ratio Chromatogram

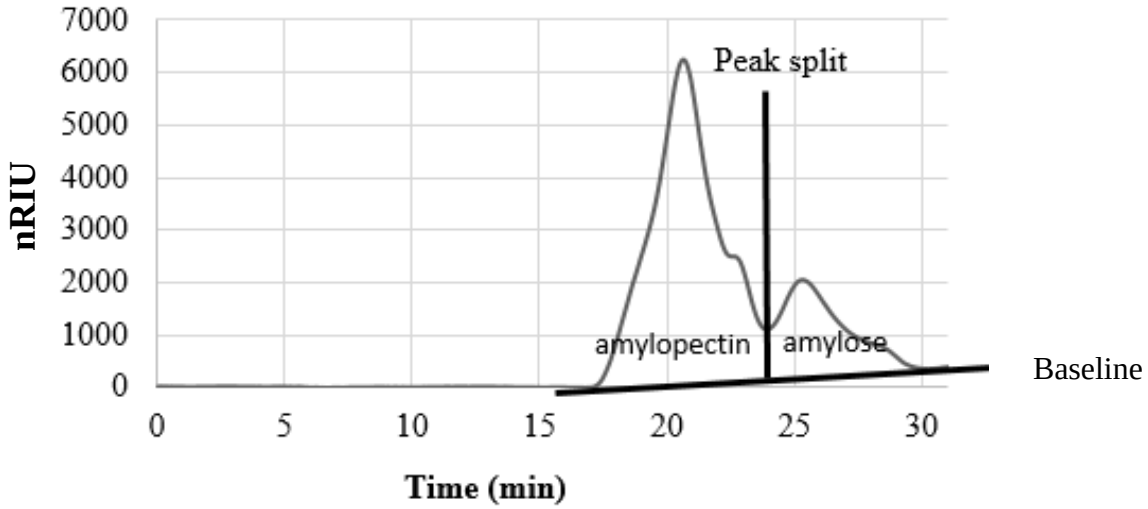

Figure 17. Hard Red Wheat (HRW) chromatogram for amylopectin (peak 1) and amylose (peak 2).

Appendix B. Soluble/Extractable and Insoluble/Unextractable Protein Example Chromatograms

