Effects of Dough Conditioners on Rheology and Bread Quality of Intermediate Wheatgrass

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Abstract

Studies have shown the detrimental effect of agricultural practices on the environment. One solution to combat those problems would be to focus on alternatives that would lead to sustainable environmental benefits, like cultivating perennial crops. While annual crops are dominating current agricultural production, cultivating perennial crops would contribute to several environmental benefits like reduced nitrogen losses, and soil erosion. With expanding global food insecurity, using perennial crops for food would offer an alternate to diminishing food supply.

Among the several perennial crops screened for domestication, intermediate wheatgrass (*Thinopyrum intermedium*, IWG) has been considered a promising crop to be used as food. The aim of this study was to evaluate IWG of same genetic material, cultivated at two location in Minnesota- Rosemount (RM) and Roseau (RS) for chemical and functional characteristics of dough and breads as affected by refinement (bran removal) and the use of dough conditioners. Five dough conditioners were used- wheat protein isolate, (WPI), vital wheat gluten (VWG), ascorbic acid (AA), Powerbake (a commercial enzyme mix) (PB) and transglutaminase (TG). While IWG kernels were studied for kernel physical properties, IWG flour at three refinement levels - 0 %bran (0B), 50 %bran (50B) and 100 %bran was investigated for proximate composition and dietary fiber following respective standard methods. Dough extensibility and resistance to extension were measured with the texture analyzer equipped with Kieffer extensibility rig, and dough stickiness was measured with a texture analyzer equipped with Chen-Hoseney stickiness cell. Baked breads were evaluated for dimensions, specific volume,

crumb firmness, and crumb grain characteristics. Controls consisted of annual hard red winter wheat (W) and IWG dough without conditioners (N).

IWG kernels were thinner, with lower weight, volume and bulk density in comparison to wheat. Results from proximate composition indicated an increased fat, protein and ash content with increasing bran concentration, and a decrease in moisture and carbohydratecontents. While there was no difference between IWG and wheat at 0B for moisture and carbohydrate, for the remaining two bran concentrations, wheat had higher moisture and carbohydrate, and lower protein, fat and ash content than IWG. IWG had higher dietary fiber content than wheat at 50 and 100B refinement levels, the difference attributed to insoluble dietary fiber, as no differences was observed in soluble fiber between wheat and IWG at all bran concentrations.

At all bran concentrations, extensibility of wheat dough was higher than for the dough made with IWG from both locations. Adding dough conditioners did not improve extensibility for any samples. Some differences were noted between the two locations- 50B-N, 50B and 100B with WPI, 100B with VWG, 50B and 100B with AA, 50B and 100B with PB and 0B and 100B with TG. At 0B and 50B, resistance to extension of wheat dough was higher than for dough made with IWG from both locations, however, for 100B, IWG from RM N, with AA, and PB and IWG from RS N, with WPI, VWG, AA and PB were different. TG increased resistance to extension for IWG from RM at 0B and 50B; for IWG from RS at all refinement levels.

At all bran concentrations, stickiness of wheat dough was lower than for dough made with IWG from both locations. Adding PB and TG to 100B IWG from RM reduced

stickiness to match values of wheat, however, no such effect was seen in IWG from RS. Adding WPI and VWG reduced stickiness of 0B IWG samples from both locations, in addition, TG also reduced stickiness of 0B RM IWG dough. While no conditioners reduced stickiness of 50B IWG from RS; WPI, VWG, PB and TG reduced stickiness of 50B IWG from RM. While dough conditioners did not reduce stickiness of 100B samples from RM; WPI, VWG and TG reduced stickiness of 100B samples from RS.

Bread results indicated a negative effect of bran on dimensions, specific volume and crumb grain characteristics. While WPI, VWG, AA and PB improved or did not change the bread dimensions, TG always reduced them. The effect of dough conditioners was more pronounced for length and width than for height; indicating IWG expanded more than rose. While none of the conditioners increased the specific volume of RS IWG samples at any refinement level, PB increased the volume of 0B IWG from RM. TG decreased the specific volume of all samples.

At 0B concentration, controls and breads with WPI and VWG demonstrated collapse when in oven. A noticeable surface smoothing effect was observed for 0B samples with AA and PB. AA and PB improved the crumb grain properties with uniform air cells distribution for 0B samples. Bran negatively affected the air cells count, and adding dough conditioners did not improve the crumb grain characteristics. While there was no effect of TG on 0B samples; cell count, cell area and cell size decreased with TG addition for higher bran contents. The breads were unacceptably dense and the effect was pronounced at higher bran concentrations.

This work provides insight on ways to improve functionality and product quality of IWG breads. AA and PB produced loaf of consistent appearance with smoother surface and uniformly distributed gas cells in the crumb. WPI and VWG exhibited expansion before the dough collapsed and thus, the loafs were unable to hold gas. Adding starch or other functional ingredients to increase viscosity would help in retaining the gas, and is thus recommended. This research would facilitate future efforts towards using IWG as a standalone flour for breads, as well as help breeders for markers selections towards developing IWG bread flour.

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Introduction, Justification and Objectives

1.1 Introduction

The world population is expected to reach 9 billion by 2050, which directly correlates to the amount of food needed to feed the growing population (Melorose et al., 2015). The necessity to feed the growing population can be fulfilled by increasing production through mechanized modern agricultural practices. However, modern agricultural practices that are offering high production are also abruptly damaging the agricultural soil and the environment. Some of these problems include soil erosion, runoff from irrigated lands, greenhouse gas emissions, and water pollution. About 11% of land on earth is arable and each year, approximately 75 billion tons of fertile soils are estimated to be lost from the world agricultural system (Pimentel & Burgess, 2013). Tilling practices in agriculture are estimated to increase soil erosion by 4 - 10 times (Pimentel et al., 2012). Rainfall on such loose soil contributes to runoff, making water less available for production. These problems have increased public awareness on how the modern agricultural system is impacting the global ecosystem, and agronomists are looking for alternatives that are more sustainable like introducing perennial crops in the agricultural system (Agricultural sustainability Institute, 2017; Kantar et al., 2016). According to FAO (2016), the alarming effect of climate change on agriculture is already increasing the food insecurity and it is very important to establish alternatives that would balance yields and ecological resiliency.

Annual crops require intense energy when weeds are removed, fertilizers are applied, and resources like water and nutrients are supplied. Perennial crops, on the other hand adapt well to the harsher environmental situation and have been proven to require

less energy inputs than annual crops which can be attributed to their deeper and extended root systems with longer growing seasons (Cox et al., 2006). Having a small shift from annual to perennial crop production can result in a significantly reduced impact on environmental footprints (Marquardt et al., 2016). As the global food insecurity continues to expand with the increasing population, having a sustainable agricultural system to addresses this challenge is of utmost importance to counteract the diminishing food supply.

1.2 Justifications and Objectives

Past research on IWG has shown it to have higher protein, fiber and certain phytochemical contents than annual winter wheat (Bunzel et al., 2014). These differences in chemical composition create functional differences during product formulation. One major difference of IWG from annual winter wheat is its deficiency in high molecular weight glutenin sub-units, a component important to form gluten. This deficiency relates to a poor gluten forming ability, resulting in an altered dough rheology (Marti et al., 2015). The poor gluten forming ability hinders the formation of viscoelastic network making it challenging for some baked products with dough rising properties like breads. Refined IWG produced by removing bran has been noted as being sticky, making it difficult to handle. Different approaches have been used to improve the functionality and bread baking quality of flours with high fiber and low functioning gluten. Some approaches are adding functional wheat proteins, using additives to modify protein and fiber profiles (Basman et al., 2002; 2003). Investigating ways to improve the gluten

forming ability and handling properties of IWG at different refinement levels is important so as to be able to use IWG as a stand-alone cereal ingredient for food applications.

The main objectives of this research, therefore, were to:

1. Screen dough conditioners at different refinement levels for their ability to improve IWG dough rheology.
2. Investigate and compare the effect of selected dough conditioners on the IWG dough rheology.
3. Investigate and compare the effect of dough conditioners on the baking quality of IWG breads.

With the results of these investigations, it is hoped that food manufacturers will have tools to improve IWG functional properties that will result in its adopting into the food chain.

Chapter 2:
Literature Review

2.1 Perennial crops

The literal meaning of perennial is “present at all seasons of the year” (Merriam-Webster, 2018). Consequently, once planted, perennials can be harvested for several years. Currently, perennial crops cover only about 19% of the global land, 15% as pasture and 4% as perennial fruits, berries and nuts (Cox et al., 2006). None of the commonly cultivated and consumed cereal grains, raw materials for staple foods around the globe, are perennial (Alston et al., 2009; Kantar et al., 2016). Compared to annual crops, perennials have deeper and more extended root systems, which help them with trapping nutrients and water. Therefore, perennials hold more carbon dioxide in the soil, preserve more of the applied nitrogen, and prevent soil erosion by wind and water (Glover et al., 2010). According to Randall and Mulla (2001), nitrogen losses from annuals are around 30-50 times greater than for perennial crops. Perennials are more disease resistant and more tolerant to drought and frost than annuals (Culman et al., 2013). Therefore, increasing the cultivation of perennial crops could lead to substantial environmental benefits (Kantar et al., 2016). However, there are numerous challenges related to incorporating perennial crops into agricultural systems. One central question is whether perennials can give competitive yields to annual crops, as they devote resources to both asexual and sexual production due to their longer life spans. As a result, perennials have smaller seeds, which lowers the yield (DeHaan, 2015; Lubofsky, 2016). Another considerable problem is asynchronized ripening, where not all seeds mature at the same time. If harvesting is delayed until all seeds have matured, shattering occurs, where seeds break upon mechanical harvesting. In addition, perennials are prone to lodging, i.e. the

condition where stems bend because of being tall but weak. This makes mechanical harvesting difficult and leads to seed loss (Lubofsky, 2016).

Commonly cultivated annuals have high productivity as a result of long term breeding efforts to enhance productivity by selecting for “increased allocation of photosynthate to the seeds and decreased intraspecific competition” (Cox et al., 2006). DeHaan and colleagues (2005) proposed that seed yield in perennials can be increased by artificial selection in a managed agricultural environment.

Given these benefits and challenges, breeders have started domesticating some perennial crops by identifying those with comparatively higher and more consistent seed production than other wild varieties (Cox et al., 2006). Several agronomic traits such as large, non-shattering and harmonious maturing seeds, as well as prospects of mechanical harvesting are being evaluated. The effort is led by The Forever Green Initiative, which aims to enhance soil, water, and wildlife resources by producing crops of food value with environmental benefits (“Forever Green,” 2018). One of the perennial crops under investigation was evaluated in this thesis, and is introduced below.

2.2 Intermediate Wheatgrass

Intermediate wheatgrass (*Thinopyrum intermedium*, IWG) is a cereal grain and a distant relative of wheat. **Table 1** shows its classification.

Table 1. Botanical classification of Intermediate Wheatgrass

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae / Gramineae
Genus	*Thinopyrum*
Species	*Thinopyrum intermedium*

Source: Intermediate wheatgrass classification, USDA, 2018.

IWG is thought to have originated in Europe, Western Asia and Southern Africa (Lawrence, 1983). IWG is currently used for hay, pasture, and forage in other regions of the world, e.g. in the Great Plains regions between U.S. and Canada (Berdahl et al., 1994; Hybner & Jacobs, 2012; Lawrence, 1983). In addition, research to evaluate the use of IWG as a biofuel is ongoing (Culman et al., 2013). The effort to domesticate IWG was started in the 1980s by the Rodale Research Center, Kutztown, PA, after characterizing numerous perennials for their agronomic properties and seed quality, and then selecting

those crops with the best properties (Becker et al., 1991; 1992; Wagoner, 1990a; 1990b; Wagoner & Schauer, 1990; Wagoner, 1995). The Land Institute, Salina, KS has been developing IWG for domestication since 2002 (Cox et al., 2010; DeHaan et al., 2005). They have trademarked the name "Kernza" to indicate their line of IWG. Like other perennial crops, IWG has an extended root system (**Figure 1**). An 86% reduction of nitrogen leaching and 13% increase in carbon sequestration over annual crops was reported for IWG in the second year after planting (Culman et al., 2013).

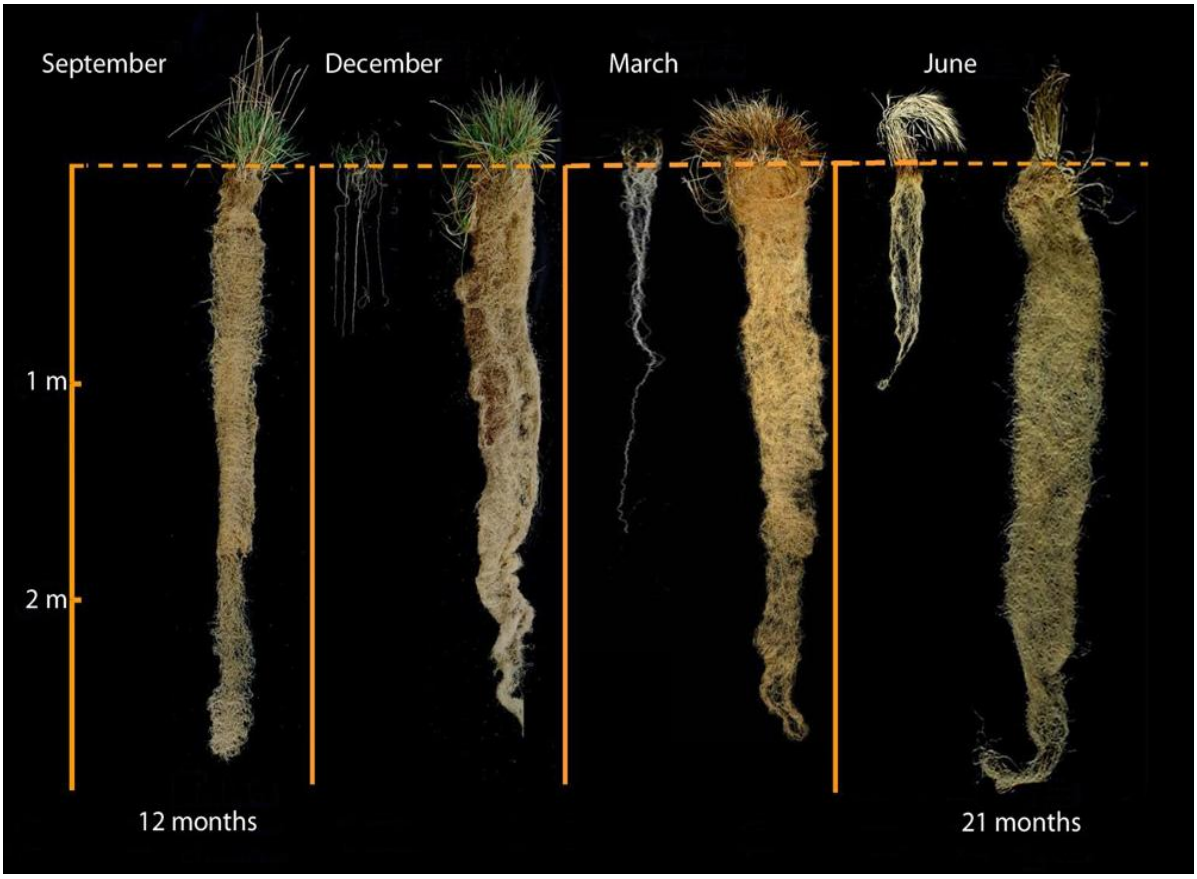

Figure 1. Comparison of root system within three months interval within a year time period between wheat (annual) and intermediate wheatgrass (perennial) (Cox et al., 2006)

IWG adapts well to regions with 30-36 cm annual rainfall and 1067- 2743 m elevation (Hybner & Jacobs, 2012). The plants grow to a height of 91-122 cm and they

have 2.5- 7.6 mm wide leaves. The leaves' color can vary from green to blue-green. Seed spikes can be 10-20 cm long and each spikelet can have about 2-6 florets (Hybner & Jacobs, 2012; Lawrence, 1983). Plants start to green up from early spring, herbage production starts late spring to early summer, while the seeds ripen by mid-August and are ready for harvest by late August (Lawrence, 1983). It produces an annual yield of 280-392 kg/ha when cultivated in dryland, and 504-617 kg/ha when cultivated in a well irrigated land (Hybner & Jacobs, 2012). However, studies on yields have shown to drop significantly after the fourth year of harvesting (Hybner & Jacobs, 2012; Lawrence, 1983).

Regardless of all the demonstrated environmental benefits, farmers will hesitate to invest in planting IWG unless there is a strong market for the crop. The following sections will discuss IWG quality traits relevant to food use.

2.3 Physical characteristics of grains

Seed size affects crop yield, and is related to milling yield, water absorption and baking quality (Morgan et al., 2000; Tsogtbayar et al., 2015). Morgan et al. (2000) demonstrated an association of kernel size with dough quality in wheat, which was also supported by Novaro et al. (2001) for kernel volume and semolina yield. Kernel size and shape characteristics have been studied for other cereals like oat, barley and rice (Ayoub et al., 2002; Fan et al., 2006; Groh et al., 2001). IWG seeds are small and the yield is lower than for wheat (DeHaan, 2015). Rahardjo (2017) reported 1000 kernel weights of several IWG lines, however, values were not compared with wheat. While annual grains have been extensively bred for desired kernel size (width and length), breeding IWG is

still in its early stages (DeHaan, 2015). Studying physical properties of IWG kernel and comparing them with wheat will facilitate genotype selection for food use.

2.4 Chemical composition of IWG and effect on functionality

Whole grain IWG flour was reported to have 46.7% starch, 20% protein and 16.9% total dietary fiber (Marti et al., 2015). The three main components of flour and their effects on the functionality during baking are discussed below.

2.4.1 Protein

The Osborne scheme classifies proteins into four types based on their solubility: albumins, soluble in water; globulins, soluble in saline; prolamins, soluble in aqueous alcohol; and glutelins, soluble in dilute acid or alkali (Osborne, 1924). In their endosperm, cereal grains contain a type of prolamin called gliadins, that can further be divided into α-, β-, γ- and ω- gliadins (Shewry and Halford, 2002). Glutenins are composed of low molecular weight glutenin subunits and high molecular weight glutenin subunits (HMW-GS). Hydrated gliadins offer viscosity and extensibility to the dough, while hydrated glutenins are cohesive and give dough strength and elasticity (Wieser, 2007). Gluten, an essential component for bread making, is a water insoluble protein network formed when gliadins and glutenins interact with water (Tuhumury et al., 2014). Gluten aggregation via covalent and non-covalent bonding gives dough its structure; non-covalent bonding includes hydrogen bonds, ionic bonds, and hydrophobic interactions, (Hoseney, 1994; Wieser et al., 2006) whereas covalent bonds include disulfide crosslinking and tyrosine crosslinking (Shewryl and Halford, 2002; Tilley et al., 2001;

Wieser, 2007). The location of cysteine is thus important for gluten functionality because of the role of disulfide bonds in forming the protein network (Grosch & Wieser, 1999; Wieser, 2003). Aggregating glutamine residues of HMW-GS through hydrogen bonding contributes to a stable gluten network (Belton et al., 1995; Wellner et al., 2005; Wrigley et al., 2006). Hydrophobic interactions and ionic bonding causes an interaction with dough biopolymers and promotes stability (Sivam et al., 2010). Belton (1999) proposed the "loop and train" model in which hydrogen bonding among HMW-GS results in loops (unbounded section) and trains (bounded sections) (**Figure 2**). High hydration would cause more loops and trains, and a balance between loops and turns provides dough elasticity.

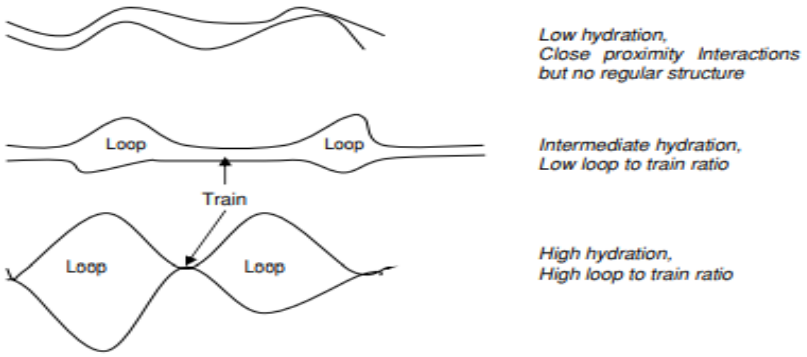

Figure 2. Loop and train model explaining the association of high molecular glutenin subunits (Belton, 1999)

These gluten-mediated viscoelastic properties are responsible for gas holding in bread (Lee & Lee, 2012). High molecular weight glutenins specifically are needed for enhancing the gas holding and elastic property in a dough (Dhaka & Khatkar, 2015; Niu et al., 2011; Wieser, 2007; Žilić, 2013).

Becker et al. (1991) found perennial grasses to have no gluten forming ability; however, results from Marti et al. (2015) showed that IWG displayed gluten aggregation properties indicating that it does possess some, albeit weak gluten forming ability. Rahardjo (2017) reported the prolamin fraction of IWG protein to include α-, β- and γ-gliadins, low molecular glutenins, as well as small amounts of HMW-GS. With the weaker gluten forming ability in IWG, strategies to improve the gluten forming ability of IWG dough are needed.

2.4.2 Starch

Starch granules are mainly composed of amylose and amylopectin, with traces of minerals and other compounds such as lipids. Starch is deposited and stored in the form of semi-crystalline insoluble granules that can only partially hydrate in cold water (Damodaran et al., 2008). When starch granules are heated in the presence of water, a process called gelatinization occurs and leads to increased viscosity. This phenomenon involves the disruption of hydrogen bonds (Alcázar-Alay & Meireles, 2015; Hoover, 2001). Starch granules swell, and starch components are leached into the solution, where they re-associate upon cooling, a phenomenon termed retrogradation (Copeland et al., 2009; Damodaran et al., 2008; Tester & Debon, 2000). Amylose undergoes retrogradation faster than amylopectin and can form gels (Ai & Jane, 2017). Starch retrogradation affects product firmness and thus the shelf-life of baked products (Copeland et al., 2009).

Starch contributes to bread quality in several ways: water absorption during dough development, gelatinization upon baking, and retrogradation upon cooling and

storage (Goesaert et al., 2005; Onyango, 2016). The viscosity built due to starch hydration and being entrapped with gluten stabilizes gas bubbles produced during yeast fermentation (Gan et al., 1995; Koehler & Wieser, 2013). In addition, starch prevents dough from collapsing after fermentation (Ahlborn et al., 2005; Hoseney and Rogers, 1990). During baking, starch gelatinization ruptures the gluten matrix that entraps the starch and leads to a porous crumb structure (Eliasson et al., 2013; Primo-Martín et al., 2007). When the product is cooled, starch molecules undergo re-association giving a porous crumb (Bloksma, 1990; Goesaert et al., 2005).

2.4.3 Dietary fiber

Dietary fibers are plant carbohydrates and associated plant substances such as lignin and waxes, that are not digested by the enzymes present in the human small intestine, but partly or completely fermented in large intestine (AACCI 2001; CODEX, 2016). Higher dietary fiber intake has been associated with a reduced risk of diabetes, cancer, ranges of cardiovascular, kidney and respiratory diseases, serum cholesterol level, and blood pressure (Krishnamurthy et al., 2012; Park et al., 2011). Since dietary fiber is primarily located in the bran and germ, whole grain flour contains higher quantities of dietary fiber than refined flour (Fardet, 2010). Dietary fiber can be soluble or insoluble in water. In cereal grains, insoluble dietary fibers (IDF) include cellulose, some arabinoxylans, resistant starch, and lignin (Dodevska et al., 2013; Gebruers et al., 2008), whereas common soluble dietary fibers (SDF) are fructans, pectin, β-glucans, and some arabinoxylans.

Arabinoxylans (AX) are the most prominent group of non-starch polysaccharides in wheat and rye and are present in both endosperm and bran (Mares & Stone, 1973a; 1973b; Ring & Selvendran, 1980). Even though the structure varies with each grain, it is primarily composed of a xylan backbone branched with arabinose branches, which in turn can be further substituted with hydroxycinnamic acids (Nandini & Salimath, 2001). Depending on the solubility, they can be classified as water extractable arabinoxylans (WEAX) and water unxtractable arabinoxylans (WUAX). WEAX form highly viscous solutions, and approximately two-thirds of the viscosity of flour may be due to WEAX (Udy, 1956) due to its ability to crosslink via ferulic acid residues (Figueroa-Espinoza, & Rouau, 1998; Izydorczyk et al., 1990; Vinkx et al., 1991). WUAX is inherently cross-linked with other cell wall components, making it insoluble while having high water holding capacity (Courtin et al., 1999; Iiyama et al., 1994). Different studies have found conflicting results on the effect of AX on dough functionality (Izydorczyk & Biliaderi, 1995). WEAX increase dough consistency by creating a viscous system and enhance the resistance to extension, but decrease the extensibility (Jelaca & Hlynca, 1971). WEAX also slow the carbondioxide diffusion rate, which has been related to improved dough gas holding capacity (Gan et al., 1995; Hoseney, 1984; Koehler & Wieser, 2013). WUAX on the other hand interfere with gluten network formation by competing with proteins for water (Courtin & Delcour, 2002). WUAX increase gas cell coalescence and reduce gas-holding capacity of the dough.