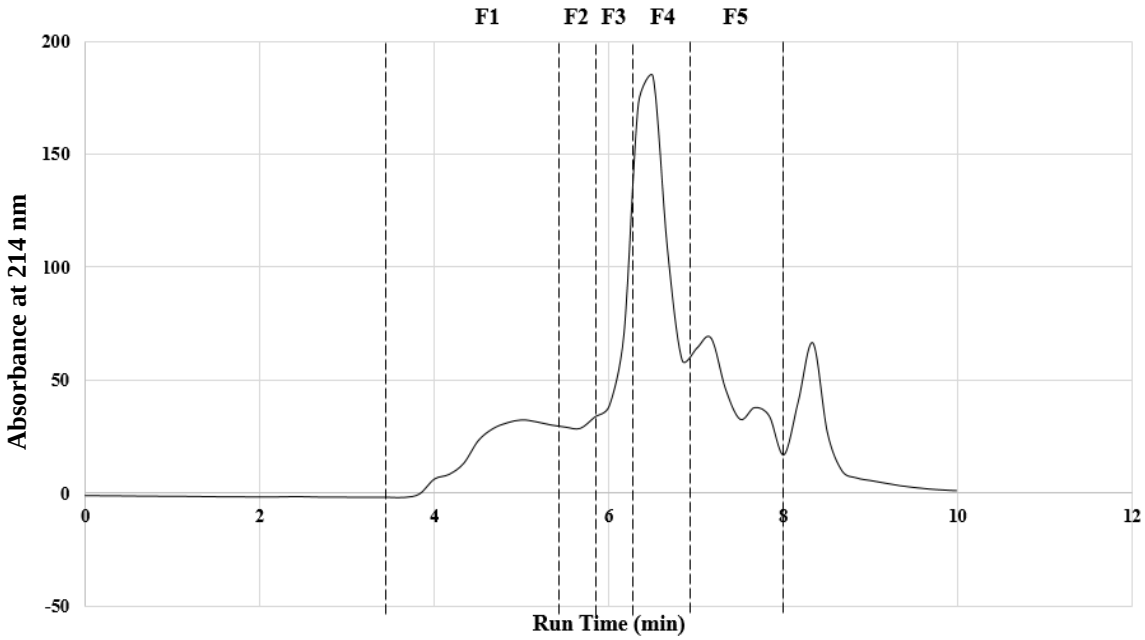

Figure 18. Hard Red Wheat (HRW) chromatogram for HMWPP (F1, extends from 3.5-5.5 min); LMWPP (F2, extends from 5.5-5.9 min); ω-gliadin (F3, extends from 5.9-6.2 min); α-, β-, and γ-gliadins (F4, extends from 6.2-6.9 min); albumins and globulins (F5, extends from 6.9-8.0 min).

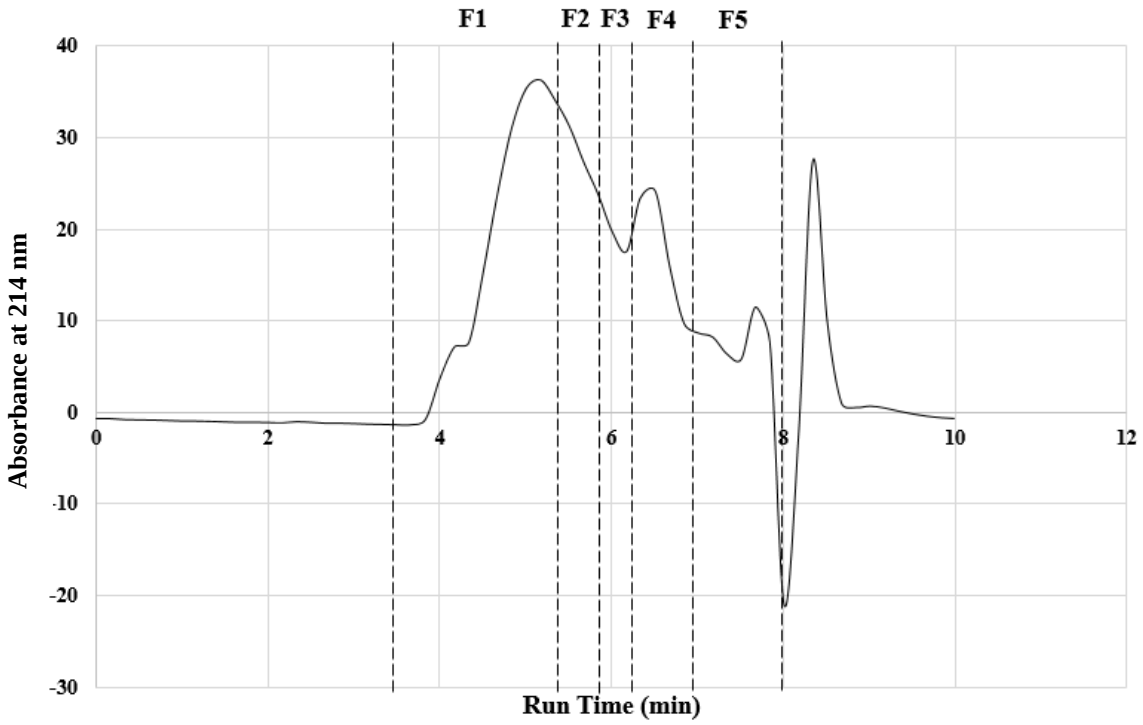

Figure 19. Hard Red Wheat (HRW) chromatogram for HMWPP (F1, extends from 3.5-5.5 min); LMWPP (F2, extends from 5.5-5.9 min); ω-gliadin (F3, extends from 5.9-6.2 min); α-, β-, and γ-gliadins (F4, extends from 6.2-6.9 min); albumins and globulins (F5, extends from 6.9-8.0 min).