Thus, dietary fiber can absorb water, making it less available to hydrate gluten or other network forming-proteins. They also dilute the gluten matrix and this consequently

affects the gas holding capacity of the dough (Gómez et al., 2003; Wang et al., 2002). Fiber reduces dough extensibility and resistance to extension (Schmiele et al., 2012). Some SDF alter dough viscosity of upon hydration (Daou & Zhang, 2014). This can be a desirable property for baking because the viscosity helps in stabilizing gas bubbles and thus improves gas holding capacity (Gan et al., 1995; Koehler & Wieser, 2013). However, excessively viscous dough impairs gluten formation, which in turn negatively affects bread volume (Izydorczyk & Biliaderi, 1995). The effects of different fibers on dough rheology were studied by Wang et al. (2002) who concluded that samples with higher IDF content had higher water absorption than samples with high SDF content. Romano et al. (2011) reported that bread made from samples with higher insoluble dietary fiber content had lower loaf volume in comparison to samples with higher soluble dietary fiber content.

Impact of flour constituents on the viscoelastic property of flour is well known. The higher fiber (Marti et al., 2015), arabinoxylans characteristics (Schendel et al., 2015), lower starch (Rahardjo, 2017), lower protein aggregation capacity (Marti et al., 2015), difference in protein profile (Marti et al., 2016) and lower HMW-GS content (Rahardjo, 2017) in IWG than wheat, all affect its viscoelastic properties. It is thus worth investigating if and how processing modifications can alter IWG's functionality in a food product that requires dough rising properties.

2.5 Bread ingredients and functionality

Bread making dates back to the start of civilization (Hoffman et al., 1941). Bread products range from flat bread of the Middle East to steamed bread of the Far East to

baguette and pan bread of North America and Europe (Cauvain, 2007). Regardless of the broad range of breads, the common processing stages are dough development, leavening and baking. Basic ingredients are flour, yeast, salt, and water; often other functional ingredients such as sugar and shortening are added. These ingredients are mixed to form a dough, which is then allowed to rise by fermentation, referred to as leavening. Finally, the dough is shaped and baked. While each ingredient affects bread quality, the effect of flour is considered to be of prime importance (DiMuzio, 2009). Ingredients from recipes used in this thesis are discussed below.

2.5.1 Flour

Flour is the structure builder for bread, and wheat is the most commonly used flour for making bread. In wheat, gliadins contribute to dough viscosity and extensibility (Khatkar et al., 1995) while glutenins are tough and impart strength and elasticity to the dough (Belton, 1999). Together, they form the gluten network that helps retain gas. When in the oven, the heat causes the gluten to lose water, which can be taken up by the starch during gelatinization.

2.5.2 Yeast

Yeast ferments certain sugars. Those sugars can be endogenous sugars present in the dough formed by amylase activity on starch, or can be added sugars in the formulation (Ali et al., 2012). Yeast produces zymase, which catalyzes the conversion of sugar to alcohol and carbon dioxide (DiMuzio, 2009). Fermentation also produces byproducts like organic acids, flavor and aroma precursors, and the reducing agent

glutathione (Verheyen & Jekle, 2016), a tripeptide composed of glutamic acid, cysteine and glycine (Damodaran et al., 2008). The sulfhydryl group in cysteine's side chain can terminate gluten polymerization by reducing the disulfide bonds between the proteins (Bloksma, 1990). The acids generated by yeast shift the dough pH closer to the isoelectric point of gluten. Thus, repulsive forces among the proteins are reduced, making the dough more elastic and less sticky (Charley & Weaver, 1998). While carbon dioxide causes the dough to rise, endogenous glutathione dehydrogenase from flour catalyzes the conversion of glutathione (G-SH), to its corresponding dimer (GSSG), which in turn strengthens the dough because glutathione is no longer available for disulfide interchange with gluten (Aamodt et al., 2003). The effect of glutathione, alcohol and the produced gases has a conditioning effect on the gluten matrix (Ali et al., 2012; Grosch & Wieser, 1999). Reduced glutathione has been reported to increase bread volume (Grosch & Wieser, 1999).

2.5.3 Sugar

Sugar, endogenous or added, is a source of fermentable carbohydrates to start the yeast activity during fermentation. Residual sugars not fermented by yeast contribute to sweetness and promote the development of crust color through caramelization and the Maillard reaction (Cauvain & Young, 2009). However, due to hygroscopicity, at levels higher than 12% of flour weight, sugars decrease fermentation rates because of absorbing more water and making the water less available for yeast (DiMuzio, 2009). Excess sugar reduces the water activity of the dough, and suppresses the yeast activity due to osmotic pressure (Jenson, 1998). It causes dough "slackening" in which the dough loses its

strength because of the interference to gluten bonds. This destabilizes the dough, which results in decreased loaf volume (DiMuzio, 2009). AACCI recommends 6% of sugar based on flour weight for optimum results (AACCI Approved methods, 2010).

2.5.4 Salt

Salt is added to dough at 1-2% concentration of flour weight (DiMuzio, 2009). Salt performs three major functions in the dough system:

1. It contributes to flavor and enhances the palatability of bread.
2. Salt also has an effect of strengthening the gluten network by reducing the electrostatic repulsion between proteins (Butow et al., 2002).
3. Salt prevents proteases from depolymerizing proteins, which would weaken the gluten network (Charley & Weaver, 1998).

Danno & Hoseney (1982) reported overmixed, and thus inelastic dough was able to regain its strength upon addition of salts. Finally, it slows down the rate of fermentation by yeast, leading to lower amounts of carbon dioxide production, which in turn prevents the dough from rising too fast (Miller & Hoseney, 2008; Tuhumury et al., 2014). Decrease in fermentation rate is due to increased osmotic pressure, and action of ions on yeasts' cell membrane. With unsalted dough, accelerated fermentation would lead to excessive gas production, producing sour dough and baked products with poor texture (Matz, 1991). Sodium chloride is the most common salt used in baking, however, potassium chloride in conjunction with sodium chloride has been used in reduced sodium

products, as potassium chloride at higher levels leaves a metallic and bitter off flavor (Matz, 1991; Salovaara, 1982).

2.5.5 Shortening

Fats with specific melting properties find use in baking applications due to their ability to shorten gluten strands during mixing, hence the name shortening. They expand the gluten network, and thus stabilize gas cells, which in turn leads to improved loaf volume (Pareyt et al., 2011; Watanabe et al., 2003). At optimum levels, shortenings lead to a tender crust and softer crumb, larger loaf volumes and improve shelf life (Pareyt et al., 2011). Shortening also acts as a lubricant and reduces dough stickiness, which improves dough machinability (Ghotra et al., 2002). AACCI suggests using ~ 3% of shortening based on the flour weight for optimum results (AACCI Approved methods, 2010). Excessive shortening will shorten the gluten strands excessively, leading to a weaker gluten network (DiMuzio, 2009).

2.5.6 Water

Water hydrates the gluten forming proteins required to entrap gas produced by the yeast activity, controls dough consistency, and enables starch gelatinization (DiMuzio, 2009). α-amylase requires water to hydrolyze starch to sugar. Yeast undergoes fermentation only after it is hydrated and activated enough, which requires water. The role of water in forming a dough is very crucial. Excessive water increases the mixing time for the dough to reach optimum consistency and lowers dough viscosity. On the other hand, reduced amount of water affects the swelling of dough components, as they

cannot hydrate fully (Stear, 1990). Excess water decreases the viscosity of the dough, turning it soft, indicating the importance of right amount of water needed to develop an optimum dough. While in the past bakers stretched the dough slowly, checking if it would break or not, to determine optimum dough development (DiMuzio, 2009), at present, a Farinograph can determine amounts of water required to form a dough at its optimum water absorption (AACCI Approved Methods, 2010). Factors that can affect the amount of water needed to develop optimum dough include amount and type of flour components, particle size of the components, and starch damage (Cauvain & Young, 2008; Stear, 1990). High starch damage increases the water absorption of dough (Goesaert et al., 2005). Water absorption relates the rehydration, swelling and solubilization that takes place during mixing, which has a direct effect dough functionality and baking performances (Pilosof et al., 1985).

2.5.7 Other Ingredients

When using flour with poor gluten forming abilities, or to improve the process ability of doughs especially in mechanized systems, bakers may use additives (Basman et al., 2003). Some commonly used additives include proteins, enzymes, oxidizing agents and reducing agents. The following paragraphs will provide a description of dough conditioners used in the course of this thesis.

2.5.7.1 Proteins

Proteins are added in dough for two reasons- to increase the nutritive content of the product or to improve the functionality of flour (Cauvain, 2017). If the concern is

nutritive value, different proteins can be used, however for functionality, wheat protein isolates or vital wheat gluten are commonly used, and the roles are these proteins are discussed in the following section.

2.5.7.1.1 Wheat Protein Isolate (WPI)

WPI is a product derived from wheat gluten. It can be produced by acidic deamidation that converts glutamine and asparagine to glutamic acid and aspartic acid, respectively, and then purified (Batey & Grass, 1983; Wu et al., 1976). Alternatively, gluten proteins are solubilized in acid or alkali, then separated and purified. WPI differs from VWG in the fact that non-protein constituents in VWG are further removed through modifications using acid, enzymes, or reducing agents. While VWG has around 75-80% total protein, WPI can have relatively higher protein content than VWG, ranging around 90-95% (US 2007/0264414A1, 2007).

Deamidation increases solubility and flexibility of proteins due to an increase in the negative charge (Kato et al., 1987). Thus, WPI has better water holding capacity and solubility than VWG (US 2007/0014914 A1, 2007). Treating VWG with sulfuric acid and phosphoric acid results in products that can absorb water 200 times their own weight (US 2005/0287267 A1, 2005). WPI is commonly used as a functional ingredient in baked products, snack bars, high protein foods, etc. (Ahmed et al., 2008). Adding WPI to flour has been demonstrated to increase dough extensibility, decrease dough mixing time, increase loaf volume, and improve bread crumb texture and structure (Ahmedna et al., 1999; MGP, 2018; US 2007/0264414A1, 2007; US 2007/0014914 A1, 2007).

2.5.7.1.2 Vital Wheat Gluten (VWG)

VWG is a commonly used ingredient by industries and bakers to improve dough rheology and bread making quality of weaker flours (Esteller et al., 2005; Marchetti et al., 2012; Weegels et al., 1994). VWG is dried insoluble gluten proteins extracted from wheat flour by a thorough washing step to remove starch and other soluble components (van der Borght et al., 2005). It is characterized by its viscoelasticity upon hydration (CODEX, 2001). In contrast, non-vital/ devitalized gluten does not demonstrate viscoelasticity upon hydration, the cause being denaturation (Tedrus et al., 2001). FDA classifies VWG as Generally Recognized as Safe under CFR184.1322 to be used as a dough strengthener, formulation aid, nutrient supplement, processing aid, stabilizer and thickener, surface finishing agent, and texturizing agent at levels not to exceed current good manufacturing practice (FDA, 2018). VWG improves dough strength and mixing tolerance (Ortolan & Steel, 2017). VWG has been shown to improve bread loaf volume and crumb texture (Borla et al., 2004). Results from Codina et al. (2008) show successive increase in resistance and extensibility parameters for alveograph between 1% - 5% VWG. VWG is also used in frozen dough formulation as prolonged freezing impairs gluten network stability (Giannou et al., 2016; Ribotta et al., 2001). In a study conducted by Giannou et al. (2016), addition of VWG above 2% improved the loaf volume, color and texture of bread made from frozen dough. They also reported VWG to be beneficial in protecting the frozen dough from damage, which has been associated to the increase in freezing point of the dough upon VWG addition. Results also indicated an improved product being obtained upon thawing and baking.

2.5.7.2 Reducing agents

Reducing agents weaken gluten bonds, reducing the molecular weight of glutenin aggregates, and thus the dough becomes soft, pliable and extensible (Angioloni & Dalla Rosa, 2007).They reduce dough development time (Henika & Rodgers, 1965). Reducing agents are used to reduce the strength of strong flours (Elkhalifa & El-Tinay, 2002) as they weaken the gluten bond, thus and make the dough more extensible. Commonly used reducing agents are L-Cysteine (Pečivová et al., 2010) and autolyzed yeast extracts (EURASYP, 2013). L-Cysteine weakens the gluten network in the dough by reducing the disulfide bonds between proteins (Bloksma, 1990). Autolyzed yeast is a source of glutathione (Foster, 2011), which hinders the gluten bonds by forming disulfide linkages with proteins, consequently weakening the gluten network (Koehler, 2003), as discussed in section **2.5.2**.

2.5.7.3 Oxidizing agents

The main role of oxidizing agents is the oxidation of sulfhydryl groups to disulfide groups, as a consequence, thiols like glutathione are not able to hinder protein cross linking (Horvat et al., 2009). Thus, oxidizing agents strengthen the dough, increase gas retention, enhance volume and crumb grain characteristics of breads (Yamada & Preston, 1994). Commonly used oxidizers are bromates, iodates, calcium peroxide, ascorbic acid and azodicarbonamide (van Oort, 2010). Potassium bromate, is the most commonly used oxidizing agent, however, due to concerns about a potential carcinogenic effect, has been in a debate to be used in many countries (van Oort, 2010). Using potassium bromate in the EU is illegal (EU, 2018), however, FDA allows 50 ppm potassium bromate in flours

(21CFR137.155) and 75 ppm for manufacturing bread (21CFR136.110) based on flour (FDA 2018). FDA allows 45 ppm azodicarbonamide in flours and as a dough conditioner for breads (21CFR172.806; FDA 2018), but is not permitted in the EU (EU, 2018).

2.5.7.3.1 Ascorbic Acid (AA)

Flour contains ascorbic acid oxidase, which oxidizes ascorbic acid to dehydro-ascorbic acid (DHA). The DHA thus produced catalyzes the oxidation of glutathione to its disulfide (Koehler, 2003). Gluten proteins polymerize via disulfide linkages, which may be intercepted by glutathione, i.e. the gluten network would be terminated. If glutathione is converted to the corresponding dimer, it is not available for disulfide interchange with gluten proteins; thus stronger gluten remains (Aamodt et al., 2003). AA also promotes the tyrosine cross-linking of glutenins (Tilley et al., 2001). AA has been reported to increase bread loaf volume, improve gas retention and increase crumb cell area (Elkassabany et al., 1980; Elkassabany & Hoseney, 1980; Horvat et al., 2007). However, high levels of AA decrease dough extensibility, forming cracks and loose structures while proofing (Hrušková & Novotná, 2003).

2.5.7.4 Enzymes

Amounts of endogenous enzymes in flour vary widely due to differences in cultivars, weather, growing location and other factors. Different amounts of enzymes have varied effects of dough properties eg. too high or too low α-amylase in wheat flour makes it unfit for bread making (Hamer, 1995). Thus, the need to correct for the effect of

endogenous enzymes in flour led to using different enzymes for bread making (Hamer, 1995). Each enzyme possesses substrate selectivity and differs in mode of action (Damodaran et al., 2008). Several enzymes have been shown to improve dough functionality and baking quality (Stear, 1990). Proteases, transglutaminase, lipoxygenase, amylases glucoamylase, glucooxidase, hemicellulases, and cellulases are commonly used enzymes in bread making (van Oort, 2010).

2.5.7.4.1 Transglutaminase (TG)

TG is a transferase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) that catalyzes polymerization and covalent crosslinking of protein through three different mechanisms:

1. Reaction between acyl donors (peptide bound glutamine residues) and acyl acceptors (primary amines)
2. Crosslinking reaction between glutamine and lysine residues of proteins
3. Deamidation of glutamine residues

(Kuraishi et al., 2001; Larré et al., 2000; Motoki & Seguro, 1998). TG can be used in a wide variety of foods like dairy (gel strength in yogurt), fish (setting surimi, a ground fish product), soybean (maintaining texture of retorted tofu), wheat (increasing strength of low grade flours), meat (binding muscles to restructure in to larger pieces) etc. (Damodaran et al., 2008; Motoki & Seguro, 1998). Microbial TG is stable over a wide range of pH and temperatures, from - 10 to 50°C but is deactivated above 70°C. TG has been reported to improve dough elasticity and strength, and the effect is comparable to

oxidizing agents (Pongjaruvat et al., 2014; Tseng & Lai, 2002). Sakamoto et al. (1996) reported the improvement of the physical properties of Chinese noodles by using microbial TG. Gerrard et al. (2000) discussed an increase in volume of yeasted croissant with TG. Basman et al. (2002) concluded the beneficial effect of TG in bread making in terms of producing stronger dough, and increased bread volume.

2.5.7.4.2 Xylanase

Xylanase (EC 3.2.1.8, β-1,4-D-xylan xylohydrolase) hydrolyzes linear β-1,4-linked polymers of xylose (Damodaran et al., 2008). Xylanase cleaves the xylan backbone of WUAX and converts them to WEAX (Laurikainen et al., 1998; Rouau et al., 1994; Wang, 2003). This mechanism releases water to the starch and gluten that increases the viscosity of the dough system, which in turn leads to an increased increasing dough elasticity and strength. If xylanase is used at higher levels or if it acts on WEAX, it results in sticky dough (Damodaran et al., 2008). Courtin et al. (2005) reported syruping effect in refrigerated dough containing xylanase, making the dough sticky and slacky, which was in conjunction with the results reported by De Schryver et al. (2008) where microbial xylanase formed visible syrup in dough. Xylanases, when used at higher level, cause excessive breakdown of AX, reducing the water holding capacity of the dough, which causes the water to migrate to the surface of dough and is seen as syrup (Courtin et al., 2005). Thus, amounts used need to be optimum.

Xylanase can be sourced from bacteria- *Bacillus*, *Erwinia*, and *Streptomyces* spp. or from fungi- *Aspergillus* and *Trichoderma* spp. (Selinheimo et al., 2006). Bacterial and fungal xylanases differ in their optimal pH range, 6.0-6.5 and 3.5-6.0 respectively, but for

both the optimum temperature range is 40-60° C (Damodaran et al., 2008). Bacterial and fungal xylanases behave differently in baking applications. Bacterial xylanases cleave xylan backbones in unsubstituted regions of AX, whereas fungal xylanases may cleave branched regions as well (Biely et al., 1997; Courtin & Delcour, 2002). The products of fungal xylanases are thus smaller than those generated by bacterial xylanases. By degrading WEAX, fungal xylanases allow formation of larger gluten aggregrates, than bacterial xylanase (Frederix et al., 2003).

Xylanase incorporation into recipes have been shown to improve bread loaf volume, shape and texture (Courtin & Delcour, 2002; Hilhorst et al., 1999; Martínez-Anaya & Jiménez, 1997; 1998; Rouau et al., 1994). In a study by Döring et al. (2017) on rye dough, the use of xylanase improved the protein network by 38% and bread loaf volume by 11% in comparison to control rye breads. Shah et al. (2006) reported 56% increase in specific volume of whole wheat breads upon using xylanase. A similar effect of increase in bread loaf volume by xylanase was reported by Jaekel et al. (2012) for both refined and whole wheat flour. Ktenioudaki et al. (2015) studied the effect of xylanase on breads made using brewer's spent grain, where they reported an improved texture, specific volume and delayed staling for sourdough and non-sourdough breads, the reason being the effect of enzyme in solubilizing non starch polysaccharides. Grossman et al. (2016) reported a positive effect of xylanase on rye bread, relating the effect of xylanase to an increased viscosity caused by increase in WEAX, as WEAX would stabilize liquid films surrounding the gas cells by creating a foam, as interpreted by Courtin & Delcour (2002).

2.5.7.4.3 α-amylase

Amylases catalyze starch hydrolysis, converting amylose and amylopectin into low molecular weight dextrins (Damodaran et al., 2008). Initially, amylases were classified as α-amylase, β-amylase and glucoamylase. However, recent findings have expanded the concept of amylases based on the effect the enzymes have on starches (Taniguchi & Honda, 2009). α- amylase (EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase) is an α→α-retaining endo-enzyme which rapidly reduces viscosity of starch suspensions by producing fragments of low molecular weight. In contrast, β-amylase (1,4-α-D-glucan maltohydrolase, EC 3.2.1.2) is an α→β-inverting exo-enzyme that produces maltose from non-reducing ends of amylose, which only marginally reduces the molecular weight of the remaining starch molecule (Taniguchi & Honda, 2009). Glucoamylase (1, 4-α-D-glucan glucanohydrolase, EC 3.2.1.3), also referred to as amyloglucosidase, is an α→β-inverting enzyme that produces glucose from non-reducing ends of linear starch strands (Damodaran et al., 2008).

Bacterial and fungal amylases dominate the industry because of the ease of genetic manipulation, and economical bulk production (de Souza & e Magalhães, 2010). α- amylase obtained from bacterial sources are mainly made with *Bacillus* spp. and are stable at 80-110° C and pH 5-7. Fungal α-amylases are produced from *Aspergillus* spp. and have an optimum activity range of 50-70° C for temperature and 4-5 for pH.

Adding α-amylase to bread dough offers more sugar to the yeast, which in turn enhances the fermentation rate and reduces viscosity (Gupta et al., 2003). This eventually leads to an improved product volume and texture. The enzyme also prevents the

interaction of protein and starch during storage so staling is delayed (Gupta et al., 2003). α- amylases that are more heat resistant than the endogenous α-amylase offer moistness and softness to the bread crumb, enhancing the shelf life of the bread (van der Maarel et al., 2002). This is attributed to the dextrins produced by α-amylase that interfere with amylopectin retrogradation (Martínez-Anaya et al., 1999). Carroll et al. (1985) discussed the anti-staling method in bread as an effect of α-amylases, the cause being accumulation of branched maltodextrins. Arora (2003) reported an extension in shelf life of tortillas from 12-28 days due to using a bacterial α-amylase. At higher levels of certain α-amylases, "keyholing", a phenomenon of caving on the sides of crusts occurs; along with producing a sticky crumb making slicing problematic (Cauvain & Young, 2009). Olesen (1991) reported the formation of gummy and sticky dough upon overdosing a bread formula with α-amylases. α-amylases have been reported to increase bread loaf volume and improve crumb structure (Martínez-Anaya et al., 1999).

2.6 Bread making

The bread making process involves mixing, fermentation/proofing, punching, shaping, proofing, and baking (AACCI Approved methods, 2010). The first step of the process is mixing of solid and liquid ingredients so that they are uniformly distributed to form a homogenous mass of dough. The dough mixing process consists of two stages. In the first stage, starch, protein and fiber from flour absorb water. Glutenins and gliadins hydrate to form interlaced/tangled chains (DiMuzio, 2009). In the second stage, continuous mixing and turning of dough forms a viscoelastic dough through inter- and intra-molecular disulfide linkages among gluten forming proteins (Stear, 1990). Gliadins

and glutenins bond through two different mechanisms (Charley & Weaver, 1998). Gliadins interactions involve hydrogen bonds and van der Waals force, making the dough cohesive. Glutenins, on the other hand, form elongated fibrils that impart elasticity to the dough (Charley & Weaver, 1998; Stauffer, 1998). If dough is over-mixed, gluten is disrupted and the dough becomes too extensible, which will negatively affect the ability of the dough to hold gas (DiMuzio, 2009). Insufficiently mixed dough has an uneven ingredient distribution, which results in lumpy and incompletely developed gluten (Pyler, 1988).

The second step in the bread making is fermentation in which the sugars are broken down by yeast into alcohol and carbon dioxide. To ensure fermentation, dough should be proofed at a temperature and humidity that supports the optimum growth of yeast (Charley & Weaver, 1998). Through the action of α-amylase and β-amylase, endogenous enzymes in flour, starch is converted to sugars fermentable by yeast and converted to carbon dioxide, which gets entrapped in the gluten network. Proteolytic enzymes hydrolyze proteins to lower molecular weight peptides. These peptides have a similar effect to reducing agents by conditioning the stronger gluten, which in turns softens the dough and enhances extensibility (Pyler, 1988; Stear, 1990). The gluten turns springy and elastic during fermentation as result of action of proteolytic enzymes from the flour, glutathione, alcohol produced during fermentation, and lowered pH of the dough (Pyler, 1988). Gluten maintains the extensible and elastic property and develops resistance against rupturing. Gas produced during fermentation causes the dough to rise as the gas molecules get trapped in the gluten (Pyler, 1988).

While the dough is proofing, it is punched several times. Punching helps release gas formed by yeast cells. This action also breaks large air holes formed in the dough which would contribute to uniform air cells in the crumbs upon baking. Punching followed by folding helps to equilibrate the temperature throughout the dough which supports uniform fermentation. Another effect of punching is increased gas holding capacity of gluten (Pyler, 1988). This is because the rate of gas production is not constant during fermentation. The production increases abruptly and later decreases. The dough volume increases remarkably during the first phase of gas production when the rate is at its maximum and the rate of volume increase of dough declines later as the rate of gas production also decreases. This difference in the rate of volume increase will cause the dough to lose a significant amount of carbon dioxide which in turn affects bread volume and crumb structure. If there is subsequent punching and folding, the gas holding capacity of gluten is retained and the rate of dough expansion accelerates again (Pyler, 1988).