Appendix C. Analysis of Variance (ANOVA) Summary Tables for Determining Significant Difference Among Samples

Table 7. Analysis of variance of proximate analysis for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Protein	Sample	18	16.750	966.880	0.000
	Error	38	0.017		
Fat	Sample	18	0.709	51.278	0.000
	Error	33	0.014		
Ash	Sample	18	0.135	22.192	0.000
	Error	38	0.006		
Moisture	Sample	18	0.583	4.004	0.000
	Error	36	0.146		

Table 8. Analysis of variance of total dietary fiber for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Total dietary fiber	Sample	18	2.647	13.200	0.000
	Error	19	0.201		

Table 9. Analysis of variance of total starch for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Total starch	Sample	18	37.532	32.638	0.000
	Error	38	1.150		

Table 10. Analysis of variance of starch damage for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Starch damage	Sample	18	12.537	327.390	0.000
	Error	37	0.038		

Table 11. Analysis of variance of molecular weight distribution of amylose and amylopectin for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Amylose MW	Sample	18	6.063E+11	12,338	0.000
	Error	38	4.914E+07		
Amylopectin MW	Sample	18	3.148E+13	57,890	0.000
	Error	38	5.439E+08		

Table 12. Analysis of variance of quantitative distribution of extractable protein fractions based of the total area for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
HMWPP	Sample	18	3.274	13.176	0.000
	Error	35	0.248		
LMWPP	Sample	18	3.259	77.339	0.000
	Error	35	0.042		
ω-gliadins	Sample	18	0.330	16.112	0.000
	Error	35	0.020		
α-, β-, and γ-gliadins	Sample	18	8.101	11.074	0.000
	Error	35	0.732		
Albumins and Globulins	Sample	18	7.260	40.513	0.000
	Error	35	0.179		

Table 13. Analysis of variance of quantitative distribution of unextractable protein fractions based of the total area for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
HMWPP	Sample	18	19.017	93.858	0.000
	Error	35	0.203		
LMWPP	Sample	18	1.652	33.845	0.000
	Error	35	0.049		
ω-gliadins	Sample	18	0.420	17.667	0.000
	Error	35	0.024		
α-, β-, and γ- gliadins	Sample	18	4.890	6.567	0.000
	Error	35	0.745		
Albumins and Globulins	Sample	18	0.876	6.718	0.000
	Error	35	0.130		

Table 14. Analysis of variance of farinograph results for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Water absorption corrected for default moisture content (14%)	Sample	18	7.686	120.310	0.000
	Error	18	0.064		
Stability	Sample	18	34,942	34.368	0.000
	Error	18	1,017		

Table 15. Analysis of variance of Kieffer results for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Resistance to extension	Sample	18	50,981	3688.7	0.000
	Error	76	14		
Extensibility	Sample	18	99.460	101.29	0.000
	Error	76	0.982		

Table 16. Analysis of variance of starch pasting profiles for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Pasting temperature	Sample	18	78.536	54.789	0.000
	Error	38	1.433		
Peak viscosity	Sample	18	748,141	1872.8	0.000
	Error	38	399		
Hold viscosity	Sample	18	243,421	955.8	0.000
	Error	38	255		
Final viscosity	Sample	18	1,108,295	1092.8	0.000
	Error	38	1,014		

Table 17. Analysis of variance of bread quality parameters for hard red wheat and Arapahoe controls, bulk IWG, and IWG experimental lines.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig
Specific volume	Sample	18	0.092	45.249	0.000
	Error	18	0.002		
Bread height	Sample	18	0.093	27.461	0.000
	Error	18	0.003		
Bread firmness	Sample	18	0.018	3.107	0.010
	Error	18	0.006		

Appendix D. Seed Size Comparison of IWG Samples

Table 18. Intermediate Wheatgrass Samples Seed Size

Samples	Seed Size (g/1000 seeds)
Bulk IWG	3.90
IWG L4-1	8.10
IWG L4-3	8.70
IWG L4-29	7.75
IWG L4-32	7.40
IWG L4-57	7.57
IWG L4-72	6.56
IWG L4-84	6.93
IWG L4-85	7.24
IWG L4-103	8.76
IWG L4-105	7.30
IWG L4-139	7.45
IWG L4-154	7.84
IWG L4-157	7.19
IWG L4-159	7.10
IWG L4-160	7.37
IWG L4-172	7.49

Appendix E. Soluble/Extractable and Insoluble/Unextractable Protein Molecular Weight Distribution