Shaping involves rounding, sheeting and rolling to form a shape that will be panned. The purpose of shaping is to give a shape to the dough as the dough will not be manipulated after this step by any means before it comes out of the oven. Shaping can alter the extensibility of the dough if too much force is applied during shaping. So, the dough should be shaped in such a way that the gluten is not too tight and can still expand in the proofing step that follows shaping (DiMuzio, 2009).

After shaping, the dough is proofed in the pan to allow for expansion and fermentation. The dough is baked after the final proofing during which the trapped water

converts to steam allowing further expansion of the dough before it cooks to bread. The yeast cells die only at 59° C, so until the center of the dough reaches that temperature, yeast cells are still active and continue to produce gas rapidly. This rapid production of gas cause increase in volume, a phenomenon called “oven spring” (DiMuzio, 2009). Extreme heat causes the formation of skin on the surface of the bread which prevents the loaf from expanding. To prevent this, one common practice is use of steam, which slows down the skin formation. Too much steam, on the other hand, reduces the rate of caramelization, producing loaves of lighter color (DiMuzio, 2009). In the initial stage, the starch gelatinizes offering rigidity to the dough and this also helps in maintaining the shape of the loaf. While the baking continues, the structure becomes rigid due to protein hardening (Charley & Weaver, 1998). Two kinds of browning reactions lead to flavor formation, the Maillard reaction and caramelization. Caramelization is due to sugars breaking down when heated. The Maillard reaction involves the reaction of proteins and reducing sugars (DiMuzio, 2009; Purlis & Salvadori, 2007).

2.7 Dough rheology

Dough rheology determines flour applications and product quality (Mohammed et al., 2012). As fiber, proteins, and starches alter the viscoelastic properties of dough, evaluating dough rheology helps product developers with choosing dough conditioners (Spies, 1989). Brabender extensigraph measures dough rheology by determining the stress-strain relationship in the dough (Preston & Hoseney, 1991). However, the lengthy testing time and the large sample size requirement are disadvantages of this method (Chen et al., 2009). The smaller scale method Kieffer dough and gluten extensibility rig

(**Figure 3**) is a modified version of extensigraph (Kieffer et al., 1998; Mann et al., 2005). It assesses rheology by measuring a dough samples' resistance to extension and extensibility. Resistance to extension is the maximum force required to break the dough, and gives a measure of dough strength (Wang et al., 2004). It is measured as the force generated by the dough when pulled by the hook attached to the texture analyzer. The development of fibrils due to the interaction of glutenins and gliadins offers resistance to extension (Charley & Weaver, 1998). Extensibility is the distance the dough travels before it breaks and related to the plasticizing effect of gliadins behaving like a plasticizer in dough (Zaidel et al., 2010). An extensible dough is desirable as it can rise upon proofing allowing the dough to expand and hold gas produced by yeast. However, it should not be so extensible that it is not able to retain its shape. Therefore, in a dough system, there should be a balance of extensibility and resistance to extension.

Figure 3. A. Dough strips formed as a result of compression of dough on the mold, B. Dough strips, C. Kieffer extensibility rig with the hook pulling the dough sample.

2.8 Stickiness

Stickiness indicates the ability of the dough to adhere to any surface and negatively affects dough machinability. Reasons for stickiness include addition of excess water, over mixing, proteolytic enzyme activity,α-amylase activity, water soluble proteins, arabinoxylans, differences in gliadin to glutenin ratio or water soluble carbohydrate (Dhaliwal & MacRitchie, 1990; Gupta et al., 1991; Hwang & Bushuk, 1972; Huang & Hoseney, 1999). Evaluating stickiness offers insights on dough machinability. With the baking industry getting more mechanized day by day, a sticky dough can affect productivity as sticky dough might interrupt the smooth flow of dough

in the process (Grausgruber et al., 2003). In addition, stickiness reduces dough strength and mixing tolerance and has a negative impact on bread quality (Graybosch et al., 1993).

Figure 4. Chen Hoseney Stickiness rig used for measuring dough stickiness

Stickiness can be measured by the Chen-Hoseney dough stickiness rig (**Figure 4**), an attachment to the texture analyzer. The rig is composed of two parts: the probe and the cell. The probe is attached to the load cell of the texture analyzer and is composed of plexiglass which is considered to be the material that dough adheres to the least. The cell is a form of extruder with a space to place the dough sample which gets pushed through the die with the screw at the bottom of the cell. When the probe comes in contact with dough, the force needed to separate the dough from the probe is measured as stickiness, i.e. the adhesive force between the surface of the dough and the probe (Chen & Hoseney, 1995). For strong doughs with high elasticity, the dough can counteract the adhesive

force and will be less sticky, while a viscous dough cannot counteract the adhesive force and will be stickier (Huang & Hoseney, 1999).

2.9 Bread characteristics

Baking transforms the dough by offering color to the crust and texture to the crumbs (DiMuzio, 2009). Shape of the baked loaf, especially dimensions, loaf volume; firmness and crumb grain are characteristics that are used to describe its quality (Stear, 1990). While loaf volume gives information on dough expansion, and relates it to the air trapped inside when the bread structure sets, dimension values provide bakers an idea of the volume increase in loaf caused due to horizontal or vertical expansion (Axford et al., 1968; Trinh et al., 2016). While horizontal expansion causes increase of length and width; height causes increase in height. Firmness is an indicator of how soft the bread is and relates bread to staling, as the bread goes firmer (Trinh et al., 2016).

Image analysis of bread crumb assesses uniformity and distribution of cell sizes (Scanlon & Zghal, 2001). **Figure 5** represents a binary image of bread used to evaluate the crumb grain features using image analysis. Formation of crumb structure is due to the series of operations taking place during bread making- namely mixing, fermentation, proofing and baking (Rosentrater & Evers, 2018). Bread crumb is composed of a solid phase (flour components) connected together with air cells (gas cells) in between (Torquato, 2000). Even though the gas cells form in the dough from the time of mixing, the structure sets upon baking due to gluten aggregation and interaction with starch (Scanlon & Zghal, 2001). Evaluating the crumb cells involves scoring the fine crumb- open vs closed cells, cell size, cell size distribution, cell area, uniformity and number of

cells (Gonzales-Barron & Butler, 2006; Pyler, 1988; Scheuer et al., 2015). Several studies have been conducted on evaluating crumb structure in wheat breads (Farrera-Rebollo et al., 2012; Gonzales-Barron & Butler, 2006), whole grain breads (Torri et al., 2013), and wheat breads supplemented with chia (Farrera-Rebollo et al., 2012), soy and almond (Lodi & Vodovotz, 2008). However, to our best knowledge, crumb grain features of IWG breads have not been studied. Given the differences between IWG and wheat (Marti et al., 2015; Rahardjo, 2017), it would be worthwhile to study the crumb grain characteristics of IWG breads, and the effect of dough conditioners on the bread.

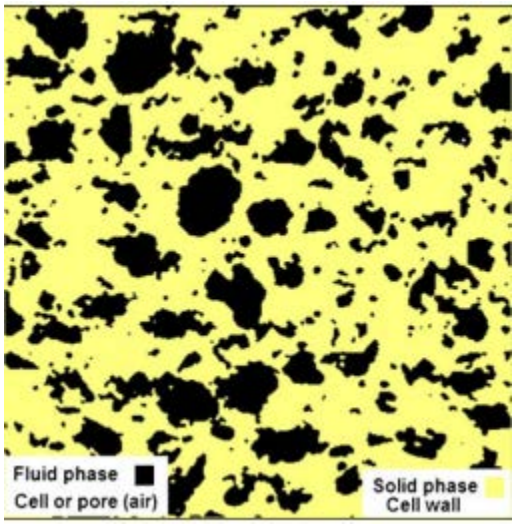

Figure 5. Bread crumb structure differentiating gas cells to non-gas cells of a binarized bread slice (Farrera-Rebollo et al., 2012)

2.10 Conclusion

With this body of knowledge taken into account, the aim of this study was to evaluate the effect of refinement and dough conditioners on IWG dough and bread properties. This study evaluated the effect of five dough conditioners on the dough rheology, particularly extensibility and resistance to extension in IWG flour at different

bran levels. These conditioners were selected based on their reported performance in rye breads and low gluten flour, since IWG bears some resemblance to rye in its chemical make-up and properties, as well as has low gluten forming ability. The levels of the various dough conditioners were determined in pre-trials for use in dough prepared for baking bread. The ultimate goal was to demonstrate the feasibility of processing conditions to facilitate the use of IWG as a stand-alone flour for food application.

Chapter 3:
Manuscript 1

Improving Intermediate Wheatgrass dough properties using dough conditioners at three flour refinement levels

3.1 Introduction

About 11% of the land on earth is arable and being used for crop production ("World Agriculture: Towards 2015/2030 - An FAO perspective," 2018). Modern agricultural practices are damaging the agricultural land, leading to soil erosion, and about 75 billion tons of soil is being lost annually (Pimentel & Burgess, 2013). Excessive use of fertilizers is affecting the environment due to nitrogen run-offs. Concern over global warming has placed pressure on agriculture to adapt sustainable agricultural systems that would balance production and support ecosystems (FAO, 2016). Perennial crops have more extended root system than annual crops (e.g., soy beans, corn, wheat), which allows them to utilize water, fertilizers, and soil nutrients more effectively (Cox et al., 2006; Dohleman & Long, 2009; Gantzer et al., 1990). Therefore, agronomists and plant breeders have been developing perennial grains as an alternative to annual crops (Wagoner & Schauer, 1990). Intermediate wheatgrass (*Thinopyrum intermedium*, IWG) is one perennial crop researched for domestication because of favorable agronomic traits such as drought and disease resistance, ease of mechanical harvesting, as well as larger seed size than what is common in perennial grains (Vogel & Jensen, 2001; Wagoner & Schauer, 1990). However, it is important to further develop this grain with the end user in mind. Use in food products offers a wider application portfolio and better prices than the currently dominating use for forage and hay (Hybner & Jacobs, 2012; Lawrence, 1983), and thus an evaluation of IWG as standalone cereal ingredient in human food is warranted.

In recent studies, IWG was shown to contain more protein and dietary fiber than wheat (Marti et al., 2015; Rahardjo, 2017). Despite these beneficial nutritional attributes, it also has characteristics that limit its functionality, most notably a lower starch content and deficiency in high molecular weight glutenin subunits (HMW-GS). This lack in HMW-GS prevents the formation of a viscoelastic network, i.e. IWG's ability to form a gluten network is poor, making it challenging to use for products requiring dough rising properties, like breads. The combination of high fiber and low HMW-GS content alters dough rheology as the gluten matrix is diluted (Coda et al., 2014). In addition, certain fibers have been shown to reduce dough elasticity and resistance to extension (Schmiele et al., 2012).

For flour with poor gluten forming ability, several strategies can be used to improve dough and bread. Since most of the dietary fiber is present in the bran (Brouns et al., 2012), a reduction of bran concentrations may lead to a stronger protein network. Another possibility that targets the fibers is use of an enzyme, xylanase, to convert water-unextractable to water extractable arabinoxylans. This releases water, which becomes available for gluten development (Courtin & Delcour, 2002; Hilhorst et al., 1999; Laurikainen et al., 1998; Martínez-Anaya & Jiménez, 1997; 1998; Rouau et al., 1994; Wang, 2003). Alternatively, additives like proteins, enzymes, and oxidizers can be used, collectively referred to as dough conditioners (Basman et al., 2002; 2003). Two commonly used conditioners, wheat protein isolate (WPI) and vital wheat gluten (VWG), supplement flour with gluten-forming proteins. WPI has been demonstrated to have a positive effect on extensibility of the dough (Ahmedna et al., 1999; MGP, 2018). VWG

has been shown to strengthen dough and improve mixing tolerance (Codina et al., 2008; Ortolan & Steel., 2017; Ribotta et al., 2001). Ascorbic acid (AA) improves dough extensibility and resistance to extension by catalyzing disulfide formation, thereby promoting cross- linking of proteins (Elkassabany et al., 1980; Elkassabany & Hoseney, 1980; Horvat et al., 2007). The enzyme transglutaminase (TG) is an acyl transferase capable of inducing protein networks (Motoki & Seguro, 1998). This has been demonstrated to transform a weaker gluten network into one that results in better dough strength (Larré et al., 2000).

Due to IWG being a novel crop, few studies on its dough properties have been conducted (Marti et al., 2015; Rahardjo, 2017), and strategies to improve dough properties have not yet been evaluated. Therefore, the objective of this study was to evaluate physical properties of IWG grain with potential relevance to bread making, and the rheology of IWG dough supplemented with dough conditioners. In addition, the influence of refinement levels and growing location were assessed. These results will further our understanding how this grain can most effectively be used in food products.

3.2 Materials and methods

3.2.1 Materials

Commercial hard red winter wheat was kindly provided by Grain Millers Inc. (Eden Prairie, MN). IWG with the same genetic make-up cultivated in 2015 at two different locations in Minnesota (Rosemount and Roseau) were obtained from crop breeding collaborators (Department of Agronomy and Plant Genetics, University of Minnesota). Dough conditioners used were WPI (Arise 8000, MGP Ingredients,

Atchison, KS), VWG (Arrowhead Mills, Melville, NY), AA (Duda Energy LLC, Decatur, AL), a commercial bakery enzyme mixture (PowerBake 960 (PB), Danisco, New Century, KS), and microbial TG (Activa TI, 1:100 blend with maltodextrin, Ajinomoto North America Inc., NJ). Concentrations of dough conditioners used are shown in **Table 2**.

Table 2. Dough conditioners and the concentrations used to study dough rheology

Dough conditioners	Concentrations (g/100g[1])
WPI	1.5, 2.5
VWG	2.5, 3.75
AA	0.0085
PB	0.00375
TG	1, 1.5, 2, 2.5

[1] Wet basis

3.2.2 Physical properties of grains

The method of Singh et al. (2005) was used to determine weight of 1000 kernel of the grains. Bulk density was calculated by taking the ratio of weight to volume of 1000 kernels and expressed as g/mL. Volume was measured by transferring kernels to a graduated measuring cylinder and tapping the cylinder 10 times before taking the reading. Length and width were measured by Neiko 01407A digital (Neiko, Taiwan, China). Length-width ratio was calculated by taking the ratio of length and width as outlined by Gayin et al. (2017).

3.2.3 Milling of the Grains

IWG kernels were milled using a Brabender Quadrumat® Junior mill (Type: 12-02-000, C. W. Brabender Instruments, Hackensack, NJ). The bran obtained from the mill

was sifted through a series of sieves (425 μm, 250 μm, 150 μm, 125 μm, and 106 μm) in a mechanical sieve shaker (model: RO-TAP, type: RX-29, serial: 2069, W. S. Tyler Inc., Gastonia, NC). Flour with particle sizes > 150 μm was collected as bran, flour with particle sizes < 150 μm as endosperm. The bran was refined using Udy Cyclone Sample Mill® (Model no: 3010-030, Udy Corporations, Fort Collins, CO) equipped with 0.25 mm screen. Wheat berries were tempered to a 15.5% target moisture content prior to milling to facilitate effective separation of bran while milling; otherwise wheat was processed the same as IWG.

Sample blends were obtained by adding back bran fractions to the endosperm based on the original bran content for each grain. Three refinement levels (blends of bran and endosperm) were used for the study, 0% bran (0B), 50% bran (50B) and 100% bran (100B). **Table 3** is the bran and endosperm content of Rosemount, Roseau and hard red wheat.

Table 3. Total bran and endosperm content of Rosemount intermediate wheatgrass (IWG), Roseau IWG and hard red winter wheat for a 100g seed sample

	Rosemount IWG	**Roseau IWG**	**Hard Red Winter Wheat**
Bran	64	46	23
Endosperm	36	54	77

3.2.4 Chemical composition of flour blends

Sample moisture was determined by infrared moisture meter (OHaus MB45, New Jersey, USA) at 130°C for 4 min. Protein content was determined by 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) and a nitrogen conversion factor of 5.70. Ash content was determined by dry ashing (AOAC 923.03, Official Methods of Analysis of AOAC International, 2016). Fat content was determined using AOAC 922.06, (Official Methods of Analysis of AOAC International, 2016). Finally, total carbohydrate was determined by difference.

Soluble and insoluble dietary fiber was quantified following AACCI 32-07.01 (AAACI Approved Methods, 2010) with a Megazyme (Wickelow, Ireland) kit.

3.2.5 Dough rheology

Dough samples were prepared with a Kitchen aid mixer (KitchenAid-KSM 900, USA) however, sample preparation differed for IWG and wheat samples. IWG dough samples for all refinement levels, with and without dough conditioners, were prepared with 46.5 % target moisture. Wheat samples were prepared with a dough of 60.1% water absorption based on level determined previously determined by Farinograph- AT (C.W. Brabender, Duisburg, Germany). Dough samples were prepared by mixing water and ingredients as shown in T**able 3** along with dough conditioners as shown in **Table 2** at speed 2 for 2 min and at speed 4 for 2 min in the 5- quart Kitchen aid mixer (Kitchen Aid - KSM 900, USA) with the dough hook, along with scraping the samples from the bowl after first mixing. Dough extensibility (mm) and resistance to extension (g) were analyzed with Kieffer dough and gluten extensibility rig (**Figure 3**) on a TA.XT-Plus Texture Analyzer (Texture Technologies, Hamilton, MA). Firstly, the fresh dough was allowed to rest for 20 min in a closed plastic box at room temperature, and then molded by Kieffer molder (TA-105) to an approximate length of 50 mm and 4 mm width. The dough was allowed to rest in the molder for 40 min at room temperature, and each strip

was clamped in the Kieffer micro-extension platform and vertically stretched with the dough hook (**Figure 3**). Extensibility and resistance to extension were evaluated by the Texture Exponent 32 version 6.0.6.0 software (pre-test speed: 2.0 mm/s; test speed: 3.3 mm/s; post-test speed: 10.0 mm/s; distance: 75 mm; trigger force: auto-5 g; data rate acquisition: 200 point per second) (Texture Technologies, Corp. Scarsdale, NY, USA). A total of 13 dough strips were tested from each batch, excluding the two strips from each ends of the molder. The SOP for analyzing dough rheology using the texture analyzer is in Appendix A.

Table 4. Ingredients and the amounts used to prepare dough to analyze dough rheology.

Ingredients	Amount (g)
IWG[1]/ Wheat	50 (bran and endosperm based on respective grain location)
Salt	1

IWG[1]- Intermediate wheatgrass

3.3Statistical Analysis

One way-Analysis of Variance (ANOVA) was done using R® (version R 3.2.2) for each location 0B, 50B, and 100B separately. When a treatment was significant ($P \leq 0.05$), differences among the means were determined using Tukey-Kramer Honest Significant Difference (HSD) test. ANOVA and Tukey HSD tables can be found in Appendix D. Two tail t-test was done in Microsoft Excel® (2013) to evaluate differences among locations.

3.4 Results and discussion

3.4.1 Physical properties of grains

Wheat kernels had higher values for width; weight, volume and bulk density of 1000 kernels than IWG from both locations (**Table 5**). Comparing the two locations, Roseau IWG was not different to Rosemount IWG for width, length/width ratio and weight of 1000 kernels. However, the bulk density of Roseau IWG was lower than for Rosemount IWG, while values for length and 1000 kernel volume were higher in Roseau IWG. Despite Roseau IWG exhibiting higher kernel volume, its value was only about one third of wheat's. These physical properties are markers of grain yield, and as such, important parameters for farmers and millers (Gayin et al., 2017; Li et al., 2004; Rukavina et al., 2002). The kernel length and width values obtained for wheat were comparable to the values reported by Mohler et al. (2016), however seed size (1000 kernel weight) values for IWG were lower than values reported by Rahardjo (2017). This discrepancy could be because of the difference in kernels in terms of environment, growing locations and genetic materials. Despite the fact that IWG from Rosemount and Roseau are composed of the same genetic material, they are fairly different in terms of physical properties. IWG kernels are thinner and longer in comparison to wheat kernels, resulting in higher bran and lower endosperm content. This causes IWG to be lower in starch and higher in total dietary fiber than wheat.

Table 5. Comparison of physical properties of Intermediate wheatgrass (IWG) and wheat kernels

Parameters	Rosemount IWG	Roseau IWG	Wheat
Length (mm)	5.36 ± 0.4 [c]	5.93 ± 0.56 [a]	5.61 ± 0.42 [b]
Width (mm)	1.06 ± 0.11 [b]	1.13 ± 0.12 [b]	2.55 ± 0.24 [a]
Length/width	5.08 ± 0.6 [a]	5.27 ± 0.56 [a]	2.22 ± 0.30 [b]
Weight of 1000 kernels (g)	5.70 ± 0.30 [b]	6.10 ± 0.04 [b]	28.13 ± 0.27 [a]
Volume of 1000 kernels (mL)	8.50 ± 0.50 [c]	10.2 ± 0.20 [b]	33.87 ± 0.12 [a]
Bulk density (g/mL)	0.67 ± 0.01 [b]	0.60 ± 0.01 [c]	0.83 ± 0.01 [a]

Lowercase letters in each row indicates significant difference among samples (p- value ≤ 0.05, n= 3)

3.4.2 Proximate composition

The proximate composition of samples at the three investigated refinement levels is shown in **Table 6**. Moisture content for wheat samples was higher than in IWG samples for respective bran concentrations. This difference can be attributed to the tempering step used for the wheat berries prior to milling. There was successive decrease in moisture content upon addition of bran for both wheat and IWG samples. From a 3-D study conducted by Song et al. (1998) on wheat grains, moisture content from the pericarp to the center of the grain increased until it reached a maximum value at the center of the grain. This suggests the moisture content is higher in endosperm than the bran, which was also observed in our case. There was no difference in the fat content between wheat and IWG at 0B, however, at 50B and 100B, both IWG samples contained more fat than wheat (**Table 6**). This is likely a consequence of most cereal lipids residing in the germ, which is part of the bran fraction (Šramková et al., 2009). As shown in **Table 4**, the bran content of IWG was higher than in wheat, and Rosemount IWG

contained more bran than Roseau IWG. This aligns with the finding that 100B Rosemount-IWG had a higher fat content than 100B Roseau-IWG.

IWG flour exhibited higher protein content than wheat samples for all three bran concentrations. With increasing bran concentrations, the protein content for IWG from both locations as well as for wheat, increased, indicating higher bran resulted in high protein content. Döring et al. (2017) also reported an increase in protein content of Rye flour upon addition of bran from 9.4% to 12.7%. For cereals, it has been reported that gliadins and glutenins are concentrated in the endosperm whereas albumins and globulins are concentrated in the germ and aleurone, which are bran fractions (Belderok et al., 2000; Šramková et al., 2009). Therefore, it is likely that 50B and 100B samples contained more albumins and globulins, which are less functional than gliadins and glutenins. Between IWG from both locations, IWG from Roseau had higher protein content than IWG from Rosemount for all bran concentrations. Even though IWG from Rosemount had a higher bran content than IWG from Roseau, the former had lower protein content indicating IWG from Roseau might have higher gliadins and glutenins than IWG from Rosemount. Further work is needed to profile protein fractions in IWG at different refinement levels.

The higher bran content also resulted in higher ash concentrations for both IWG samples than wheat, in agreement with previous findings (Marti et al., 2015; Rahardjo, 2017). Between IWG from both locations, IWG from Roseau had higher ash content than IWG from Rosemount for 0B concentrations. This could mean the endosperm of IWG from Roseau had a higher mineral concentration than IWG from Rosemount, or that more

bran was retained during milling. However, for 50B and 100B, IWG from Roseau had less ash than IWG from Rosemount. This can be linked to the higher bran level in IWG from Rosemount.

As a consequence of IWG's higher protein, ash, and fat contents, the total carbohydrates were higher in wheat than IWG samples for all refinement levels, likely because starch, the main carbohydrate is located in endosperm (Šramková et al., 2009). IWG has a smaller seed size and higher length/width ratio than wheat (**Table 5**) indicating less endosperm, which justifies the lower carbohydrate content than wheat.