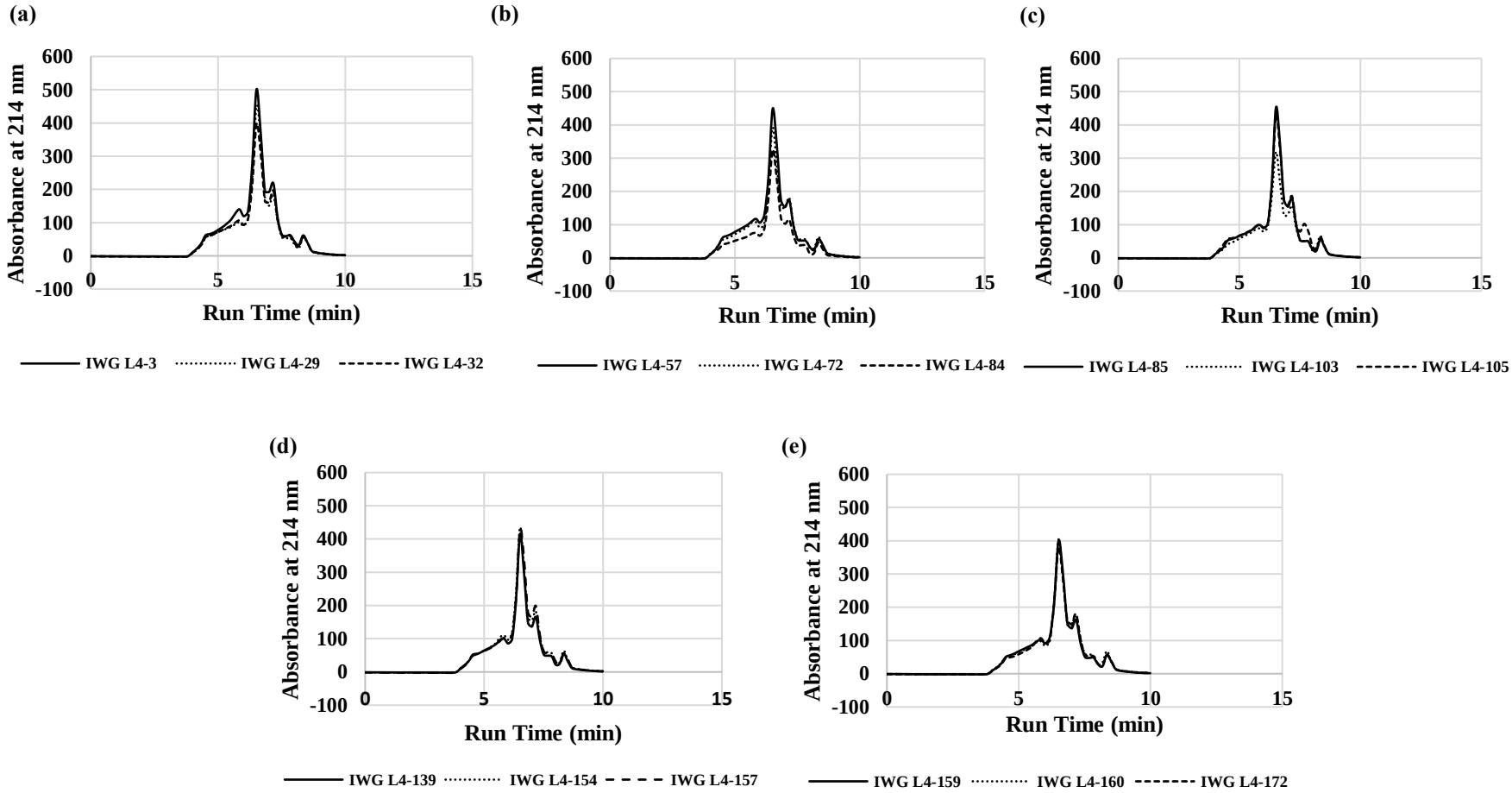

Figure 20. Soluble/extractable protein molecular weight distribution by SE-HPLC for IWG experimental lines L4- (a) 3,29, 32; (b) 57,72,84; (c) 85,103,105; (d) 139, 154, 157; and (e) 159, 160, 172.