Table 6. Proximate composition of wheat (W) and Intermediate wheatgrass (IWG) from Rosemount (RM) and Roseau (RS) at different bran (B) content

B	Sample	Moisture (%)	Fat (%)	Protein(%)	Ash (%)	CHO (%)
0	RM	10.23±0.13 ^{b}	1.97±0.07 ^{a}	14.38±0.09 ^{*b}	0.57±0.02 ^{*b}	72.85±0.27 ^{*a}
	RS	10.05±0.15 ^{b}	1.82±0.00 ^{a}	16.65±0.04 ^{a}	0.74±0.01 ^{a}	70.74±0.10 ^{b}
	W	13.53±0.16 ^{a}	1.83±0.03 ^{a}	10.62±0.09 ^{c}	0.51±0.01 ^{c}	73.50±0.20 ^{a}
50	RM	8.53±0.07 ^{b}	3.59±0.09 ^{a}	14.88±0.08 ^{*b}	1.88±0.05 ^{*a}	71.14±0.31 ^{b}
	RS	8.83±0.20 ^{b}	3.030.00 ^{a}	16.92±0.07 ^{a}	1.67±0.02 ^{b}	69.53±0.26 ^{b}
	W	12.43±0.00 ^{a}	1.88±0.24 ^{b}	11.18±0.18 ^{c}	0.93±0.01 ^{c}	73.57±0.06 ^{a}
100	RM	6.91±0.00 ^{c}	5.19±0.02 ^{*a}	15.58±0.00 ^{*b}	3.26±0.02 ^{*a}	69.07±0.04 ^{*b}
	RS	7.76±0.13 ^{b}	4.57±0.13 ^{b}	17.06±0.03 ^{a}	2.55±0.01 ^{b}	68.00±0.12 ^{c}
	W	11.68±0.08 ^{a}	2.38±0.11 ^{c}	11.75±0.08 ^{c}	1.30±0.02 ^{c}	72.89±0.09 ^{a}

Lowercase letters in each column indicates significant difference among samples (p-value $\leq$ 0.05, n= 2) for respective bran content and asterisks indicates significant differences between Rosemount and Roseau locations (p- value $\leq$ 0.05, n= 2) for respective bran content. CHO- Carbohydrate by difference

3.4.3 Dietary fiber

Total dietary fiber of 50B and 100B IWG samples was higher than for wheat (**Table 7**), as a consequence of higher bran contents (**Table 4**), where dietary fiber is concentrated (Brouns et al., 2012). Also, with increasing bran concentrations, the dietary fiber content increased for both IWG as well as wheat. Total dietary fiber ranged from 4.37 to 22.7 % in IWG and from 3.37 to 11.05 % in wheat samples. All samples contained less soluble than insoluble dietary fiber and there was no difference in soluble fiber between IWG and wheat at respective bran concentrations, in agreement with Rahardjo (2017). Thus, the differences in total dietary fiber are the effect of higher insoluble dietary fiber content in IWG. Rosemount-IWG contained more insoluble dietary fiber and total dietary fiber than Roseau-IWG for 50B and 100B samples. The values for soluble, insoluble and total dietary fiber for IWG are higher than the values reported by Marti et al. (2015). This discrepancy could be related to sample preparation (reconstituted flour) and raw material. However, the values were in conjunction to some IWG samples among the 13 different samples reported by Bunzel et al. (2014). Fiber has a negative effect on the dough functionality as fiber dilutes the gluten matrix by competing with the water in the dough system. The result of that is an alteration of viscoelastic properties of dough which affects dough rheology and final product quality (Schmiele et al., 2012).

Table 7. Insoluble (IDF), soluble (SDF) and total dietary fiber (TDF) for wheat (W) and Intermediate wheatgrass (IWG) from Rosemount (RS) and Roseau (RS) at different bran (B) content

Bran content	Sample	IDF (%)	SDF (%)	Sum of IDF and SDF (TDF, %)
0	RM	3.10 ± 0.73 [a]	1.75 ± 0.02 [a]	4.85 ± 0.75 [a]
	RS	3.57 ± 0.22 [a]	0.79 ± 0.63 [a]	4.37 ± 0.41 [a]
	W	1.76 ± 0.12 [a]	1.60 ± 0.33 [a]	3.37 ± 0.21 [a]
50	RM	11.96 ± 0.05 [*a]	2.6 ± 1.19 [a]	14.56 ± 1.23 [*a]
	RS	9.04 ± 0.29 [b]	1.59 ± 0.66 [a]	10.64 ± 0.37 [b]
	W	4.56 ± 0.00 [c]	0.89 ± 0.29 [a]	5.45 ± 0.29 [c]
100	RM	19.79 ± 0.84 [*a]	2.91 ± 0.16 [a]	22.7 ± 1.01 [*a]
	RS	16.02 ± 0.28 [b]	2.79 ± 1.06 [a]	18.81 ± 0.78 [b]
	W	7.97 ± 0.00 [c]	3.08 ± 0.36 [a]	11.05 ± 0.35 [c]

Lowercase letters in each column indicates significant difference among samples (p-value ≤ 0.05, n= 2) for respective bran content and asterisks indicates significant differences between Rosemount and Roseau locations (p- value ≤ 0.05, n= 2) for respective bran content

3.4.4 Dough rheological properties

3.4.4.1 Extensibility

Extensibilities of all samples are shown in **Figure 6**. Wheat dough had higher extensibility than any IWG dough sample, regardless of bran content or location. Gluten drives the extensibility and wheat has a good balance of gluten forming proteins (Wieser, 2007; Žilic, 2013) whereas IWG does not (Marti et al., 2015), resulting in greater extensibility values for wheat than IWG. Upon bran addition, extensibility decreased for all samples. This result is in conjunction with research conducted by Lai et al. (1989) where he reported that fiber from bran dilutes the gluten matrix and negatively affects the dough extensibility. Insoluble dietary fiber competes with gluten for water and thus less water is available for hydrating proteins, which impairs dough strength (Wang et al., 2002; Gómez et al., 2003). Soluble fiber increases the viscosity of the dough and retards

the gluten network formation (Izydorczyk & Biliaderi, 1995). IWG being deficient in HMW-GS (Rahardjo, 2017) and having more dietary fiber than wheat (**Table 7**) must be the reasons for the difference in the extensibility.

No dough conditioner improved IWG extensibility when compared to the control from the same growing location. However, several Roseau IWG dough samples had higher extensibility than samples from Rosemount IWG: 50B control, 50B and 100B with WPI; 100B with VWG; 50B and 100B with AA; 50B and 100B with PB; 0B and 100B with TG.

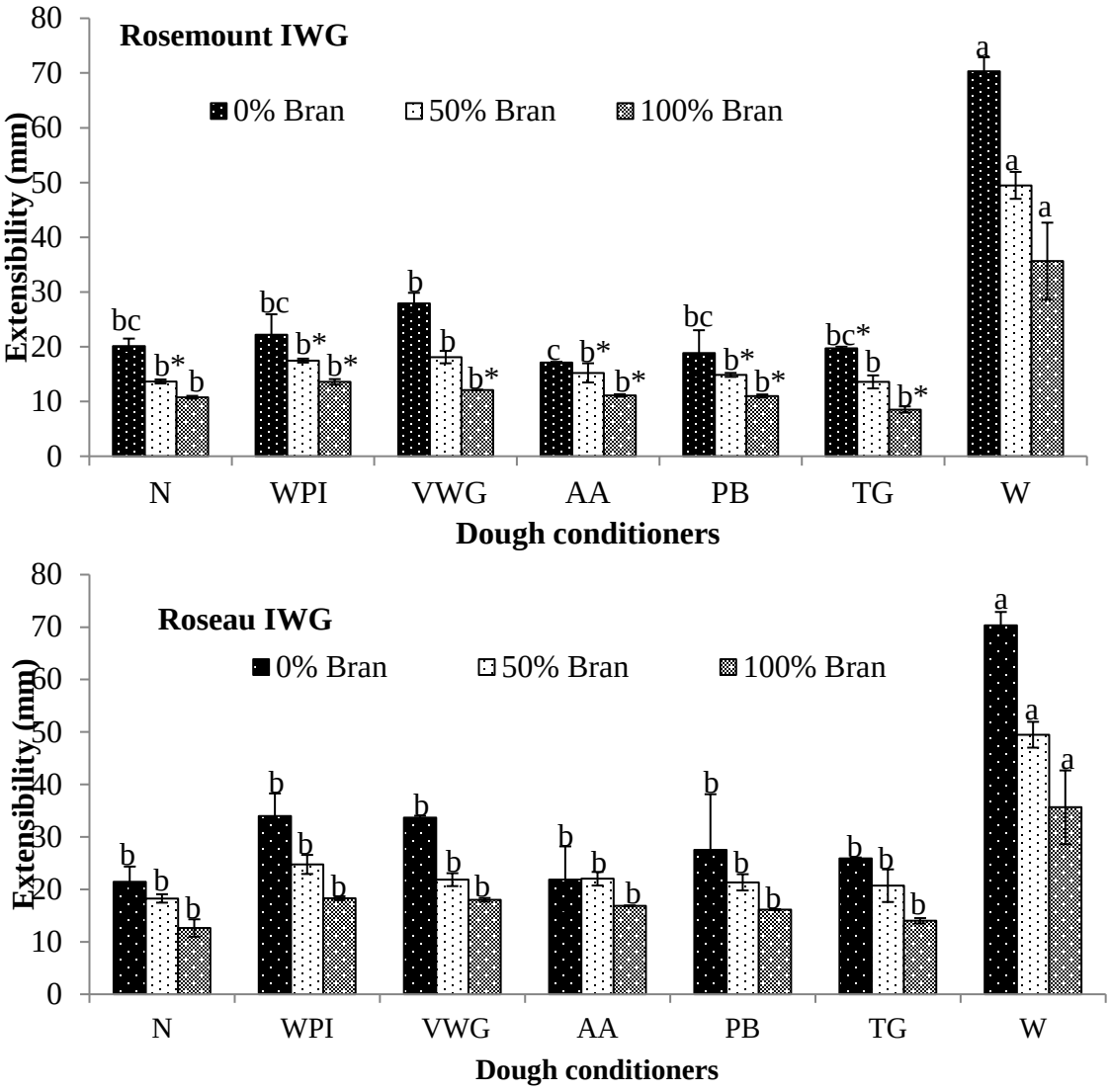

Figure 6. Extensibility for Intermediate Wheatgrass from Rosemount (top) and Roseau (bottom) using different dough conditioners. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2) with the same bran content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content. N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

Between the two locations, difference in extensibility was evident for samples at 50 B and 100B concentrations except for 100B control, 50B VWG and 50B TG. One reason for extensibility values always being higher for IWG from Roseau than from

Rosemount is the fact that IWG from Rosemount had a higher bran content to start with (**Table 3**) and bran causes a reduced extensibility in the dough (Schmiele et al., 2012).

3.4.4.2 Resistance to extension

Resistance to extension for all samples is shown in **Figure 7**. Wheat dough had higher resistance to extension values than all IWG samples regardless of the bran concentrations. Dough should be extensible; however, it should not be too extensible that it cannot hold its shape upon expansion. Hence, there should be a balance of extensibility and resistance to extension which is accomplished with a balance of gluten forming proteins. The balanced profile of gluten forming proteins in wheat (Wieser, 2007; Žilic, 2013) likely leads to these results (Marti et al., 2015). Upon bran addition, there was an increase in resistance to extension for all samples.

The only dough conditioner to affect resistance to extension was TG (**Figure 7**). For IWG from Rosemount, resistance to extension increased for 0B and 50B samples with TG; however no effect was seen for any 100B samples. For IWG from Roseau, resistance to extension increased with addition of TG at 0B; VWG and TG at 50B; and TG at 100B concentrations.

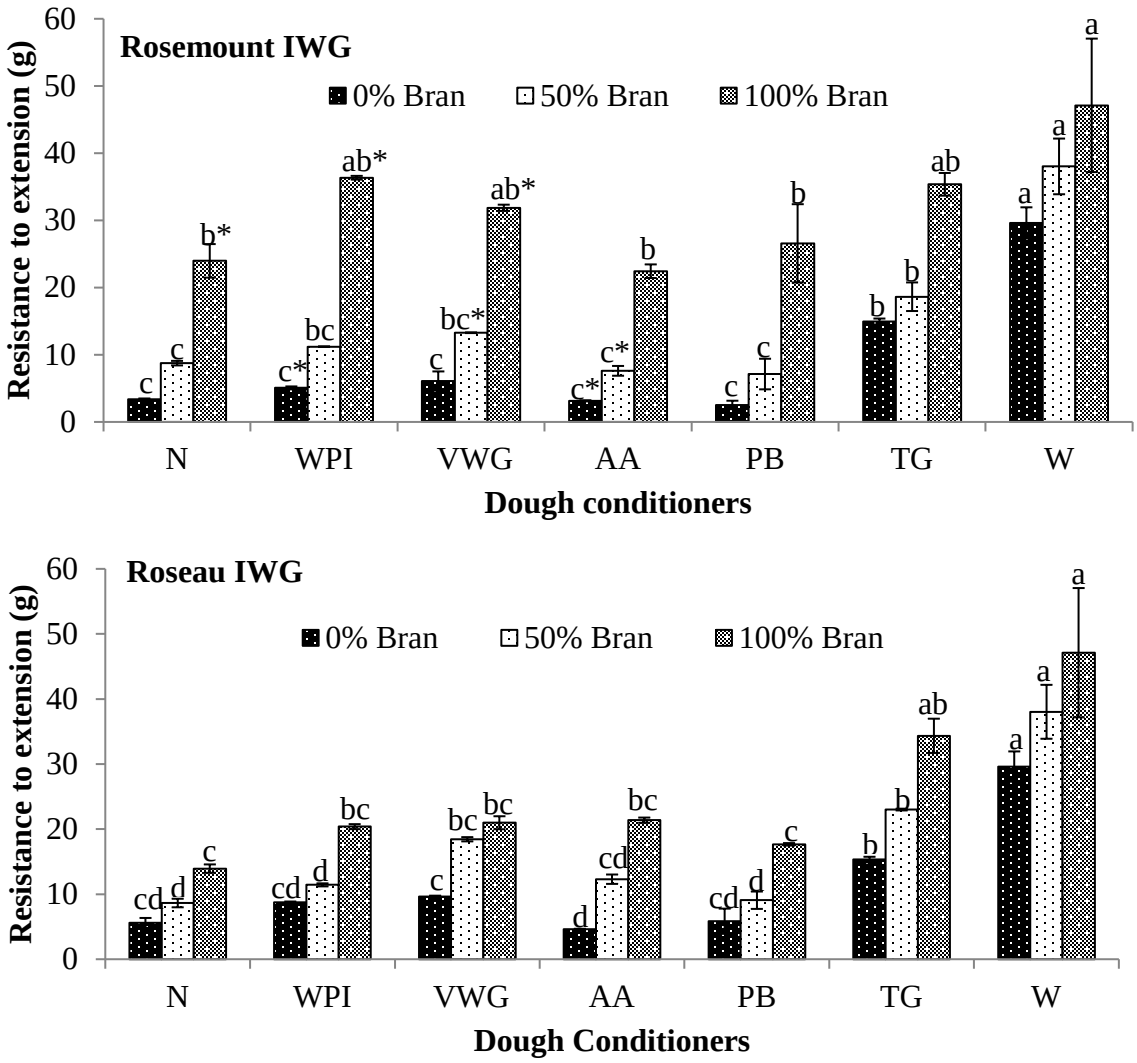

Figure 7. Resistance to extension for Intermediate Wheatgrass from Rosemount (top) and Roseau (bottom) using different dough conditioners. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2) with the same bran content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content. N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

Comparing the two locations Rosemount and Roseau, the resistance to extension values were higher for 0B and 50B for samples from Roseau, while for 100B samples, resistance to extension values were higher for Rosemount. This could be because of Rosemount IWG's higher fiber content (**Table 7**), as bran has a positive effect on the

resistance to extension (Schmiele et al., 2012). Samples different among the two locations were 100B control, 0B and 100B with WPI; 50B and 100B with VWG; 0B and 50B with AA. Resistance to extension is the measure of dough strength which is contributed by the grain proteins (Wieser et al., 2006) and Roseau having more protein (**Table 6**) than Rosemount could be the reason for higher resistance to extension at lower bran concentrations. However, further investigation on the types of proteins in Roseau and Rosemount IWG and the protein secondary structures is needed to understand the cause of the differences in the resistance to extension. The reason why this was not the same for higher bran concentration could be because the bran diluted the gluten matrix reducing the protein functionality.

Since WPI, VWG and TG increased the values of resistance to extension and extensibility for both locations at several refinement levels, their effect on rheology was also evaluated at higher concentrations.

3.4.4.3 Effect of different concentrations of WPI on extensibility and resistance to extension

The effect of different concentrations of WPI on extensibility is shown in **Figure 8**. There was no difference in extensibility for 0B samples at both concentrations of WPI for IWG from both locations. At 50B and 100B bran concentrations both 1.5 % and 2.5 % WPI had increased extensibility when compared to the control, however, there was no difference between the two concentrations. This was the case for both the locations. The 0B control, 0B and 50B with 1.5% WPI and 50B and 100B with 2.5% samples of Roseau-IWG had higher extensibilities than Rosemount - IWG. The difference in

extensibility at the higher bran content between the two locations might be an effect of the bran because IWG from Rosemount has higher bran content (**Table 3**), and bran has a negative effect on extensibility (Lai et al., 1989). A similar result was reported by Sulieman et al. (2016) where the extensibility of wheat flour decreased upon addition of fibrous material from pomegranate peels. The results indicated to us that 2.5% WPI was a suitable choice for further experiments.

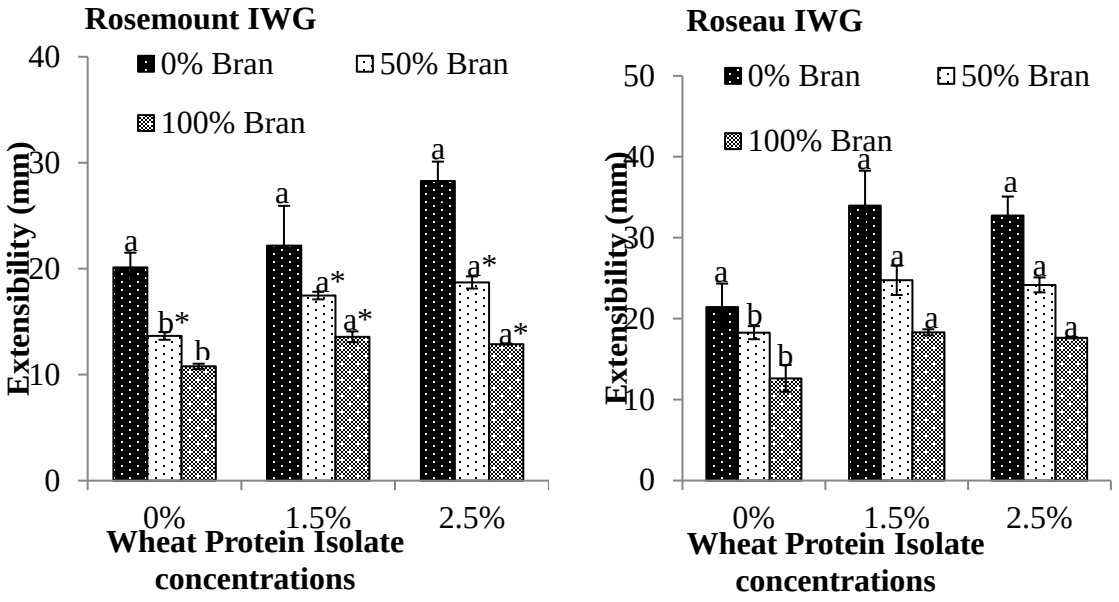

Figure 8. Extensibility for Intermediate Wheatgrass from Rosemount (left) and Roseau (right) at two Wheat Protein Isolate concentrations. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2) with the same bran content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content**.**

The effect of different concentrations of WPI on resistance to extension for IWG from both locations is represented by **Figure 9**. For IWG samples from Roseau location, resistance to extension of dough was increased by both concentrations of WPI for all bran concentrations, however, for IWG from Rosemount, resistance to extension was

increased by both concentrations of WPI at 0B, by only 2.5% at 50B and by only 1.5% at 100B. Comparing the two locations, Roseau had higher resistance to extension for 0B samples without WPI, with 1.5% and 2.5% WPI, however, Rosemount had higher resistance to extension for 100B samples without WPI, with 1.5% and 2.5% WPI. Looking at the results of both extensibility and resistance to extension, 2.5% WPI was shown to improve the dough properties more than 1.5% WPI, and was selected for further study.

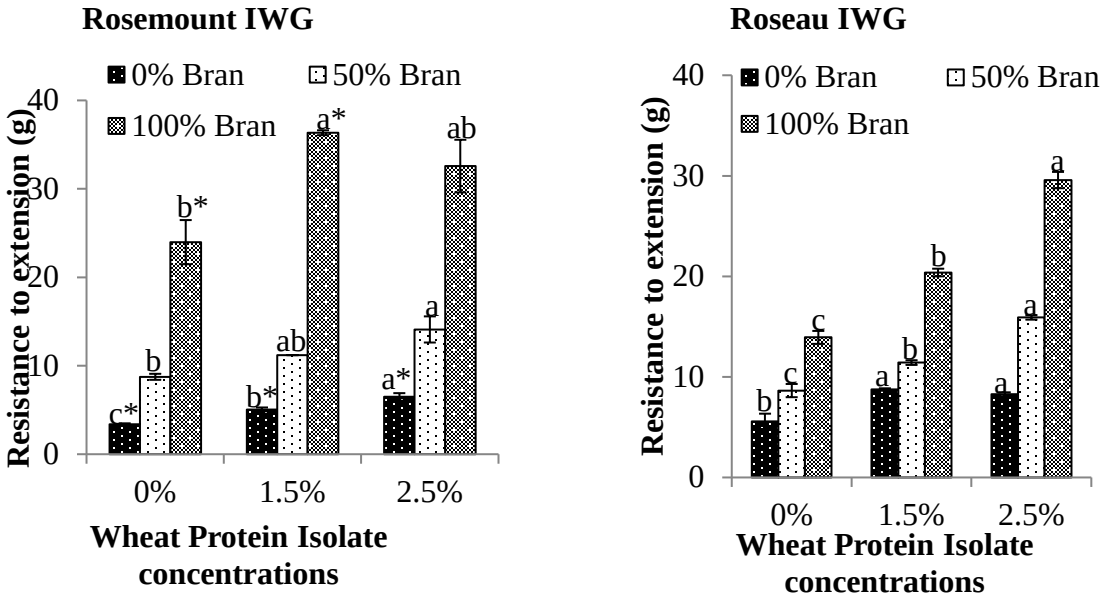

Figure 9. Resistance to extension for Intermediate Wheatgrass from Rosemount (left) and Roseau (right) using two Wheat Protein Isolate concentrations. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2) with the same bran content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content.

3.4.4.4 Effect of different concentrations of VWG on extensibility and resistance to extension

The effect of different concentrations of VWG on extensibility and resistance to extension is presented in **Figure 10** and **Figure 11** respectively. In comparison to IWG

samples without VWG, extensibility for 0B sample from Rosemount increased when using 3.75% VWG, while both concentrations of VWG increased extensibility for Roseau location, but there was no difference in extensibility between the two VWG concentrations for both locations. For 50B samples from Rosemount, extensibility was increased by both VWG concentrations, but for samples from Roseau only 3.75% VWG had an effect and there was no difference between the two VWG concentrations for both the locations at 50B. For 100B samples from Rosemount, extensibility was increased by both VWG concentrations and there was difference between the two VWG concentrations as well. For 100B samples from Roseau only 2.5% VWG had an effect on extensibility.

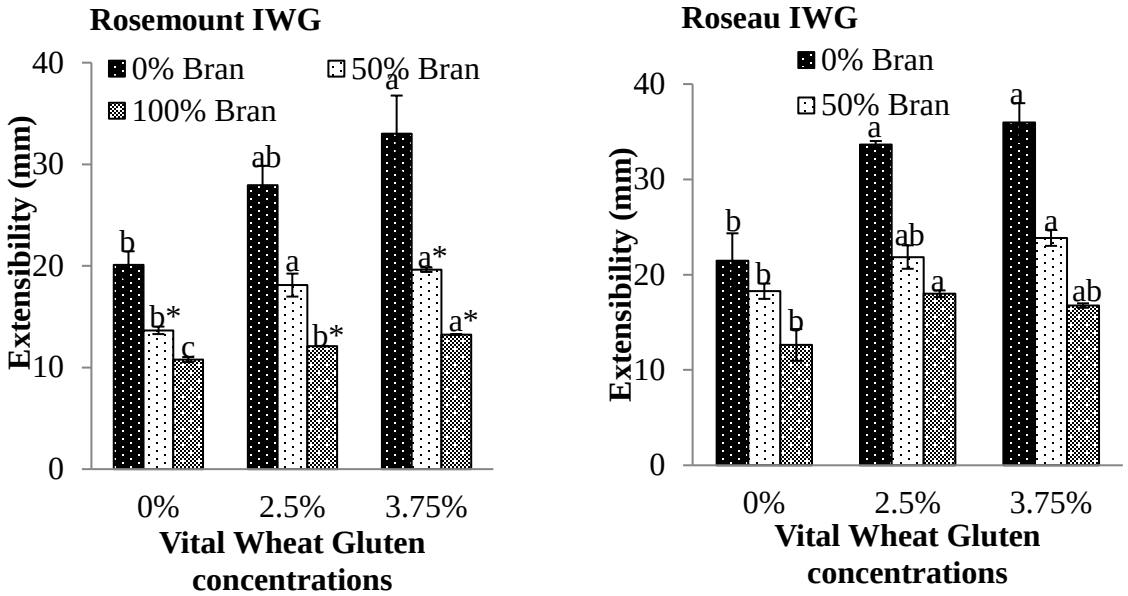

Figure 10. Extensibility for Intermediate Wheatgrass from Rosemount (left) and Roseau (right) using two Vital Wheat Gluten concentrations. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2) with the same bran content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content.

Both concentrations of VWG increased the resistance to extension for 0B samples from Roseau while 0B samples from Rosemount had an increased extensibility only at 3.75% concentration. For both Roseau and Rosemount locations, the resistance to extension did not differ between two VWG concentrations at 0B. At 50B, resistance to extension for samples from Rosemount differed from without VWG as well as between two concentrations. The case was the same for samples from Roseau too. At 100B condition, samples from Rosemount had higher resistance to extension with 3.75% but resistance to extension at 2.5% VWG Rosemount was neither different with samples without VWG or samples with 3.75% VWG indicating a relatable effect by both VWG concentrations. Both concentrations increased the resistance to extension for 100B samples from Roseau, however, there was no difference in resistance to extension between the two concentrations.

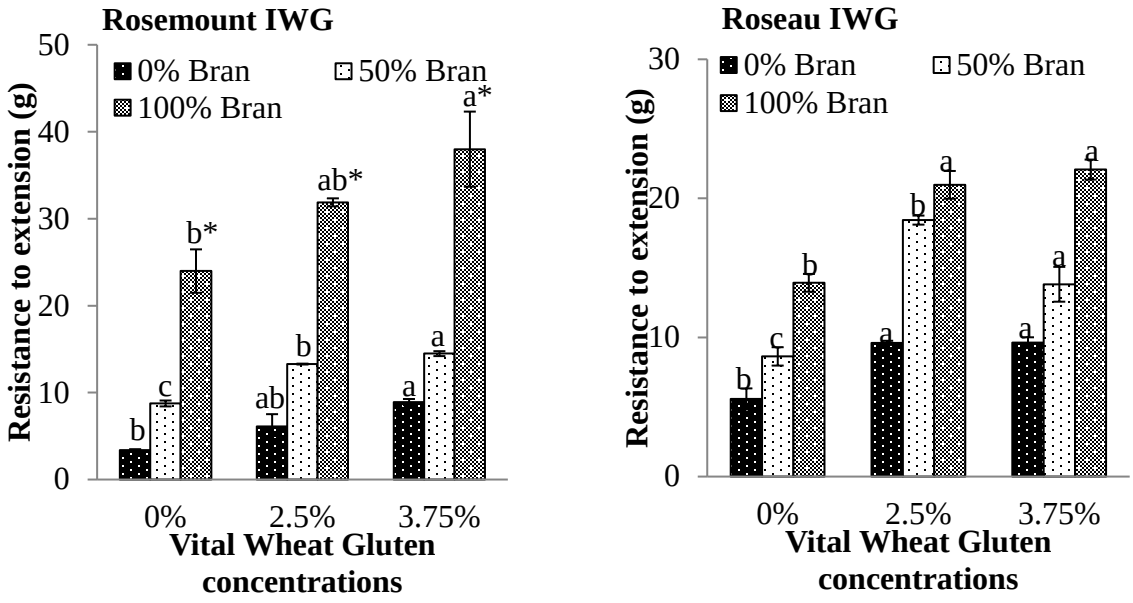

Figure 11. Resistance to extension for Intermediate Wheatgrass from Rosemount (left) and Roseau (right) using different dough conditioners. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2) with the same bran content, and

asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content.

Comparing the two locations for extensibility, there was a difference in extensibility for samples without conditioner at 50B, sample with 2.5% VWG at 50B and sample with 3.75% at 50B and 100B with Roseau always having higher extensibility than Rosemount. Evaluating the resistance to extension between locations, there was difference only for 100B samples for 0%, 2.5% and 3.75% VWG. Rosemount IWG had higher resistance to extension for those samples indicating the pronounced effect of bran over the conditioners, which may be because Rosemount IWG had higher bran content than Roseau IWG (**Table 3**). However, the protein fractions, and structures which affect the resistance to extension (Žilic, 2013) should also be studied to conclude the differences between Rosemount and Roseau IWG.

As the effects of 2.5% VWG and 3.5% VWG were similar, 2.5% VWG was the concentration chosen to improve IWG dough properties for further experiments.

3.4.4.4 Effect of different concentrations of TG on extensibility and resistance to extension for refined flour from Roseau location

Due to sample limitation and time constraints, extensibility and resistance to extension was analyzed only for 0B IWG samples from Roseau to determine a concentration of TG to use, which is discussed in the following. The reason for choosing IWG from Roseau was because of its higher protein, lower fiber, and that the effect of conditioners was more prominent for IWG from Roseau than from Rosemount.

The effect of different concentrations of TG on extensibility and resistance to extension is represented in Figure 12. It is clearly seen that extensibility increased at one

point (1%) and then decreased. There was an increase in extensibility at 1% TG; however extensibility decreased at the 2% and 2.5% concentrations.

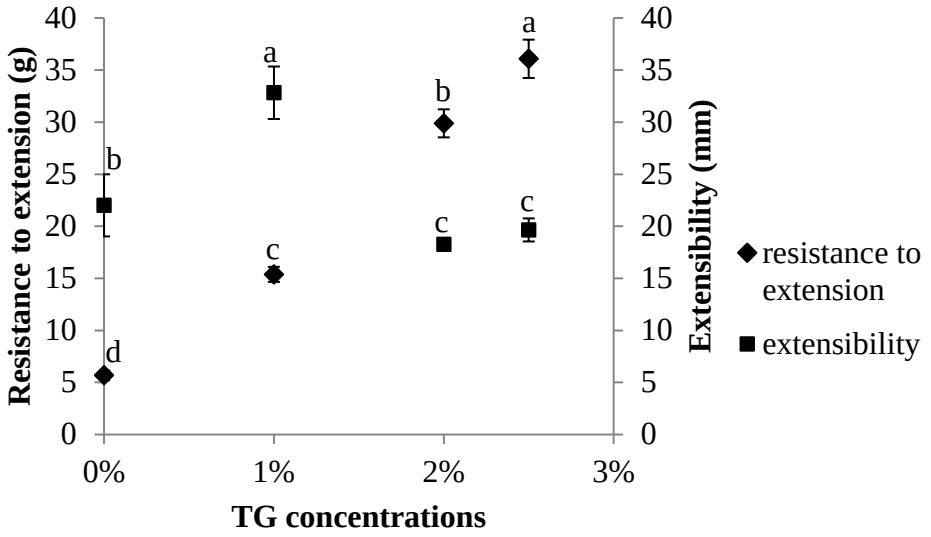

Figure 12. Extensibility and resistance to extension for refined Intermediate Wheatgrass (IWG) flour (0% bran) from Roseau at different Transglutaminase concentrations. Different letters represent significant differences for samples (p-value ≤ 0.05, n=2).

On the other hand, resistance to extension increased almost in a linear fashion with increasing TG concentrations. There was an increase in resistance to extension for all concentrations in comparison to samples without TG, however, extensibility did not vary among 2% and 2.5%. There should be a balance of extensibility and resistance to extension in a dough system. 2% TG concentration was chosen for further experiments because extensibility values formed a plateau and resistance to extension had greatly increased from control.

3.5 Conclusion

For some IWG samples, VWG, WPI and TG improved dough extensibility and resistance to extension. Evaluating the effects of these dough conditioners on protein

profile and secondary structure will offer insight to identify more approaches to improve IWG dough functionality. Understanding the rheological properties offers insight on how the dough behaves during mixing, but the effect of these differences on final product functionality is important. The next chapter will describe the investigation of this in a bread system.

Protein profiles affect dough rheology. Gliadins and glutenins are essential for developing the gluten network (Žilić, 2013). Secondary structures of proteins- β - sheets and β - turns affect the elasticity and gas holding capacity in dough (Bock & Damodaran, 2013; Bock et al., 2013; Mejia et al., 2007). Evaluating the protein profiles, and changes in secondary structure, with addition of bran and dough conditioners should be investigated. Starch damage also affects the functional properties of flour (Hackenberg et al., 2018) and future studies should evaluate the effect of starch damage on IWG rheological properties.

Chapter 4:
Manuscript 2

Effects of dough conditioners on Intermediate Wheatgrass breads at three refinement levels

4.1 Introduction

Consumers are developing interest in products that support sustainable agriculture as they are considering health effects, environment protection, animal welfare in addition to fulfilling calorie needs when purchasing foods (Soler et al., 2013; Essoussi & Zahaf, 2009; Schleenbecker & Hamm, 2013). Multiple organizations such as the American Dietetic Association, Food and Agricultural Organization, United States Department of Agriculture, and American Society of Agronomy are encouraging sustainability practices in agriculture as well as food consumption behavior (Soler et al., 2013; Robinson & Smith, 2003). Domesticating perennial crops for food production is one novel approach for sustainable agriculture. Perennial crops offer numerous environmental benefits like reduced nitrogen leaching, carbon sequestration, soil erosion prevention, and drought resistance, and low energy requirements for production (Cox et al., 2006; Dohleman & Long, 2009; Gantzer et al., 1990). Intermediate wheatgrass (*Thinopyrum intermedium*, IWG) is a perennial relative of wheat and explored for food applications such as breads due to its agronomic traits (DeHaan & Ismail, 2017; Wagoner & Schauer, 1990). Retail sales of bread and baked goods amounted to approximately 27.46 billion USD in 2015 (“Bread and bakery products- Statistics & amp; Facts,” 2018) and the majority of bread is made of wheat. Higher contents of protein, dietary fiber and certain antioxidants than for wheat have been reported for IWG (Bunzel et al., 2014; Marti et al., 2015; Rahardjo, 2017; Schoenfuss et al., 2014). However, despite these nutritional advantages, IWG has the drawback of being deficient in high molecular weight glutenin subunits (HWM-GS) and thus differs significantly in gluten forming ability to wheat (Bunzel et al., 2014;

Rahardjo, 2017). During the bread making process, both types of gluten-forming proteins, glutenins and gliadins, become hydrated and form a viscoelastic network capable of gas holding. Deficiency in HMW-GS results in dough with low viscoelasticity and bread of inferior quality (Dhaka & Khatkar, 2015; Niu et al., 2011; Wieser, 2007; Žilić, 2013). Moreover, dietary fiber may dilute the gluten matrix and compete for water with gliadins and glutenins, which in turn reduces gas retaining capacity (Bock & Damodaran, 2013; Gómez et al., 2003; Wang et al., 2002).

The deleterious effect of certain fibers and inferior protein quality can be minimized by addition of proteins such as vital wheat gluten (VWG), or by using additives that modify proteins and fibers (Basman et al., 2002; 2003). Some commonly used additives to modify the protein and fiber profile in flours are wheat protein isolate (WPI), vital wheat gluten (VWG), ascorbic acid (AA), transglutaminase (TG), xylanases, and α-amylases. These additives fall under the category of dough conditioners. Xylanases cleave the xylan backbone of water unextractable arabinoxylans, transforming them to water extractable arabinoxylans (Laurikainen et al., 1998; Rouau et al., 1994). IWG arabinoxylans bear some similarity to rye (Schendel et al., 2015). Xylanase has been shown to improve bread loaf volume, shape and texture in wheat breads (Courtin & Delcour, 2002; Hilhorst et al., 1999; Martínez-Anaya & Jiménez, 1997; 1998; Rouau et al., 1994) and rye breads (Grossmann et al., 2016). Certain α-amylases form dextrins that can reduce amylopectin retrogradation, thus delaying staling (Martínez-Anaya et al., 1999). WPI is a derivative of wheat gluten obtained through acidic deamidation, converting glutamine and asparagine to glutamic acid and aspartic acid respectively or

through solubilizing gluten protein in an acidic or alkaline medium (Batey & Grass, 1983; Wu et al., 1976). WPI being a derivative of wheat gluten, has high protein content (90-95%) than VWG (75-80%) (US 2007/0264414A1, 2007). Adding WPI to flour has been reported to increase bread loaf volume, improve crumb texture and cell structure (Ahmedna et al., 1999; MGP, 2018). VWG is commonly added to weak flours to strengthen the protein and improve loaf volume (Borla et al., 2004; Giannou et al., 2016; Ortolan & Steel, 2017). AA is oxidized to dehydro-ascorbic acid which catalyzes glutathione to its disulfide (Koehler, 2003). Thus, glutathione is no longer available to bond with proteins, allowing the proteins to bond, strengthening the gluten network (Horvat et al., 2007). Researchers have shown that AA can increase bread loaf volume, improve gas retention and increase crumb cell area in some cases (Elkassabany et al., 1980; Elkassabany & Hoseney, 1980; Horvat et al., 2007). TG modifies protein crosslinking through an acyl transfer reaction (Motoki & Seguro, 1998) by catalyzing the formation of “inter- and intra- molecular lysine cross links in wheat proteins (Larré et al., 2000). TG has been reported to improve dough strength, which can be related to improved bread quality (Pongjaruvat et al., 2014; Tseng & Lai, 2002).

Very few studies have evaluated the bread making ability of IWG, and all used it in the form of whole-grain flour (Marti et al., 2015; Rahardjo, 2017). While tremendous progress in IWG breeding has been made, optimizing processing operations has the potential to further improve the quality of IWG-based foods. Upon refinement, IWG dough was noticeably sticky, and this handle-ability issue could cause problems in large-scale manufacturing. Effect of bran removal and dough conditioners on stickiness of

IWG has not been studied. Thus, the objective of this study was to evaluate the effects of refinement and five dough conditioners on IWG dough stickiness and bread quality.

4.2 Materials and methods

IWG of the same genetic material grown in Roseau (RS) and Rosemount (RM), Minnesota, in 2015 and was obtained from crop breeding collaborators (Department of Agronomy and Plant Genetics, University of Minnesota). Hard red winter wheat was provided by Grain Millers Inc. (Eden Prairie, MN). The five dough conditioners used were WPI, (Arise 8000, MGP Ingredients, Atchison, KS), VWG (Arrowhead Mills, Melville, NY), Ascorbic Acid (Duda Energy LLC, Decatur, AL) (AA), commercial bakery enzymatic dough conditioner (PowerBake 960 (PB), Danisco, New Century, KS), and microbial TG (Activa TI, 1:100 blend with maltodextrin, Ajinomoto North America Inc, NJ). Their concentrations used in the formula are shown in **Table 8**.

Table 8. List of dough conditioners and the concentrations used for the study

Dough conditioners	Concentrations (g/100g[1])
Wheat Protein Isolate, (WPI)	2.5
Vital Wheat Gluten (VWG)	2.5
Ascorbic acid (AA)	0.0085
Powerbake (PB)	0.00375
Transglutaminase (TG)	2

[1] Wet basis

IWG and wheat kernels were milled with a Brabender Quadrumat® Junior mill (Type: 12-02-000, C.W. Brabender Instruments, Hackensack, NJ). Bran was refined with Udy Cyclone Sample Mill® (Model no: 3010-030, Udy Corporations, Fort Collins, CO) equipped with a 0.25 mm screen. For wheat milling, berries were tempered to 15.5% moisture content before milling to facilitate separation of bran. To the best of our

knowledge, optimum tempering conditions for IWG have not been evaluated yet, which is why this step was not included for IWG. Bran obtained after refinement was re-combined with endosperm at 0%, 50% and 100% of its original content leading to samples 0B, 50B and 100B respectively. **Table 9** lists bran and endosperm contents of RM-IWG, RS-IWG and hard red wheat.

Table 9. Percent of bran and endosperm present in Rosemount intermediate wheatgrass (IWG), Roseau IWG and hard red winter wheat

	Rosemount IWG	Roseau IWG	Hard Red Winter Wheat
Bran	64	46	23
Endosperm	36	54	77

4.2.1 Sample preparation

For baking bread, AACCI 10-10.03 (AACCI Approved Methods, 2010), was used with slight modifications. Yeast was mixed with sugar and water, kept at 30° C and 85% relative humidity for 20 min and then combined with the remaining ingredients (**Table 10**) and mixed using a kitchen aid mixer, with the dough hook (Kitchen Aid - KSM 900, USA). Mixing speed 2 was used for 2 min and speed 4 for 2 min, with scraping the samples from the bowl after the first mixing. For IWG dough samples, a final moisture of 46.5% was targeted, and for wheat, dough of 60.1% water absorption was prepared based on the level previously determined by Farinograph - CT (Model No: 810162, C.W. Brabender, Duisburg, Germany).

Table 10. Ingredients and the amounts used to prepare a dough to analyze dough rheology and for breads

Ingredients	Amount (g)
Flour	60 (bran and endosperm based on respective grain location)
Salt	0.9
Shortening	1.8
Sugar	3.6
Yeast	3.18
Water	46.5% target moisture for IWG[1], 60.1% water absorption for wheat
Dough conditioners	as in table 3.1

[1]IWG- Intermediate wheatgrass

4.2.2 Dough stickiness

Dough samples (n = 2) were analyzed for stickiness (g) using a Chen Hoseney stickiness rig (**Figure 4**) on a TA.XT-Plus Texture Analyzer (Texture Technologies, Hamilton, MA) equipped with Texture Exponent 32 version 6.0.6.0 software. The internal screw of the Chen Hoseney stickiness cell (TA-100) was unscrewed to allow maximum space for the dough. Approximately 10 g of dough was placed in the chamber and levelled with a spatula. The extruder lid was screwed into the cell completely and the internal screw rotated to allow small amounts of dough to extrude through the die. The first extrudate was discarded and the internal screw rotated to extrude approximately 1mm of dough. The dough samples were allowed to rest for 30 seconds to release the stress produced upon extrusion. The 25 mm cylindrical probe attached to the texture

analyzer was then used to measure the stickiness of the dough with the following settings: pre-test speed: 0.5 mm/s; test speed: 0.5 mm/s; post-test speed: 10.0 mm/s; distance: 4 mm; force: 40 g; time: 0.1 s; trigger type: auto-5 g; data rate acquisition: 500 point per second) (Texture Technologies, Corp. Scarsdale, NY, USA). The SOP of the method can be found in Appendix A.

4.2.3 Baking

The remaining dough samples were split into two equal parts of 47.5 g and put into the proofer (Baxter PW2E, Orting, WA, USA) in a container, without covering, at 30°C and 85% relative humidity (RH) for 52 min. After the first proofing, samples were punched by hand 10 times and underwent a second proofing for 25 min at 30°C and 85% RH, then, punched again for 10 times and proofed for 13 min under the same conditions as above. Dough was then sheeted to 4.8 mm thickness 5 times using an automated sheeter (Sheeter-moulder, National Manufacturing, Division of TMCO Inc., Lincoln, NE), hand-rolled to a ball, re-sheeted 5 times with the same adjustment, and then rolled to 53 mm length to fit into a Freshware CB-308RB silicon trapezoidal pan of size 71 × 38 mm top, 53 × 31 mm bottom, and 28 mm depth (Freshware Inc., LA, USA) that was sprayed with pan release oil (PAM, Conagra, IL). The pan was placed in the proofer set at 30°C and 85% RH for 33 minutes. Then the pan was put into a preheated baking oven and baked at 218° C for 14 min with steam in the first 10 sec of baking (Baxter OV500E1, Orting, WA, USA). The bread removed from the oven, and allowed to cool for one hour. Bread was sliced to 12.5 mm thick slices using an automatic Bread Slicer (Oliver Products Company, Grand Rapids, MI, USA).

4.2.3.1 Physical measurements of breads

The weight of the bread was measured using an electronic balance (Scout Pro SP602), after allowing it to cool for an hour. Length, width and height were measured using a Neiko 01407A digital caliper (Neiko, Taiwan, China). Length was measured as the maximum length measured from the top of the loaf. Width was measured as the maximum width from the top of the loaf and height was measured vertically as the highest point of the loaf.

4.2.3.2 Loaf specific volume

Loafs were cooled for an hour, and specific volume was measured using the rapeseed displacement method AACC method 10-05.01 (AACCI Approved Methods, 2010) with slight modifications. A bowl was overfilled with rapeseeds, and a ruler was used to level it to straight edge. Then, the same bowl was filled half with rapeseed, the bread was placed and then the bowl was again filled with rapeseed and leveled similarly like before. The rapeseeds displaced by the bread were collected and their volume measured with a graduated cylinder. The specific loaf volume was determined by dividing the loaf volume by its weight (mL/g).

4.2.3.4 Crumb firmness analysis

Bread firmness was measured using a TA.XT-Plus Texture Analyzer (Texture Technologies, Hamilton, MA) following AACCI method 74-09.01 (AACCI Approved Methods, 2010), calibrated for a load cell of 5 kg (setting- pre-test speed: 1.0 mm/sec; test speed: 1.7 mm/sec; post-test speed: 10.0 mm/sec; target mode: distance; distance: 5

mm; trigger type: auto-5 g; data rate acquisition: 250 point per second). One 12.5 mm bread slice at a time was used to measure the firmness. Four sub duplicates of each sample were analyzed. The attachment to the TA-XT Plus was TA-5, a cylindrical probe of 1.3 cm diameter and 3.5 cm length. The force (g) by which the probe deformed the bread was measured. The data was generated using Texture Exponent 32 version 6.0.6.0 software (Texture Technologies, Corp. Scarsdale, NY, USA) was used for data analysis.

4.2.3.5 Crumb grain analysis

The central slice (12.5 mm) from each bread loaf was scanned with an EPSON scanner (Model no: C462B, software: vers. 3.7.9. OUS) with the 8-bit grayscale, 600 dpi setting. Images were analyzed with ImageJ 1.50i version (National Institute of Health, MD, USA). Three rectangles of width 584 mm and height 520 mm from the center top, and the lower left-hand and right-hand corners were analyzed, after converting from grayscale to binary by setting the auto threshold to Otsu as outlined by Gonzales- Barron and Butler (2006). Gas cells were analyzed setting the size range from 0.0001 to infinity. A representation of a binarized image is shown in Figure 3.1. Several crumb grain features were extracted: cell count, cell area, and average cell size. The SOP of the method can be found in Appendix A.

4.3 Statistical analysis

One way-Analysis of Variance (ANOVA) was done using R® (version R 3.2.2) for 0B, 50B, and 100B from each location separately. Differences among means were determined using Least Significant Difference (LSD) test and $P \leq 0.05$ values were

considered significant. ANOVA tables can be found in Appendix D. Two tailed z-test was done in Microsoft Excel® (2013) to evaluate differences among location at respective bran contents.

4.4 Results and discussion

4.4.1 Stickiness

The stickiness of IWG dough was higher than wheat dough for samples from both locations at 0B and 50B levels (**Figure 13**). For 100B, all samples from RS-IWG were more sticky that wheat dough, but for 100B RM-IWG, all doughs except with PB had higher stickiness than wheat. When bran was added, the stickiness decreased, and the trend was the same for all samples. As the fiber in bran absorbs water, dough viscosity decreases as it is removed (Schmiele et al., 2012) and it can overcome the adhesive force, and thus the dough is not sticky (Huang and Hoseney, 1999). For RM-IWG 0B samples, the stickiness decreased with WPI, VWG and TG; at 50B stickiness decreased with addition of all dough conditioners except AA. At 100B there was no differences between control and samples with dough conditioners. For RS-IWG samples at 0B, WPI and VWG reduced the stickiness, while at 50B AA enhanced the stickiness and at 100B WPI, VWG and TG reduced the stickiness. The effect of reduced stickiness seen by WPI and VWG could be because these are proteins, and would have absorbed water during mixing. The TG was used in higher amounts than AA and PB (**Table 8**), and mainly contained maltodextrin, which could have absorbed water.

Between the two IWG locations, control-50B, VWG-0B and PB-100B had different amounts of stickiness. For 100B sample, RS-IWG had higher stickiness, while for 0B and 50B, the stickiness was higher for RM-IWG. RS-IWG has less bran than RM-IWG (**Table 9**), which may explain the higher stickiness of 100B RS-IWG. As the insoluble fiber competes with water while forming a dough in a sample with high bran (Schmiele et al., 2012), water soluble components cannot hydrate as much, which would otherwise enhance stickiness (Huang & Hoseney, 1999). Differences in stickiness between RM-IWG and RS-IWG could be related to the different amounts of water-soluble components (Huang & Hoseney, 1999), gliadin-gluten ratios (Dhaliwal & MacRitchie, 1990), alpha-amylase activity (Rasper et al., 1992) or proteolytic activity (Hwang & Bushuk, 1972). Therefore, future studies should evaluate these factors, and compare them to wheat.