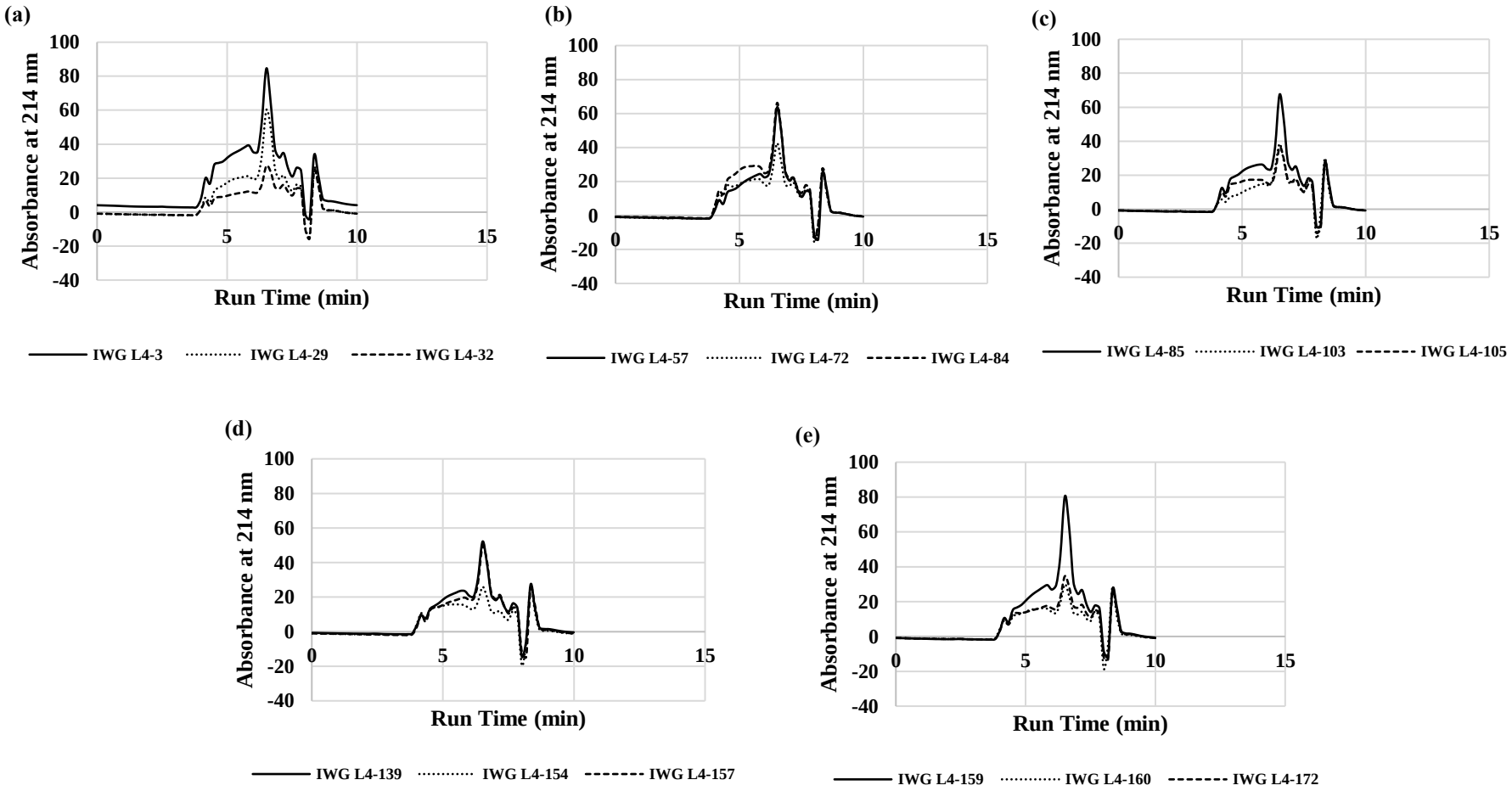

Figure 21. Insoluble/unextractable protein molecular weight distribution by SE-HPLC for IWG experimental lines L4- (a) 3,29, 32; (b) 57,72,84; (c) 85,103,105; (d) 139, 154, 157; and (e) 159, 160, 172.

Appendix F. Farinograms of IWG experimental lines

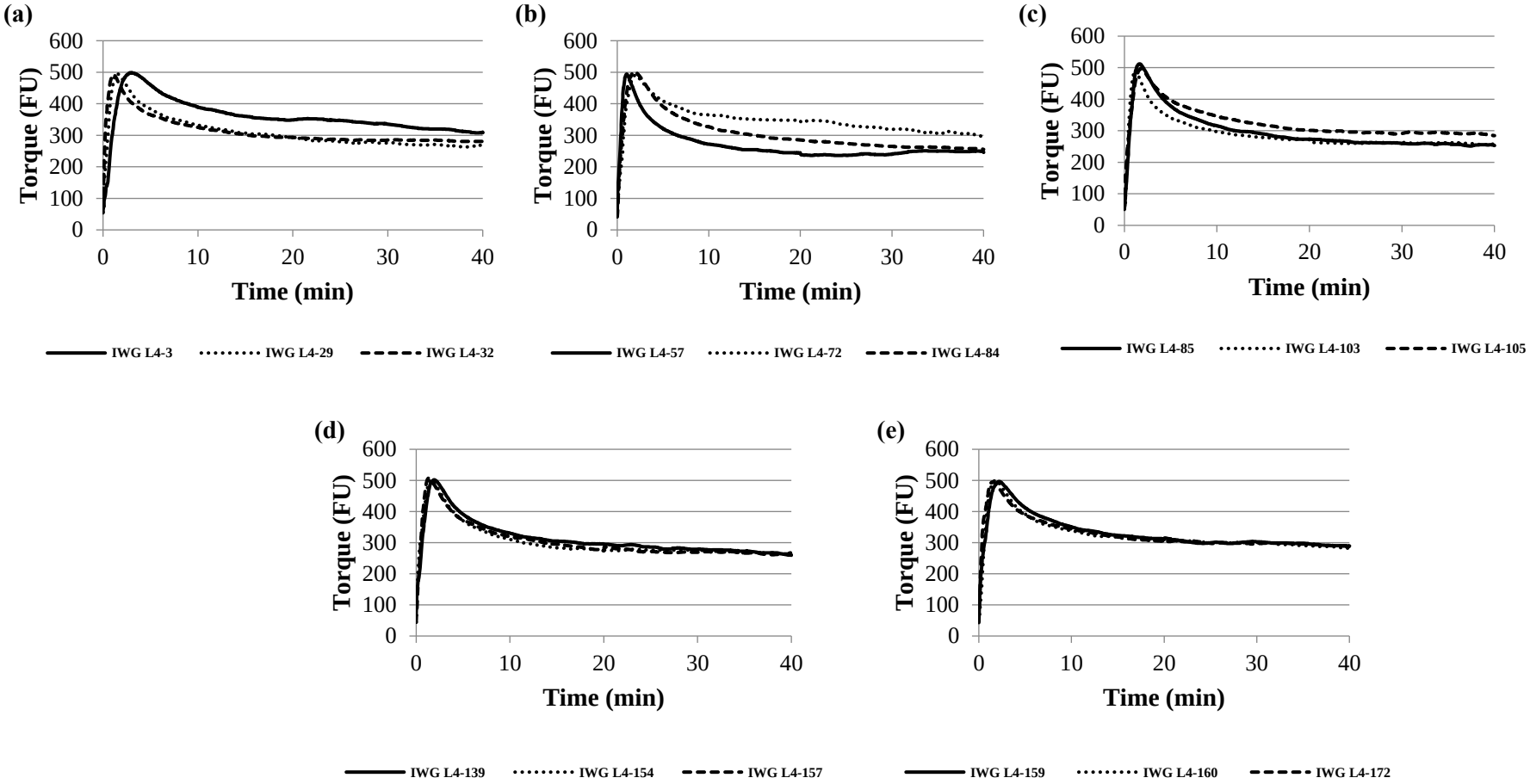

Figure 22. Farinograms of IWG experimental lines L4- (a) 3,29, 32; (b) 57,72,84; (c) 85,103,105; (d) 139, 154, 157; and (e) 159, 160, 172.