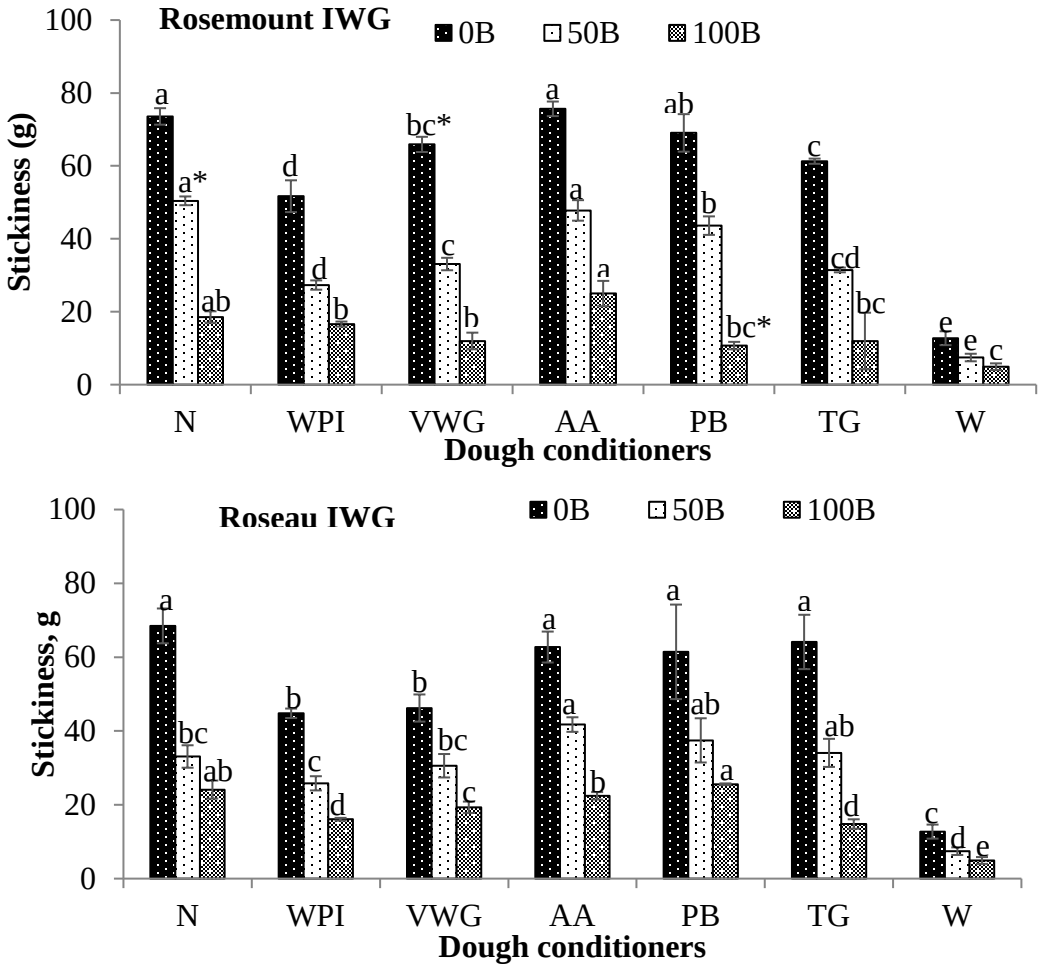

Figure 13. Stickiness for Intermediate wheatgrass (IWG) Rosemount (top) and Roseau (bottom). Different letters represent significant differences for samples (n=2) with the same bran content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran (B) content. N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

4.4.2 Bread quality

Breads made from IWG were compared against wheat controls at respective refinement levels. Breads were evaluated for dimensions, specific volume, crumb firmness and crumb cell analysis.

4.4.2.1 Length, Width, Height

The length, width, and height of all samples are shown in **Table 11**. The length of breads ranged from 64.0 mm to 76.6 mm, while the width ranged from 34.1 mm to 55.4 mm and the height ranged from 30.4 mm to 47.2 mm. When bran was added, the values for dimensions of breads decreased for all samples. Wheat breads had higher values for length, width and height than IWG control from both locations at 0B and 100B. For 50B, there was no difference in length between any of the samples; however, width and height were higher for wheat breads.

Both WPI and VWG improved length and width for 0B RM-IWG, however, only WPI led to an increased height and width at 50B and 100B respectively. However, for RS-IWG the width and height increased at all bran contents with both WPI and VWG. It was noted that upon addition of WPI and VWG, 0B samples demonstrated reasonably good proofing, but while wheat dough increased in height and maintained its shape, IWG dough expanded both horizontally and vertically. The sideward expansion could be due to the viscous dough not being able to rise, but spread instead. Gliadins offer viscosity to the dough (Žilić, 2013), and the higher amount of gliadins than glutenins in IWG than wheat (Rahardjo, 2017) might have increased the length and width of IWG breads.

Table 11. Mean length (mm), width (mm), and height(mm) for breads made from Intermediate wheatgrass without conditioner (N), with wheat protein isolate (WPI), vital wheat gluten (VWG), ascorbic acid (AA), powerbake (PB), transglutaminase (TG) and wheat (W) at three refinement levels

Samples		RM 0B	RS 0B	RM 50B	RS 50B	RM 100B	RS 100B
N	Length	70.7±0.6 ^{b}	70.9±0.9 ^{c}	70.4±0.4 ^{a}	70.5±0.3 ^{a}	68.7±0.6 ^{b}	68.6±0.8 ^{b}
	Width	53.7±0.8 ^{cd*}	51.3±1.5 ^{c}	49.1±0.7 ^{cd*}	45.5±0.3 ^{d}	39.4±0.5 ^{d*}	40.5±0.4 ^{c}
	Height	39.1±0.6 ^{b}	39.0±0.6 ^{c}	35.0±0.71.8 ^{d}	35.6±0.7 ^{e}	31.8±0.4 ^{bc*}	32.6±0.4 ^{d}
WPI	Length	74.5±2.3 ^{a*}	71.5±3.4 ^{c}	66.5±1.5 ^{c*}	69.0±0.6 ^{b}	64.0±0.9 ^{e*}	66.6±2.1 ^{d}
	Width	54.9±2.6 ^{ab}	53.7±2.4 ^{b}	48.4±0.4 ^{de*}	47.3±0.9 ^{c}	40.9±0.3 ^{b*}	43.2±2.2 ^{a}
	Height	38.6±0.6 ^{b*}	41.6±0.8 ^{b}	36.5±0.7 ^{b*}	37.5±0.8 ^{b}	32.3±0.9 ^{b*}	34.7±0.8 ^{b}
VWG	Length	74.4±1.2 ^{a*}	76.6±2.3 ^{a}	70.5±1.2 ^{a}	71.2±0.8 ^{a}	66.9±1.4 ^{c}	68.0±1.4 ^{bc}
	Width	55.4±1.4 ^{a*}	53.0±0.5 ^{b}	48.4±0.8 ^{e*}	49.2±1.0 ^{b}	34.1±0.6 ^{d*}	43.0±1.1 ^{ab}
	Height	38.9±1.0 ^{b*}	41.2±1.3 ^{b}	35.5±0.3 ^{cd*}	36.5±0.3 ^{c}	31.7±0.3 ^{c*}	33.4±0.3 ^{c}
AA	Length	68.5±0.8 ^{c*}	69.9±1.5 ^{c}	69.9±1.2 ^{a}	69.3±0.9 ^{b}	67.0±1.3 ^{c}	66.1±1.2 ^{d}
	Width	52.7±0.4 ^{d*}	51.2±0.6 ^{c}	50.8±1.4 ^{a*}	46.7±0.2 ^{c}	40.6±0.5 ^{bc}	40.5±1.1 ^{c}
	Height	39.0±0.5 ^{b}	39.3±0.7 ^{c}	35.0±0.7 ^{c}	36.1±0.7 ^{d}	31.7±0.5 ^{c*}	33.3±0.5 ^{c}
PB	Length	69.0±0.7 ^{c*}	71.1±2.8 ^{c}	67.7±1.4 ^{bc}	68.1±1.3 ^{c}	67.4±0.5 ^{c}	67.0±2.1 ^{cd}
	Width	54.0±0.8 ^{bc*}	53.2±1.0 ^{b}	49.9±1.4 ^{bc*}	48.8±0.9 ^{b}	40.1±1.2 ^{c*}	42.1±0.9 ^{b}
	Height	39.2±0.9 ^{b}	39.6±1.4 ^{c}	35.0±0.8 ^{d}	35.2±0.3 ^{e}	31.7±0.6 ^{c*}	32.6±0.5 ^{d}
TG	Length	69.0±1.8 ^{c*}	66.7±1.6 ^{d}	67.7±0.2 ^{b*}	68.5±0.8 ^{bc}	65.0±0.9 ^{d*}	66.6±0.4 ^{d}
	Width	47.4±1.1 ^{e*}	46.3±0.8 ^{d}	40.0±0.8 ^{f*}	39.1±0.8 ^{e}	34.1±0.4 ^{e*}	36.0±0.4 ^{d}
	Height	33.8±0.7 ^{c*}	35.0±0.9 ^{d}	31.5±0.3 ^{e*}	31.9±0.3 ^{f}	30.4±0.3 ^{d*}	30.9±0.5 ^{e}
		0B	0B	50B	50B	100B	100B
W	Length	73.9±2.9 ^{a}	73.9±2.9 ^{b}	70.3±1.8 ^{a}	70.3±1.8 ^{a}	70.5±1.5 ^{a}	70.5±1.5 ^{a}
	Width	55.3±1.1 ^{a}	55.3±1.1 ^{a}	50.1±0.8 ^{ab}	50.1±0.8 ^{a}	43.8±1.1 ^{a}	43.8±1.1 ^{a}
	Height	47.2±0.6 ^{a}	47.2±0.6 ^{a}	44.9±0.4 ^{a}	44.9±0.4 ^{a}	39.9±0.9 ^{a}	39.9±0.9 ^{a}

Each result is the average of 4 breads ± standard deviation. Lowercase letters indicate significant difference among samples of same refinement levels and significant difference between two locations Roseau (RS) and Rosemount (RM) at same bran (B) content determined according to the LSD means comparison test ($P \leq 0.05$).

Thus, the increase in length and width of IWG breads with dough conditioners, in comparison to the control, could be because of the low viscosity leading to expansion rather than rising. Pans used for baking were made of silicon, which is flexible. This might have allowed the dough to expand, resulting an increase in length and width of breads. However, once the breads were placed in the oven, a collapse was observed for 0B breads made without any conditioners, with WPI, VWG and TG. This phenomenon leads to a possibility that these dough conditioners improved the gas holding ability of the flour, but due to the low amount of starch and low gluten strength due to deficiency of HMW-GS (Marti et al., 2015; Rahardjo, 2017) could not hold the structure upon baking. Thus, further studies on looking at the protein structures will help establish the effect the conditioners had. Almeida and Chang (2014) reported French rolls that were proofed longer collapsed upon baking, which was related to the loss of strength to hold the gas. All breads were baked with the process optimized for wheat, and thus it could be possible that IWG requires a different proofing time than wheat. Thus, optimizing the proofing would offer insight on understanding the cause of collapse in 0B IWG breads. Sarabhai et al. (2017) evaluated the biscuit making potential of gluten free flours, and found the biscuits to be having higher spreads than wheat controls due to weaker protein networks. Another noticeable effect seen with these dough conditioners was the non-smooth surface of the crust and the holes on the walls of the bread (**Figure 14**), which was possibly caused due to the collapse of air cells.

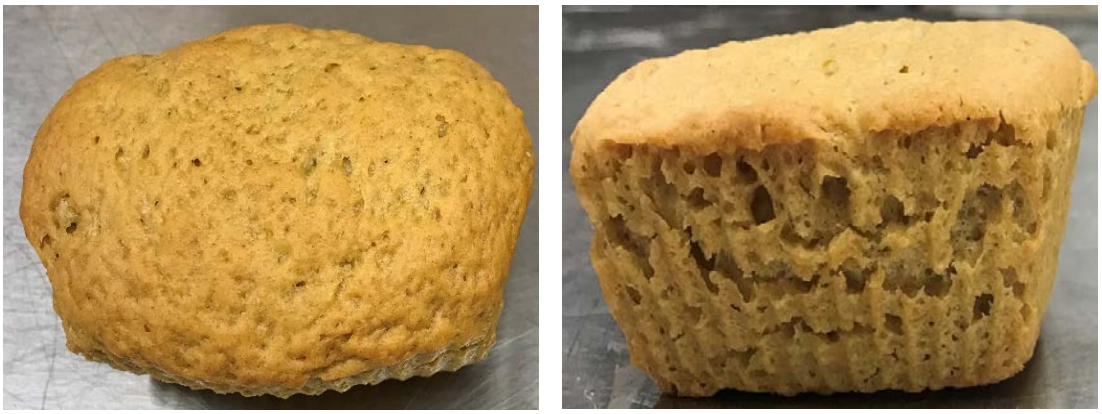

Figure 14. Breads made from refined Intermediate wheatgrass flour, top view (left) and side view (right).

While AA led to a decrease in length for 0B RM-IWG bread and 100B breads from both locations, the width and height were increased for 50B breads for IWG from both locations. An increase in width for RM-IWG and height for RS-IWG was noted at 100B. Though the effect of AA on dimensions was minor, AA caused a remarkable change in the appearance of the bread in comparison to IWG controls at 0B. The top surfaces of the bread loaves were smooth, and the walls did not have as many holes as the control breads had (**Figure 15**). Addition of PB either decreased or did not alter the length, and increased or did not alter the width, but did not affect height. While the bread length decreased only for RM-IWG at 0B, the length decreased for both location at 50B and 100B. Similarly, the width increased only for RS-IWG at 0B, but at 50B and 100B, the width increased for breads from both locations. Regardless of the fact that the effect of PB on bread dimensions was less pronounced in comparison to WPI and VWG, the appearance of breads at 0B was improved in a similar manner as for AA.

Figure 15. Surface appearance of breads made from refined Intermediate wheatgrass (IWG) flour (left) and refined IWG flour with Ascorbic acid (right).

For IWG from both locations, TG decreased all dimensions at all refinement levels and was especially apparent at 100B level (**Figures 16** and **17**).

Comparing the two locations for length, WPI and TG caused difference at all bran contents with RM-IWG being longer than RS-IWG at 0B, and RS-IWG being longer at 50B and 100B than RM-IWG. VWG, AA and PB also resulted in longer loafs than control at 0B; and breads from RS-IWG were longer than from RM-IWG. RM-IWG breads were wider than from RS-IWG at 0B and 50B. But for 100B, RS-IWG was wider than RM, which can be related to higher fiber content in RM-IWG than RS-IWG (**Table 7**) as bran fiber dilutes the gluten matrix (Schmiele et al., 2012) that would affect expansion. Samples different between two locations at 0B were control, samples with VWG, AA, PB and TG; for 50B were control, WPI, AA, PB and TG; and for 100B were control, WPI, VWG, PB and TG. Samples different in height between two locations at 0B were samples with WPI, VWG and TG; for 50B were WPI, VWG and TG; and for 100B

were control, WPI, VWG, AA, PB and TG. For those the samples significantly different, RS-IWG had higher height than RM-IWG.

Figure 16**.** Bread slices of hard red wheat, Intermediate wheatgrass (IWG) from Rosemount location without dough conditioner (control) and with five dough conditioners at respective bran (B) contents

Figure 17. Bread slices of hard red wheat, Intermediate wheatgrass (IWG) from Roseau location without dough conditioner (control) and with five dough conditioners at respective bran (B) contents

4.4.2.2. Specific volume

The specific volume of IWG breads was lower than wheat bread volumes for all refinement levels (**Figure 18**), i.e. breads were denser. When bran was added, the specific volume decreased, and the trend was same for all samples. While WPI, VWG, AA and PB either increased or did not alter the specific volume, TG always resulted in lower volumes at 0B and 50B refinement levels, but no difference was observed at 100B. For 0B samples from both locations, PB led to an increase in specific volume, but for 50B and 100B no difference was observed in specific volume for both locations when WPI, VWG, AA or PB were incorporated. This result is in accordance with findings of Grossmann et al. (2016) where TG decreased and xylanase increased the specific volume of rye breads. However, Schoenlechner et al. (2013) reported 25% increase in volume when a combination of TG and xylanase was added to bread made with 50:50 wheat/millet. The difference in the effect of TG between our study and theirs could be due to the amount used, the different raw material (millet/wheat blend), the combination of TG and xylanase, or other factors.

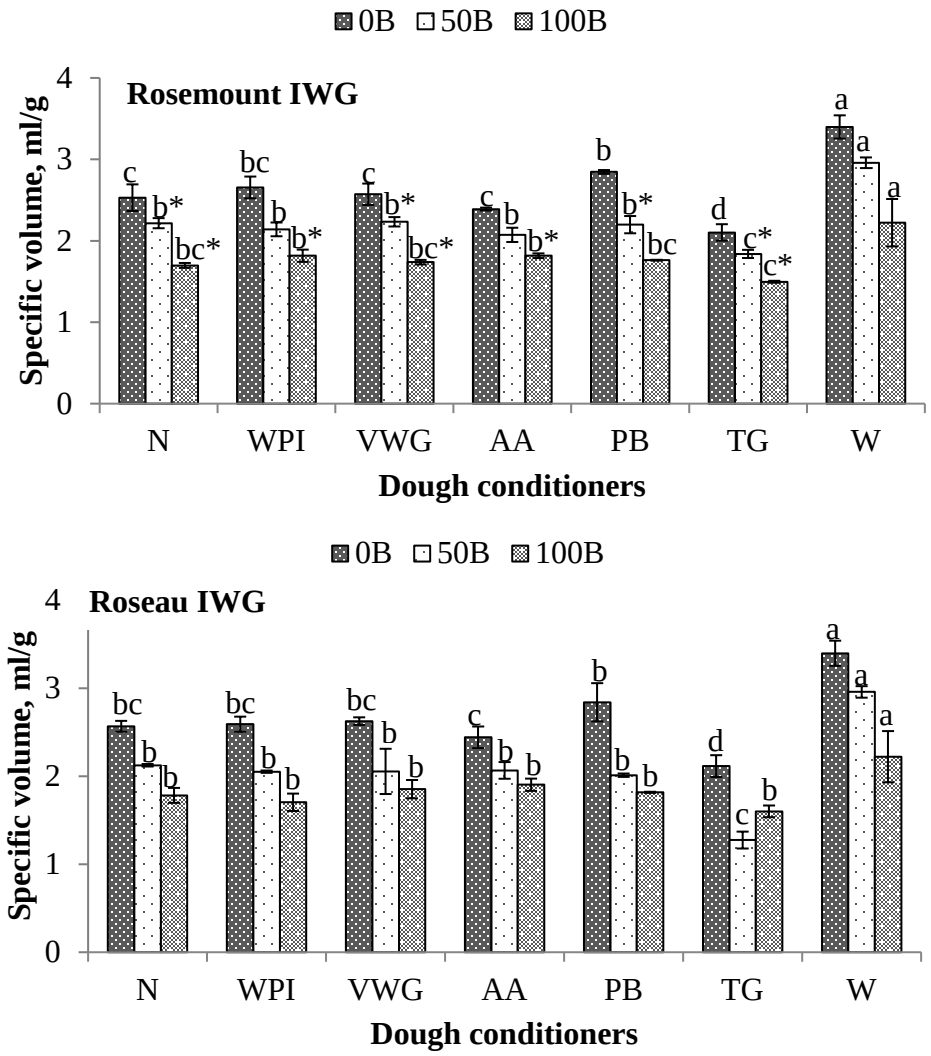

Figure 18. Specific volume for Intermediate wheatgrass (IWG) Rosemount (top) and Roseau (bottom). Different letters represent significant differences for samples (n=2) with the same bran (B) content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content. N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

Comparing the two locations, there were no differences within 0B samples. For 50B samples, control, IWG with VWG, PB, and TG were different. For all these cases, RM-IWG had higher specific volume than IWG-RS. Due to the fact that RM-IWG has higher bran content than RS-IWG (**Table 9**), its specific volume would be expected to be

RM-IWG lower because bran fiber dilutes the gluten matrix (Schmiele et al., 2012) which disrupts gas cells and negatively affects the bread quality (Lai et al., 1989). However, for 100B samples, control, WPI, VWG, AA, and TG were different between the two locations. In case of differences among locations, RS-IWG had higher specific volumes with the exception of 100B WPI, where specific volume for RM-IWG was higher.

4.4.2.3 Bread crumb firmness

In general, firmness values were similar among all samples (**Figure 19**), though there were some differences. For samples from RM-IWG at 0B, VWG increased the firmness. At 50B, samples with VWG, AA, as well as wheat had lower firmness than RM-IWG control, however, no difference in firmness was observed at 100B level.

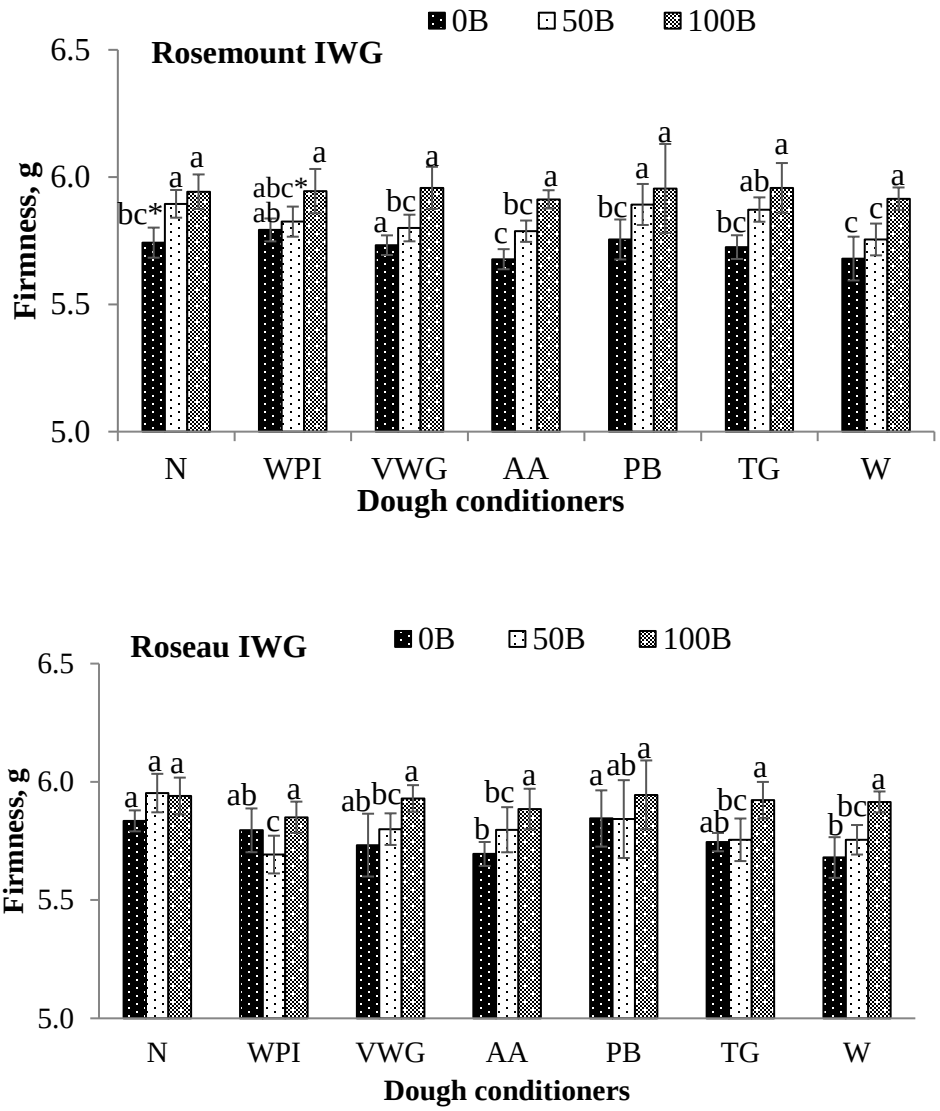

Figure 19. Crumb firmness of breads made from Intermediate wheatgrass (IWG) Rosemount (top) and Roseau (bottom). Different letters represent significant differences for samples (n=2) with the same bran (B) content, and asterisks indicate differences between dough from Roseau IWG and Rosemount IWG at the same bran content. N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

For samples from RS, AA and wheat had lower firmness than control at 0B, while other dough conditioners did not affect the firmness. However, for 50B samples, all

dough conditioners except PB had lower firmness than the control. There was no difference among 100B samples, indicating two possible conclusions - either no conditioners had an effect on firmness, or the effect of bran was so prominent that the effect of conditioner was not observed.

Comparing the two locations, only 0B IWG control and 50B WPI had different firmness, and RS-IWG had higher firmness. Rahardjo (2017) reported higher firmness in IWG breads in comparison to hard red wheat, the firmness being attributed to higher fiber in IWG in comparison to wheat. However, results from this research shows there is no difference in firmness at 100B level for IWG (with and without dough conditioners) and wheat. This difference could be because Rahardjo (2017) utilized novel breeding IWG populations grown at a different location than the IWG used in this study.

4.4.2.4 Crumb cell structure

The crumb cell count, average area and average cell size for different breads at different refinement levels are shown in **Table 12**. A higher number of alveoli represents a larger percentage of air trapped in the crumb (Salinas & Puppo, 2015). Higher counts in combination with smaller areas denote denseness. A porous crumb with uniform distribution of air cells is desirable.