Appendix G. Starch pasting profiles of IWG experimental lines

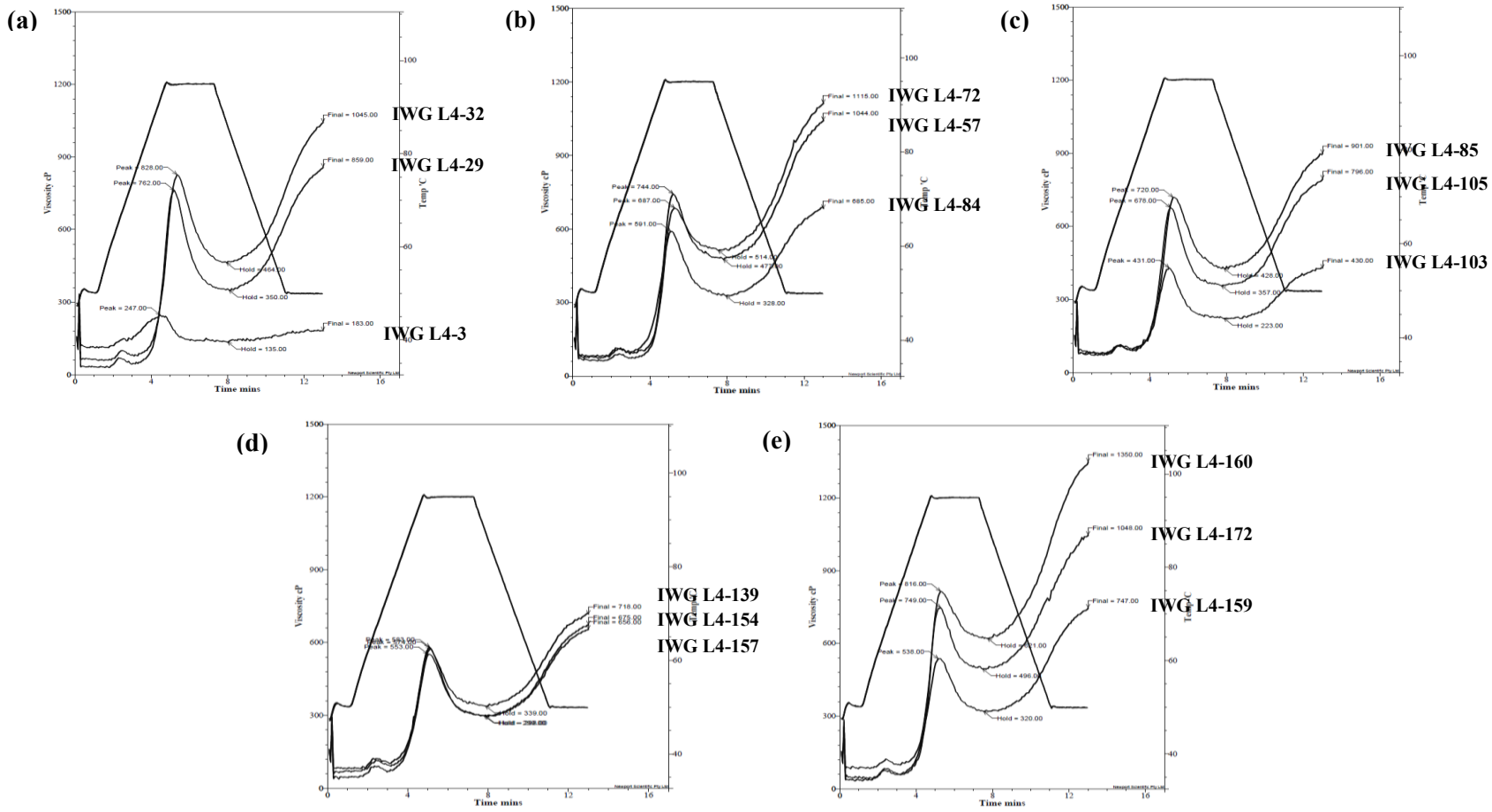

Figure 23. Starch pasting properties performed by rapid visco analyzer for IWG experimental lines L4- (a) 3,29, 32; (b) 57,72,84; (c) 85,103,105; (d) 139, 154, 157; and (e) 159, 160, 172.