Table 12. Effect of dough conditioners and bran refinement on crumb grain features of breads made with Intermediate wheatgrass from two locations Rosemount (RM) and Roseau (RS) and wheat (W)

B	Sample	N	WPI	VWG	AA	PB	TG	W
				Count				
0B	RM	247±3^{c*}	335±41^{a*}	366±2^{a}	129±6^{d}	292±21^{d}	265±13^{bc}	226±10^{c}
	RS	356±23^{b}	565±80^{a}	325±41^{bc}	104±4^{d}	309±13^{bc}	305±88^{bc}	226±10^{c}
50B	RM	403±2^{a}	347±53^{ab}	377±50^{ab}	333±9^{b}	316±9^{b*}	121±5^{c}	364±33^{ab}
	RS	393±29^{ab}	364±58^{ab}	357±42^{ab}	129±6^{c}	422±28^{a}	236±157^{bc}	364±33^{ab}
100B	RM	237±16^{bc*}	266±30^{bc}	286±63^{b}	234±2^{bc}	186±65^{c*}	93±22^{d}	421±1^{a}
	RS	311±28^{bc}	310±9^{bc}	264±98^{bc}	244±48^{c}	363±4^{ab}	86±7^{d}	421±1^{a}
				Cell area				
0B	RM	1.3± 0.2^{a}	1.4±0.1^{a}	1.4±0.0^{a}	1.2±0.1^{a}	1.3±0.0^{a}	1.2±0.0^{a}	1.3±0.0^{a}
	RS	1.3± 0.2^{a}	1.6±0.1^{a}	1.4±0.0^{a}	1.1±0.1^{a}	1.3±0.1^{a}	1.2±0.1^{a}	1.3±0.0^{a}
50B	RM	1.6± 0.0^{ab}	1.6±0.2^{ab}	1.7±0.1^{a*}	1.6±0.0^{ab}	1.6±0.2^{a}	1.1±0.2^{c}	1.3±0.1^{bc}
	RS	1.5± 0.0^{a}	1.3±0.2^{a}	1.4±0.1^{a}	1.2±0.1^{a}	1.5±0.0^{a}	1.3±0.4^{a}	1.3±0.1^{a}
100B	RM	2 ±0.0^{a*}	1.9±0.1^{a}	1.9±0.1^{a}	2±0.1^{a}	2.1±0.0^{a*}	1.5±0.3^{b}	1.5±0.0^{b}
	RS	1.8± 0.0^{ab}	1.8±0.0^{ab}	1.9±0.1^{ab}	1.9±0.0^{a}	1.7±0.0^{b}	1.1±0.1^{d}	1.5±0.0^{c}
				Average cell size				
0B	RM	0.006±0.001^{bc}	0.004±0.000^{cd*}	0.004±0.000^{d}	0.01±0.000^{a}	0.005±0.000^{cd}	0.005±0.000^{bcd}	0.006±0.000^{b}
	RS	0.004±0.001^{c}	0.003±0.000^{c}	0.004±0.001^{bc}	0.011±0.001^{a}	0.004±0.000^{bc}	0.004±0.001^{c}	0.006±0.000^{b}
50B	RM	0.004±0.000^{b}	0.005±0.000^{b}	0.005±0.001^{b}	0.005±0.000^{b}	0.005±0.001^{b*}	0.009±0.002^{a}	0.004±0.000^{b}
	RS	0.004±0.000^{c}	0.004±0.000^{bc}	0.004±0.000^{c}	0.01±0.001^{a}	0.004±0.000^{c}	0.007±0.002^{ab}	0.004±0.000^{c}
100B	RM	0.009±0.000^{ab*}	0.008±0.002^{b}	0.013±0.010^{ab}	0.009±0.000^{ab}	0.013±0.004^{ab*}	0.021±0.007^{a}	0.004±0.000^{b}
	RS	0.006±0.001^{b}	0.006±0.000^{b}	0.009±0.004^{b}	0.009±0.002^{b}	0.005±0.000^{b}	0.019±0.007^{a}	0.004±0.000^{b}

Each parameter is the average of three repetitions of two sub-replicates per sample. Lowercase letters in respective rows indicate significant differences according to the Least Significant Differences mean comparison test ($P \leq 0.05$) at same bran (B) content and asterisks represent significant difference between the two locations according to two tailed t-test ($P \leq 0.05$) at same bran content. N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

Addition of bran and using dough conditioners influenced the number of alveoli (**Figure 20**). Despite the differences observed on the number of alveoli, similar trends could be generated when the counts were divided into three cell ranges: greater than 0.1mm^2, between 0.1 and 4 mm^2 and between 0.04 and 4 mm^2 based on Farrera-Rebollo et al. (2012). The results for count, area and average cell size for those three ranges can be found in Appendix B. The reason for selecting these cell size ranges is because particles smaller than 0.2 mm cannot be distinguished by the human eye, and may not be perceived as crumb cells (Farrera-Rebollo et al., 2012).

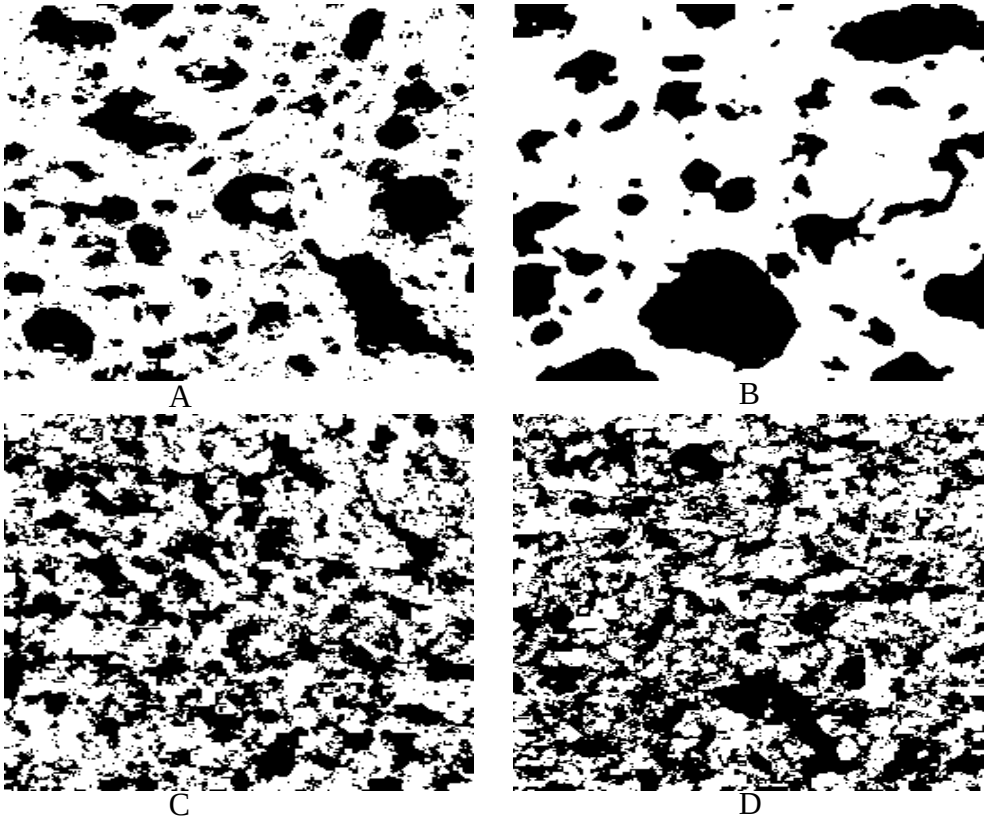

Figure 20. Binarized images of bread slices that differentiates gas cells and non-gas cells as produced by imageJ. A- 0 %bran intermediate wheatgrass (IWG) from Roseau; B- 0 %bran IWG from Roseau with Ascorbic acid (AA); C- 100 %bran IWG from Roseau; D- 100 %bran IWG from Roseau with AA

There were no differences in count at 0B between wheat and RM-IWG control, however, RS-IWG had higher counts than wheat. No difference was seen at 50B, but IWG controls had lower count than wheat breads at 100B. There were no differences in the cell area between wheat and IWG controls at 0B and 50B, however, at 100B wheat had lower count than IWG controls from both locations. Average cell size between wheat and IWG control from both locations was not different for any refinement level. While the count increased for both locations with WPI addition at 0B, no other differences was observed at any refinement level for count, cell area and average cell size. When VWG was used, the count for 0B RM-IWG increased. The same sample had a reduced average cell size indicating the numerous and smaller gas cells. As explained earlier, WPI and VWG dough expanded and collapsed upon baking which might have caused the disruption of air cells causing an increased number of counts.

Ascorbic acid lead to a decrease in count for 0B and 50B samples from both locations, consequently increasing the average cell size in 0B samples from both locations and 50B RS-IWG. This indicates a more open, porous crumb, also visible in **Figures 16** and **17**. A similar effect was reported by Horvat et al. (2007) when adding ascorbic acid to flour with medium dough strength. PB caused an increase in count only for 0B and 50B RM-IWG, with no change observed in average cell size.

TG did not affect crumb grain characteristics at 0B. However, 50B RM-IWG, 100B RM-IWG and RS-IWG had a lower count, lower cell area, and higher average cell size. Generally the expectation is that when the count decreases, the cell area increases. However, for TG this was not the case because the crumb was dense and very few gas

cells were present. The tighter crumb would also mean the gas cells are very small, which is supported by the data for TG at higher bran level. This is supported by the results from lower bread dimensions and specific volume. The combined effect of protein cross linking by TG and high fiber could have caused the breads to be denser for 100B breads. Another cause could be because TG we used mainly contained maltodextrin, which could have competed with water, making water unavailable for proteins.

Overall, there was a wide distribution in crumb grain characteristics for TG, especially at 100B levels. However, the distribution was narrow for AA and PB, indicating the crumb grain uniformity that was facilitated by the gas cell growth during fermentation.

4.5 Conclusion

The deficiency of starch and HMW-GS, and high fiber content, affected IWG bread properties. Several dough conditioners improved the workability by reducing the stickiness (WPI, VWG and TG) and also improved IWG bread properties (height- WPI, VWG; specific volume- PB; smooth surface- AA, PB; crumb characteristics- AA, PB) with effect most evident at 0B content. As refinement would cause a loss compounds with beneficial health effects like dietary fiber, a 50% bran level could be a balanced compromise between the nutritional and functional aspects. The complexity of the bread baking mechanisms due to the interaction of starch, proteins and dough conditioners should further be evaluated to consider using IWG as a standalone flour for breads.

Chapter 5:
Concluding Remarks and Next Steps

This work was the first to analyze the effect of bran content in combination with dough conditioners on IWG dough rheology and bread quality.

As a consequence of IWG kernels being thinner and lighter than wheat, IWG had different bran to endosperm contents. This caused differences in fat, protein, ash, fiber and starch content from wheat at respective refinement levels. In addition, the growing location influenced bran to endosperm ratios as well as protein and insoluble dietary fiber contents. These variations were associated with differences in functionality.

Each dough conditioner affected certain parameters of IWG. However, no conditioner improved both dough properties as well as bread quality at all refinement levels. Dough with TG displayed the most improved properties over the control dough, in terms of stickiness and resistance to extension. However, TG resulted in unacceptably dense breads, and thus using TG at the tested level for bread is not recommended. While addition of wheat proteins, either in the form of WPI or VWG, resulted in some improvements in dough properties and bread loafs, the effects were most pronounced in samples at lower bran content, and from the Roseau location. However, a noticeable collapse of breads when in the caused loafs to have an uneven appearance and rough surface. In contrast, AA and PB addition neither altered dough viscoelasticity nor workability, but produced loafs of consistent appearance, smoother surfaces and open crumb with uniformly distributed gas cells. These improvements were noticeable only at refined and partially refined flour in comparison to whole IWG flour, indicating the large influence of bran on bread quality.

Roseau-IWG consistently displayed better functional properties than Rosemount-IWG. These differences can be traced back to its higher protein, and lower dietary fiber content. However, differences among protein types (albumin/globulins vs. prolamins), and protein secondary structures were not investigated, and the content and structure of the fiber is not known. Future works could investigate the effect of dough conditioners on these above mentioned parameters to understand the effect of dough conditioners better. Future work to improve IWG breads could also investigate the effect of the combination of dough conditioners, and optimize it. This work investigated the effect of dough conditioners at single concentration. Studying bread properties at several concentrations of each dough conditioners will help in determining the best concentration for IWG breads. Modeling systems could be used to optimize dough conditioners' concentration. Evaluating IWG breads for consumer acceptance through sensory analysis could be another step towards introducing IWG breads in the current food supply chain.

We observed that IWG had a tendency to expand horizontally instead of rising vertically, due to insufficient gluten strength. A horizontal expansion would still lead to an increased specific volume, however, it does not indicate improved bread quality. This highlights the necessity to report multiple aspects of bread quality, including the overall dimensions, when processing conditions, particularly baking equipment (silicon pans in our case), allow for horizontal expansion. This importance of using a multi-dimensional approach to characterize dough and bread, and overall IWG performance, was evident throughout our work. Analyzing dough properties only would have suggested that AA and PB are not suitable for use in IWG breads, while, analyzing bread only would not

have captured the beneficial effects of TG on rheology. Thus, a multi-dimensional approach is needed to explore the possibility of novel grains like IWG to be used as standalone flour for food applications.

Overall, our results indicate that with optimized processing conditions, IWG has the potential of being used for bread-making. With respect to current breeding lines, at least partially refined IWG is recommended considering the nutritional and functional synergy. While this would reduce the amount of dietary fiber and protein that whole IWG can provide, it is worth noting that even 0B samples contained 14.4 - 16.7% protein and 4.4 - 4.9% dietary fiber. Considering the nutritional and the environmental benefits that IWG offers, it is advisable integrating IWG into food processing and commercialization, which would lead to a win-win situation of environmental welfare and a sustainable alternative to annual grains.

Chapter 6:
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Chapter 7:
Appendices

7.1 Appendix A. Standard Operating Procedures (SOP)

7.1.1 Extensibility and resistance to extension with Kieffer dough and gluten extensibility rig

Preparing the dough:

1. The dough can be prepared either on Brabender Farinograph or a kitchen aid (lab 95)
2. For doughs made with Farinograph, take the dough once it reaches the 500BU line
3. Once the dough is ready, place the dough in the plastic box (which is with the Kieffer rig box in lab 5) for 20 minutes. The aim is to keep the dough at a constant temperature, allowing it to hydrate
4. While waiting, use the paint brush to oil the molder and put the paper strips in each section of the molder. Use mineral oil that is in the tube (placed in the plastic box)

Molding the dough:

5. After 20 minutes, roll the dough by hand and placed it on the Kieffer molder (TA-105)
6. Slide the molder through the black holder and screw it tight such that the dough seeps out of the grooves of the molder
7. Remove the excess dough that seeped out of the molder
8. Put the molder in a zip lock bag, and allow it to rest for 40 minutes
9. After 40 minutes, analyze the dough with texture analyzer.

Texture analyzer:

10. Turn on the texture machine with the power switch (back of the machine)
11. Turn on the computer
12. Turn on the TA.XT exponent file, log in with your name and password, and the exponent loads
13. Close the “tip of the day” window
14. In the exponent window, go to index, and find “Kieffer Extensibility Rig”
15. Another window opens, and select “Extensibility of dough and measure of gluten quality”
16. Select display, and load the project
17. A new window opens
18. Go to settings-> calibrate -> calibrate force using both loads 2kg and 5 kg
19. Make sure you have the 5 kg load cell attached to the TA head
20. Go to settings-> advanced option on-> trigger force-> 0 for IWG and 5 for wheat

21. Attach the kieffer dough and gluten extensibility rig to the second opening of the TA head
22. Place the kieffer platform as a base for the machine, making the screw handle facing the front
23. Make sure the tip of the kieffer rig is below the open section of the platform such that once the dough strips are placed, the rig can pull the strip to measure extensibility and resistance to extension
24. Take the molder, unscrew it, and slide the molder to expose the dough strips
25. Discard the first two strips and take third strip with the spatula from the Kieffer dough and gluten extensibility rig box
26. Place the dough strip on the tray and peel the paper strip with care so as to avoid any deformation to the dough strips
27. Push the handle of the platform so that a space form in between, and place the tray with dough into that space
28. Run a test and name the file correctly and save it in proper folder
29. Exclude the last two strips from the molder
30. After all the tests, select all-> macro-> run
31. A new window opens with all the results. Save the outputs by copying the results in the Excel.

7.1.2 Dough stickiness with Chen – Hoseney stickiness cell

Preparing the dough:

1. The dough can be prepared either on Brabender Farinograph or a kitchen aid (lab 95)
2. For doughs made with Farinograph, take the dough once it reaches the 500BU line

Texture analyzer:

3. Turn on the texture machine with the power switch (back of the machine)
4. Turn on the computer
5. Turn on the TA.XT exponent file, log in with your name and password, and the exponent loads
6. Close the “tip of the day” window
7. In the exponent window, go to index, and find “Stickiness”
8. Another window opens, and select “Measurement of dough stickiness”
9. Select display, and load the project
10. A new window opens
11. Go to settings-> calibrate -> calibrate force using both loads 2kg and 5 kg
12. Make sure you have the 5 kg load cell attached to the TA head.

13. Attach the 25 mm cylinder probe (TA-11) to the second opening of the TA head.

Using the stickiness cell:

14. Unscrew the cell so that the extruder lid comes off.
15. Rotate the internal screw such that the piston goes all the way down, and there is space to put the dough
16. Place about 10g of dough into the space created.
17. Remove excess of the dough by scraping with spatula such that it levels off with the cell.
18. Screw the extruder lid
19. Rotate the internal screw a little such that small amount of dough is extruded out through the holes.
20. Discard that extrudate, and repeat the process to extrude about 1 mm dough.
21. Place the perspex cap over the exposed sample to minimize sample moisture loss.
22. Allow the dough to rest for 30 seconds to release the pressure produced through extrusion
23. Remove the perspex cap after 30 seconds, and place the cell under the 25 mm cylinder probe that is attached to the texture analyzer

Texture analyzer:

24. Run a test and name the file correctly and save it in proper folder.
25. After all the tests, select all-> macro-> run
26. A new window opens with all the results. Save the outputs by copying the results in the Excel

7.1.3 Bread crumb firmness with Texture analyzer AACCI method 74-09.01

Slicing of bread:

1. Slice the bread on the bread slicer in lab 95.
2. Put all the slices in a zip lock bag and label them properly

Texture analyzer:

3. Turn on the texture machine with the power switch (back of the machine)
4. Turn on the computer
5. Turn on the TA.XT exponent file, log in with your name and password, and the exponent loads.
6. Close the “tip of the day” window.
7. In the exponent window, go to index, and find “Bread”
8. Another window opens, and select “Determination of bread firmness using AACCI 74-09 standard method”
9. Select display, and load the project

10. A new window opens
11. Go to settings-> calibrate -> calibrate force using both loads 2kg and 5 kg
12. Make sure you have the 5 kg load cell attached to the TA head.
13. Use the acrylic probe (similar to TA-510A - not sure with number but the dimensions are similar- 10mm diameter, 45 mm long) with dimension 13mm diameter and 35 mm length.
14. Place the probe on the second opening of the TA head.
15. Place the square platform as a base for the test
16. Go to settings-> advanced settings on-> target mode-> select distance instead of strain
17. Place each slice on the platform such that the probe lands around the center of the bread slice.
18. Run a test and name the file correctly and save it in proper folder.
19. After all the tests, select all-> macro-> run
20. A new window opens with all the results. Save the outputs by copying the results in the Excel.

7.1.4 Bread crumb cell analysis with ImageJ

Scanning the bread:

1. Scan the bread in the scanner at lab 107 or an equivalent (e.g. in the computer labs)
2. Save the image as a color, as well as 8-bit grayscale, 600dpi.

Setting up ImageJ:

3. Download ImageJ for free at https://imagej.nih.gov/ij/download.html , ij150.zip
4. Open ImageJ (select the microscope icon- application file)
5. Run the program (it asks you every time you open the file)
6. Go to Plugins-> Macro-> Record
7. A new window will open, which has a section for Name. Choose a file name, e.g. Breadimage
8. Paste the following codes in the blank section:

```
run("Set Scale...", "distance=140 known=1 unit=cm global");
makeRectangle(2520, 4232, 584, 520);
run("Specify...", "width=2 height=2 x=18 y=30  scaled");
run("Crop");
setAutoThreshold("Otsu");
//run("Threshold...");
setOption("BlackBackground", false);
run("Convert to Mask");
run("Analyze Particles...", "size=0.0001-Infinity display summarize");
```

9. Hit the “create” button, and this will lead you to a new window with the same name and the codes you specified.

10. Go to File-> Save As and save it in the plugins folder as "Breadimage.ijm". The file extension should be .ijm

Analyzing the bread slice:

11. Once saved, go to ImageJ, File-> Open. Select the saved scanned image you want to analyze.
12. The image you want to analyze opens in a new ImageJ window.
13. Go to ImageJ, File-> Open. Select the macro ("Breadimage.ijm" in our example) from the plugins folder (step 10)
14. The macro opens in a new ImageJ window as ."Breadimage.ijm"
15. Select the code, go to Macros-> Run Macros or press Ctrl+R.
16. Save all the outputs by copying the results to Excel.

7.2 Appendix B. Mean gas cell count and area in crumb of breads made with wheat and intermediate wheatgrass from Rosemount and Roseau along with different level dough conditioners at three refinement levels

Table 13. Mean crumb cell count and area greater than 0.1 mm^2 for breads made from Intermediate wheatgrass (IWG) and wheat (W) at different refinement levels

Samples	Parameters	0B		50B		100B	
		RM	RS	RM	RS	RM	RS
N	count	68±3 ^{b}	72±4 ^{bc}	90±2 ^{a}	88±7 ^{a}	21±1 ^{d*}	53±4 ^{bc}
	area	1.95±0.34 ^{a}	1.72±0.24 ^{cd}	1.63±0.18 ^{bc}	1.53±0.28 ^{a}	9.87±0.65 ^{a*}	3.33±0.31 ^{c}
WPI	count	78±6 ^{a}	72±5 ^{bc}	78±6 ^{b}	77±5 ^{b}	26±5 ^{d*}	50±3 ^{cd}
	area	1.75±0.16 ^{a*}	2.19±0.31 ^{abc}	1.79±0.37 ^{bc}	1.92±0.22 ^{a}	7.99±1.53 ^{ab*}	3.54±0.25 ^{bc}
VWG	count	81±5 ^{a}	83±5 ^{a}	72±3 ^{bc*}	89±4 ^{a}	24±2 ^{cd*}	56±4 ^{b}
	area	1.63±0.13 ^{a}	1.66±0.10 ^{d}	2.21±0.11 ^{b*}	1.54±0.15 ^{a}	8.74±1.12 ^{a*}	3.09±0.28 ^{c}
AA	count	69±6 ^{b*}	49±3 ^{e}	79±7 ^{b}	70±3 ^{bc}	37±4 ^{b*}	42±2 ^{e}
	area	1.75±0.21 ^{a*}	2.49±0.24 ^{a}	1.86±0.43 ^{bc}	2.00±0.23 ^{a}	5.40±0.71 ^{c*}	4.27±0.22 ^{a}
PB	count	70±4 ^{b*}	60±1 ^{d}	58±4 ^{d*}	88±6 ^{a}	32±5 ^{b*}	48±4 ^{d}
	area	1.80±0.16 ^{a*}	2.32±0.24 ^{ab}	2.99±0.31 ^{a*}	1.60±0.31 ^{a}	6.36±1.05 ^{bc*}	3.67±0.31 ^{abc}
TG	count	64±2 ^{b*}	76±4 ^{b}	69±4 ^{c*}	66±3 ^{c}	26±2 ^{c*}	46±3 ^{de}
	area	1.64±0.32 ^{a}	1.45±0.24 ^{d}	1.79±0.27 ^{bc}	1.82±0.51 ^{a}	7.74±1.00 ^{ab*}	3.78±0.31 ^{ab}
W	count	67±6 ^{b}	67±6 ^{c}	77±5 ^{b}	77±5 ^{b}	86±3 ^{a}	86±3 ^{a}
	area	1.87±0.15 ^{a}	1.87±0.15 ^{bcd}	1.54±0.19 ^{c}	1.54±0.19 ^{a}	1.67±0.08 ^{d}	1.67±0.08 ^{d}

N = Sample without dough conditioner, WPI = Wheat Protein Isolate, VWG = Vital Wheat Gluten, AA = Ascorbic Acid, PB = Powerbake and TG = Transglutaminase. Each result is the average of 4 slices ± standard deviation. Lowercase letters indicate significant difference among samples of same refinement levels from the same location and asterisks indicate significant difference between two locations determined by the LSD means comparison test ($P \leq 0.05$). N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

Table 14. Mean crumb cell count and area between 0.1 and 4 mm^2 for different kinds of breads made from Intermediate wheatgrass (IWG) and wheat (W) at different refinement levels

Samples	Parameters	0B		50B		100B	
		RM	RS	RM	RS	RM	RS
N	count	58±7 ^{b}	64±4 ^{bc}	82±3 ^{a}	81±7 ^{a}	18±2 ^{c*}	46±4 ^{b}
	area	0.79±0.01 ^{ab}	0.80±0.04 ^{b}	0.83±0.10 ^{a}	0.87±0.07 ^{a}	0.53±0.10 ^{a*}	0.70±0.05 ^{a}
WPI	count	69±5 ^{a}	64±4 ^{bc}	69±8 ^{b}	68±3 ^{b}	23±4 ^{c*}	44±5 ^{bc}
	area	0.82±0.02 ^{ab}	0.77±0.08 ^{b}	0.91±0.05 ^{a}	0.85±0.11 ^{a}	0.61±0.16 ^{a}	0.68±0.18 ^{a}
VWG	count	73±4 ^{a}	73±6 ^{a}	63±5 ^{bc*}	79±5 ^{a}	22±2 ^{c*}	46±4 ^{b}
	area	0.89±0.09 ^{ab}	0.78±0.06 ^{b}	0.83±0.14 ^{a}	0.82±0.09 ^{a}	0.58±0.20 ^{a}	0.85±0.08 ^{a}
AA	count	61±5 ^{b*}	39±5 ^{e}	70±8 ^{b}	64±3 ^{bc}	33±3 ^{b*}	39±1 ^{c}
	area	0.93±0.08 ^{a}	1.00±0.10 ^{a}	0.88±0.09 ^{a}	0.99±0.03 ^{a}	0.65±0.23 ^{a}	0.67±0.13 ^{a}
PB	count	60±5 ^{b*}	51±1 ^{d}	49±4 ^{d*}	79±7 ^{a}	28±5 ^{b*}	40±4 ^{c}
	area	0.80±0.07 ^{ab}	0.82±0.05 ^{ab}	0.79±0.13 ^{a}	0.83±0.07 ^{a}	0.75±0.13 ^{a}	0.75±0.12 ^{a}
TG	count	58±3 ^{b*}	69±3 ^{ab}	60±5 ^{c}	59±3 ^{c}	23±3 ^{c*}	39±1 ^{c}
	area	0.72±0.10 ^{b}	0.84±0.14 ^{ab}	0.81±0.08 ^{a}	0.85±0.15 ^{a}	0.83±0.26 ^{a}	0.77±0.04 ^{a}
W	count	60±5 ^{b}	60±5 ^{c}	70±3 ^{b}	70±3 ^{b}	78±5 ^{a}	78±5 ^{a}
	area	0.87±0.07 ^{ab}	0.87±0.07 ^{ab}	0.77±0.04 ^{a}	0.77±0.04 ^{a}	0.74±0.11 ^{a}	0.74±0.11 ^{a}

N = Sample without dough conditioner, WPI = Wheat Protein Isolate, VWG = Vital Wheat Gluten, AA = Ascorbic Acid, PB = Powerbake and TG = Transglutaminase. Each result is the average of 4 slices ± standard deviation. Lowercase letters indicate significant difference among samples of same refinement levels from the same location and asterisks indicate significant difference between two locations determined by the LSD means comparison test ($P \leq 0.05$). N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

Table 15. Mean crumb cell count and area between 0.04 and 4 mm^2 for breads made from Intermediate wheatgrass (IWG) and wheat (W) at different refinement levels

Samples	Parameters	0B		50B		100B	
		RM	RS	RM	RS	RM	RS
N	count	125±20 ^{cd}	143±14 ^{b}	168±22 ^{a}	172±5 ^{a}	55±8 ^{d*}	103±12 ^{b}
	area	0.40±0.03 ^{c}	0.40±0.05 ^{bc}	0.44±0.04 ^{ab}	0.45±0.02 ^{b}	0.21±0.02 ^{b*}	0.35±0.03 ^{bc}
WPI	count	147±17 ^{b}	173±20 ^{a}	150±7 ^{ab*}	132±10 ^{bc}	67±11 ^{bc*}	113±14 ^{b}
	area	0.42±0.03 ^{bc*}	0.32±0.03 ^{c}	0.46±0.04 ^{ab}	0.47±0.05 ^{b}	0.25±0.05 ^{b}	0.30±0.07 ^{c}
VWG	count	172±8 ^{a}	163±12 ^{a}	154±17 ^{ab}	156±8 ^{ab}	66±10 ^{bcd*}	102±20 ^{b}
	area	0.40±0.06 ^{bc}	0.40±0.03 ^{bc}	0.38±0.05 ^{b}	0.46±0.06 ^{b}	0.24±0.08 ^{b*}	0.43±0.05 ^{ab}
AA	count	98±5 ^{e*}	64±10 ^{d}	144±6 ^{b*}	103±22 ^{d}	76±2 ^{b*}	99±6 ^{b}
	area	0.60±0.04 ^{a}	0.65±0.15 ^{a}	0.47±0.06 ^{ab*}	0.66±0.11 ^{a}	0.32±0.11 ^{ab}	0.31±0.06 ^{c}
PB	count	133±18 ^{bc}	119±10 ^{c}	121±5 ^{c*}	178±19 ^{a}	61±5 ^{cd*}	113±9 ^{b}
	area	0.40±0.04 ^{c}	0.39±0.04 ^{bc}	0.36±0.05 ^{b}	0.41±0.05 ^{b}	0.38±0.07 ^{ab}	0.31±0.03 ^{c}
TG	count	118±6 ^{cd*}	143±17 ^{b}	100±11 ^{d}	117±22 ^{cd}	41±7 ^{e*}	64±4 ^{c}
	area	0.39±0.04 ^{c}	0.44±0.04 ^{bc}	0.52±0.06 ^{a}	0.47±0.05 ^{b}	0.49±0.15 ^{a}	0.49±0.05 ^{a}
W	count	108±11 ^{de}	108±11 ^{c}	159±11 ^{ab}	159±11 ^{a}	172±6 ^{a}	172±6 ^{a}
	area	0.51±0.04 ^{ab}	0.51±0.04 ^{ab}	0.38±0.03 ^{b}	0.38±0.03 ^{b}	0.38±0.06 ^{ab}	0.38±0.06 ^{bc}

N = Sample without dough conditioner, WPI = Wheat Protein Isolate, VWG = Vital Wheat Gluten, AA = Ascorbic Acid, PB = Powerbake and TG = Transglutaminase. Each result is the average of 4 slices ± standard deviation. Lowercase letters indicate significant difference among samples of same refinement levels from the same location and asterisks indicate significant difference between two locations determined by the LSD means comparison test ($P \leq 0.05$). N- None, (Intermediate wheatgrass without dough conditioner), WPI- Wheat Protein Isolate, VWG- Vital Wheat Gluten, AA- Ascorbic Acid, PB- Powerbake, TG- Transglutaminase and W- Wheat

7.3 Appendix C. Sample of R code

```
X<-(name of your .csv file that you load in R)
X$Treatments <-as.factor(paste(X$Treatment you have- usually category variable))
var <-X$`variable you have`
test.aov <- aov(var ~ Treatments, data=X)
summary(test.aov)
TukeyHSD(test.aov)
library(agricolae)
LSD<-LSD.test(test.aov, 'Treatments')
LSD
```

Example:
Name of the file: RM50B_cell_area

treatment	total area
0	22
0	25
0	21
.	.
.	.
.	.
5	11

```
rs<-(RM50B_cell_area)
rs$Treatments <-as.factor(paste(rs$treatment))
var <-rs$`total area`
test.aov <- aov(var ~ Treatments, data=rs)
summary(test.aov)
TukeyHSD(test.aov)
library(agricolae)
LSD<-LSD.test(test.aov, 'Treatments')
LSD
```

7.4 Appendix D. Analysis of Variance (ANOVA) summary tables for determining significant differences among samples

Table 16. Analysis of Variance summary table for physical properties of wheat, intermediate wheatgrass from Rosemount and Roseau

Parameters analyzed	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
Length	Treatment	2	8.180	4.088	18.810	< 0.001
	Residuals	147	31.950	0.217		
Width	Treatment	2	70.360	35.180	1264.000	0.000
	Residuals	147	4.090	0.030		
Length/width	Treatment	2	292.110	146.050	575.000	0.000
	Residuals	147	37.340	0.250		
Weight of 1000 kernels	Treatment	2	988.100	494.100	9045.000	< 0.001
	Residuals	6	0.300	0.100		
volume of 1000 kernels	Treatment	2	1204.700	602.300	6776.000	< 0.001
	Residuals	6	0.500	0.100		
Bulk density	Treatment	2	0.088	0.044	495.400	< 0.001
	Residuals	6	0.001	0.000		

Table 17. Analysis of Variance summary table for the proximate analysis for wheat, intermediate wheatgrass from Rosemount and Roseau at three refinement levels

Parameters analyzed	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
Moisture	0B	Treatment	2	15.380	7.690	369.400	<0.001
		Residuals	3	0.062	0.021		
	50B	Treatment	2	18.840	9.420	639.400	<0.001
		Residuals	3	0.044	0.015		
	100B	Treatment	2	25.915	12.958	1540.000	<0.001
		Residuals	3	0.025	0.008		
Fat	0B	Treatment	2	0.026	0.013	8.113	0.062
		Residuals	3	0.005	0.002		
	50B	Treatment	2	3.025	1.513	68.490	0.003
		Residuals	3	0.066	0.022		
	100B	Treatment	2	8.987	4.494	393.800	<0.001
		Residuals	4	0.046	0.011		
Protein	0B	Treatment	2	37.060	18.531	3063.000	<0.001
		Residuals	3	0.020	0.006		
	50B	Treatment	2	33.890	16.946	1168.000	<0.001
		Residuals	3	0.040	0.015		
	100B	Treatment	2	30.048	15.024	5516.000	<0.001
		Residuals	3	0.008	0.003		
Ash	0B	Treatment	2	0.080	0.040	239.400	<0.001
		Residuals	6	0.001	0.000		
	50B	Treatment	2	1.490	0.745	753.400	<0.001
		Residuals	6	0.006	0.001		
	100B	Treatment	2	5.883	2.942	9129.000	<0.001
		Residuals	6	0.002	0.000		
CHO	0B	Treatment	2	8.341	4.171	81.090	0.002
		Residuals	3	0.154	0.051		
	50B	Treatment	2	16.514	8.257	150.900	0.001
		Residuals	3	0.164	0.055		
	100B	Treatment	2	26.509	13.255	1767.000	<0.001
		Residuals	3	0.023	0.008		

Table 18. Analysis of Variance summary table for the insoluble, soluble and total dietary fiber in wheat, intermediate wheatgrass from Rosemount (RM) and Roseau (RS) at three refinement levels

Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
0B	Treatment	2	3.523	1.761	8.758	0.056
	Residuals	3	0.603	0.201		
50B	Treatment	2	55.580	27.791	971.100	< 0.001
	Residuals	3	0.090	0.029		
100B	Treatment	2	145.740	72.870	278.800	< 0.001
	Residuals	3	0.780	0.260		
0B	Treatment	2	1.059	0.530	3.136	0.184
	Residuals	3	0.507	0.169		
50B	Treatment	2	2.938	1.469	2.286	0.249
	Residuals	3	1.928	0.643		
100B	Treatment	2	0.085	0.042	0.099	0.908
	Residuals	3	1.277	0.426		
0B	Treatment	2	2.294	1.147	4.379	0.129
	Residuals	3	0.786	0.262		
50B	Treatment	2	83.530	41.760	72.120	0.003
	Residuals	3	1.740	0.580		
100B	Treatment	2	140.710	70.360	121.400	0.001
	Residuals	3	1.740	0.580		

Table 19. Analysis of Variance summary table for extensibility of wheat, intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	4314.000	718.900	114.600	<0.001
		Residuals	7	44.000	6.300		
	50B	Treatment	6	2017.400	336.200	192.600	<0.001
		Residuals	7	12.200	1.700		
	100B	Treatment	6	1053.600	175.610	24.490	<0.001
		Residuals	7	50.200	7.170		
RS	0B	Treatment	6	3459.000	576.500	21.620	<0.001
		Residuals	7	187.000	26.700		
	50B	Treatment	6	1387.300	231.220	64.040	<0.001
		Residuals	7	25.300	3.610		
	100B	Treatment	6	713.000	118.830	15.780	0.001
		Residuals	7	52.700	7.530		

Table 20. Analysis of Variance summary table for resistance to extension of wheat, intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	1181.700	196.960	170.100	< 0.001
		Residuals	7	8.100	1.160		
	50B	Treatment	6	1433.300	238.880	60.730	< 0.001
		Residuals	7	27.500	3.930		
	100B	Treatment	6	887.900	147.980	7.241	0.010
		Residuals	7	143.000	20.440		
RS	0B	Treatment	6	936.800	156.140	109.500	< 0.001
		Residuals	7	10.000	1.430		
	50B	Treatment	6	1332.000	222.000	77.360	< 0.001
		Residuals	7	20.100	2.870		
	100B	Treatment	6	1607.200	267.860	17.460	0.001
		Residuals	7	107.400	15.340		

Table 21. Analysis of Variance summary table for extensibility of intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different levels of wheat protein isolate at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	2	72.050	36.030	5.558	0.098
		Residuals	3	19.450	6.480		
	50B	Treatment	2	27.645	13.823	69.890	0.003
		Residuals	3	0.593	0.198		
	100B	Treatment	2	8.512	4.256	39.190	0.007
		Residuals	3	0.326	0.109		
RS	0B	Treatment	2	190.600	95.300	8.801	0.056
		Residuals	3	32.490	10.830		
	50B	Treatment	2	51.410	25.707	16.030	0.025
		Residuals	3	4.810	1.603		
	100B	Treatment	2	38.740	19.370	19.970	0.019
		Residuals	3	2.910	0.970		

Table 22. Analysis of Variance summary table for resistance to extension of intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different levels of wheat protein isolate at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	2	9.602	4.801	60.190	0.004
		Residuals	3	0.239	0.080		
	50B	Treatment	2	28.742	14.371	18.750	0.020
		Residuals	3	2.299	0.766		
	100B	Treatment	2	160.790	80.400	15.900	0.025
		Residuals	3	15.170	5.060		
RS	0B	Treatment	2	11.667	5.834	27.600	0.012
		Residuals	3	0.634	0.211		
	50B	Treatment	2	53.960	26.981	151.300	0.001
		Residuals	3	0.530	0.178		
	100B	Treatment	2	247.430	123.710	303.900	< 0.001
		Residuals	3	1.220	0.410		

Table 23. Analysis of Variance summary table for extensibility of intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different levels of vital wheat gluten at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	2	168.550	84.280	12.790	0.034
		Residuals	3	19.760	6.590		
	50B	Treatment	2	38.450	19.226	39.420	0.007
		Residuals	3	1.460	0.488		
	100B	Treatment	2	6.088	3.044	134.900	0.001
		Residuals	3	0.068	0.023		
RS	0B	Treatment	2	243.520	121.760	28.900	0.011
		Residuals	3	12.640	4.210		
	50B	Treatment	2	32.120	16.060	16.780	0.024
		Residuals	3	2.870	0.957		
	100B	Treatment	2	31.670	15.830	16.150	0.025
		Residuals	3	2.940	0.980		

Table 24. Analysis of Variance summary table for resistance to extension of intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different levels of vital wheat gluten at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	2	30.530	15.265	21.040	0.017
		Residuals	3	2.176	0.725		
	50B	Treatment	2	36.510	18.254	277.100	< 0.001
		Residuals	3	0.200	0.066		
	100B	Treatment	2	197.620	98.810	11.810	0.038
		Residuals	3	25.110	8.370		
RS	0B	Treatment	2	21.762	10.881	43.150	0.006
		Residuals	3	0.756	0.252		
	50B	Treatment	2	96.250	48.120	68.590	0.003
		Residuals	3	2.100	0.700		
	100B	Treatment	2	78.160	39.080	60.800	0.004
		Residuals	3	1.930	0.640		

Table 25. Analysis of Variance summary table for extensibility and resistance to extension of refined intermediate wheatgrass flour from Roseau with different levels of transglutaminase

Parameters	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
Extensibility	Treatment	3	1704.9	568.3	136.3	0.0
	Residuals	48	200.1	4.2		
Resistance to extension	Treatment	3	7415.0	2471.6	1663.0	0.0
	Residuals	48	71.0	1.5		

Table 26. Analysis of Variance summary table for stickiness of wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	5672.000	945.300	104.800	< 0.001
		Residuals	7	63.000	9.000		
	50B	Treatment	6	2616.300	436.100	139.900	< 0.001
		Residuals	7	21.800	3.100		
	100B	Treatment	6	491.500	81.920	6.953	0.011
		Residuals	7	82.500	11.780		
RS	0B	Treatment	6	4497.000	749.500	18.960	0.001
		Residuals	7	277.000	39.500		
	50B	Treatment	6	1492.800	248.800	22.240	< 0.001
		Residuals	7	78.300	11.190		
	100B	Treatment	6	602.300	100.380	58.320	< 0.001
		Residuals	7	12.000	1.720		

Table 27. Analysis of Variance summary table for specific volume of breads made of wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	1.971	0.329	24.590	< 0.001
		Residuals	7	0.094	0.013		
	50B	Treatments	6	1.435	0.239	41.870	< 0.001
		Residuals	7	0.040	0.006		
	100B	Treatments	6	0.573	0.095	7.174	0.010
		Residuals	7	0.093	0.013		
RS	0B	Treatments	6	1.871	0.312	19.670	< 0.001
		Residuals	7	0.111	0.016		
	50B	Treatments	6	2.856	0.476	37.310	< 0.001
		Residuals	7	0.089	0.013		
	100B	Treatments	6	0.460	0.077	4.396	0.037
		Residuals	7	0.122	0.017		

Table 28. Analysis of Variance summary table for length of breads made of wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	540.800	90.130	31.890	0.000
		Residuals	77	217.600	2.830		
	50B	Treatment	6	206.200	34.360	23.080	< 0.001
		Residuals	77	114.600	1.490		
	100B	Treatment	6	335.500	55.920	48.310	0.000
		Residuals	77	89.100	1.160		
RS	0B	Treatment	6	692.300	115.390	21.010	< 0.001
		Residuals	77	422.900	5.490		
	50B	Treatment	6	95.070	15.845	14.640	< 0.001
		Residuals	77	83.360	1.083		
	100B	Treatment	6	167.700	27.955	12.640	< 0.001
		Residuals	77	170.300	2.212		

Table 29. Analysis of Variance summary table for width of breads made of wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	551.90	91.99	51.94	0.00
		Residuals	77	136.40	1.77		
	50B	Treatment	6	981.30	163.55	183.90	0.00
		Residuals	77	68.50	0.89		
	100B	Treatment	6	614.30	102.38	196.10	0.00
		Residuals	77	40.20	0.52		
RS	0B	Treatment	6	592.70	98.78	60.01	0.00
		Residuals	77	126.70	1.65		
	50B	Treatments	6	977.60	162.94	296.60	0.00
		Residuals	77	42.30	0.55		
	100B	Treatment	6	513.70	85.62	63.63	0.00
		Residuals	77	103.60	1.35		

Table 30. Analysis of Variance summary table for height of breads made of wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	1122.50	187.08	360.90	0.00
		Residuals	77	39.90	0.52		
	50B	Treatment	6	1211.70	201.96	429.80	0.00
		Residuals	77	36.20	0.47		
	100B	Treatment	6	728.80	121.46	310.80	0.00
		Residuals	77	30.10	0.39		
RS	0B	Treatment	6	970.10	161.68	177.10	0.00
		Residuals	77	70.30	0.91		
	50B	Treatment	6	1141.60	190.27	676.80	0.00
		Residuals	77	21.60	0.28		
	100B	Treatment	6	593.80	98.96	277.60	0.00
		Residuals	77	27.40	0.36		

Table 31. Analysis of Variance summary table for crumb firmness of breads made of wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatment	6	0.084	0.014	4.043	0.008
		Residuals	21	0.073	0.003		
	50B	Treatment	6	0.076	0.013	3.748	0.011
		Residuals	21	0.071	0.003		
	100B	Treatment	6	0.008	0.001	0.150	0.987
		Residuals	21	0.188	0.009		
RS	0B	Treatment	6	0.103	0.017	2.227	0.081
		Residuals	21	0.161	0.008		
	50B	Treatment	6	0.163	0.027	2.892	0.033
		Residuals	21	0.197	0.009		
	100B	Treatment	6	0.028	0.005	0.642	0.696
		Residuals	21	0.150	0.007		

Table 32. Analysis of Variance summary table for gas cells count in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	72461.000	12077.000	37.420	<0.001
		Residuals	7	2259.000	323.000		
	50B	Treatments	6	104325.000	17387.000	15.220	0.001
		Residuals	7	7995.000	1142.000		
	100B	Treatments	6	119297.000	19883.000	14.100	0.001
		Residuals	7	9873.000	1410.000		
RS	0B	Treatments	6	233481.000	38913.000	16.370	0.001
		Residuals	7	16644.000	2378.000		
	50B	Treatments	6	131309.000	21885.000	4.863	0.029
		Residuals	7	31505.000	4501.000		
	100B	Treatments	6	134845.000	22474.000	12.170	0.002
		Residuals	7	12925.000	1846.000		

Table 33. Analysis of Variance summary table for gas cells area in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	0.085	0.014	1.435	0.322
		Residuals	7	0.069	0.010		
	50B	Treatments	6	0.609	0.101	4.780	0.030
		Residuals	7	0.149	0.021		
	100B	Treatments	6	0.692	0.115	5.457	0.021
		Residuals	7	0.148	0.021		
RS	0B	Treatments	6	0.253	0.042	3.664	0.057
		Residuals	7	0.080	0.011		
	50B	Treatments	6	0.123	0.020	0.481	0.804
		Residuals	7	0.297	0.042		
	100B	Treatments	6	0.909	0.152	39.340	< 0.001
		Residuals	7	0.027	0.004		

Table 34. Analysis of Variance summary table for average gas cells size in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	< 0.001	< 0.001	24.170	<0.001
		Residuals	7	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	8.545	0.006
		Residuals	7	< 0.001	< 0.001		
	100B	Treatments	6	< 0.001	< 0.001	2.258	0.200
		Residuals	7	< 0.001	< 0.001		
RS	0B	Treatments	6	< 0.001	< 0.001	33.010	<0.001
		Residuals	7	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	7.481	0.009
		Residuals	7	< 0.001	< 0.001		
	100B	Treatments	6	< 0.001	< 0.001	5.292	0.020
		Residuals	7	< 0.001	< 0.001		

Table 35. Analysis of Variance summary table for gas cell count greater than 0.1 mm^2 in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	878.900	146.490	6.651	< 0.001
		Residuals	21	462.500	22.020		
	50B	Treatments	6	2285.500	380.900	16.480	< 0.001
		Residuals	21	485.500	23.100		
	100B	Treatments	6	12257.000	2042.900	155.000	< 0.001
		Residuals	21	277.000	13.200		
RS	0B	Treatments	6	3053.900	509.000	28.090	< 0.001
		Residuals	21	380.500	18.100		
	50B	Treatments	6	2050.600	341.800	13.970	< 0.001
		Residuals	20	489.400	24.500		
	100B	Treatments	6	5014.000	835.700	73.970	< 0.001
		Residuals	21	237.000	11.300		

Table 36. Analysis of Variance summary table for gas cell count between 0.1-4 mm^2 in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	881.900	146.980	6.088	0.001
		Residuals	21	507.000	24.140		
	50B	Treatments	6	2530.700	421.800	14.420	< 0.001
		Residuals	21	614.300	29.300		
	100B	Treatments	6	10477.000	1746.200	143.700	< 0.001
		Residuals	21	255.000	12.200		
RS	0B	Treatments	6	3303.000	550.600	32.010	< 0.001
		Residuals	21	361.000	17.200		
	50B	Treatments	6	1775.600	295.930	12.570	< 0.001
		Residuals	20	470.900	23.550		
	100B	Treatments	6	4625.000	770.900	56.500	< 0.001
		Residuals	21	287.000	13.600		

Table 37. Analysis of Variance summary table for gas cell count between 0.04-4 mm^2 in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	14987.000	2497.800	13.920	<0.001
		Residuals	21	3768.000	179.400		
	50B	Treatments	6	13623.000	2270.500	14.320	<0.001
		Residuals	21	3329.000	158.500		
	100B	Treatments	6	44843.000	7474.000	130.500	<0.001
		Residuals	21	1203.000	57.000		
RS	0B	Treatments	6	33336.000	5556.000	29.270	<0.001
		Residuals	21	3985.000	190.000		
	50B	Treatments	6	19251.000	3209.000	13.370	<0.001
		Residuals	20	4801.000	240.000		
	100B	Treatments	6	24557.000	4093.000	31.040	<0.001
		Residuals	21	2769.000	132.000		

Table 38. Analysis of Variance summary table for gas cell area greater than 0.1 mm^2 in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	< 0.001	< 0.001	1.025	< 0.001
		Residuals	21	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	12.290	< 0.001
		Residuals	21	< 0.001	< 0.001		
	100B	Treatments	6	0.017	0.003	30.990	< 0.001
		Residuals	21	0.002	< 0.001		
RS	0B	Treatments	6	< 0.001	< 0.001	11.630	< 0.001
		Residuals	21	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	1.801	0.150
		Residuals	20	< 0.001	< 0.001		
	100B	Treatments	6	0.002	< 0.001	38.880	< 0.001
		Residuals	21	< 0.001	< 0.001		

Table 39. Analysis of Variance summary table for gas cell area between 0.1-4 mm^2 in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	< 0.001	< 0.001	3.541	0.014
		Residuals	21	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	1.055	0.419
		Residuals	21	< 0.001	< 0.001		
	100B	Treatments	6	< 0.001	< 0.001	1.405	0.259
		Residuals	21	< 0.001	< 0.001		
RS	0B	Treatments	6	< 0.001	< 0.001	3.193	0.022
		Residuals	21	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	2.526	0.055
		Residuals	20	< 0.001	< 0.001		
	100B	Treatments	6	< 0.001	< 0.001	1.202	0.344
		Residuals	21	< 0.001	< 0.001		

Table 40. Analysis of Variance summary table for gas cell area between 0.04-4 mm^2 in crumb of breads made with wheat and intermediate wheatgrass from Rosemount (RM) and Roseau (RS) along with different level dough conditioners at three refinement levels

Sample	Bran content	Source of variation	Degree of freedom	Sum of squares	Mean Square	F-value	Pr(>F)
RM	0B	Treatments	6	< 0.001	< 0.001	13.780	<0.001
		Residuals	21	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	5.480	0.002
		Residuals	21	< 0.001	< 0.001		
	100B	Treatments	6	< 0.001	< 0.001	5.176	0.002
		Residuals	21	< 0.001	< 0.001		
RS	0B	Treatments	6	< 0.001	< 0.001	9.989	< 0.001
		Residuals	21	< 0.001	< 0.001		
	50B	Treatments	6	< 0.001	< 0.001	8.594	< 0.001
		Residuals	20	< 0.001	< 0.001		
	100B	Treatments	6	< 0.001	< 0.001	7.838	< 0.001
		Residuals	21	< 0.001	< 0.001		