Effects of Bran Content, Thermal Treatment, and Storage on Flavor Development and Functionality in Intermediate Wheatgrass Flour

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Dedication

To my mother, Bebay Luu, who has given so much for my brother and me.

And to my fur-child, Elio, for his endless support and love.

Abstract

Intermediate wheatgrass (IWG, *Thinopyrum intermedium*) is a perennial crop that has garnered attention for its environmental and nutritional benefits. Selected as a promising candidate among other perennial crops for food use, IWG has good flavor, breeding potential, and superior environmental benefits due to its extensive root system and long growing season. Understanding the storage stability of IWG will further improve its likelihood of integration into the food market. Grains, including wheat, are typically processed into flour and stored until use. Grains can be stable for up to 8-12 years; however, flour has a significantly lower shelf-life. While the storage stability of IWG groats has been investigated, the storage stability of IWG flour have not yet been addressed. Thermal treatment may be used to increase grain shelf life by inactivating enzymes that are involved in lipid rancidity, which is a major pathway for the formation of odor active volatile odor active compounds (VOAC). On the other hand, thermal treatment may negatively impact functionality over storage. Reducing the bran content may also increase shelf life by reducing fat content; however, it would be at the expense of reducing the dietary fiber content. Understanding the storage stability of IWG and identifying methods to improve its stability will not only enhance commercialization potential, but will also incentivize farmers to plant IWG.

The objectives for this research study were to: (1) Evaluate the effects of prior grain storage, bran content, and steam treatment on the development of flavor in IWG flour over storage at 43% relative humidity and (2) Evaluate the effects of prior grain storage, bran content, and steam treatment on the functionality of IWG flour over storage at 43% and 65% relative humidity.

Prior to storage, compositional analysis of IWG and hard red wheat (HRW, control), from two growing seasons, were carried out following official AOAC and AACCI methods. IWG groats were subjected to steam treatment directly above a boiling water bath at 100°C for 2 minutes. After equilibration at room temperature for 24 hours, IWG groats were milled into refined, partially refined (75% bran), and

whole flour, while HRW groats were milled into whole and refined flour. Flour samples were stored at ambient temperature at 43% and 65% relative humidity (RH) for up to 9 months of storage. Samples were analyzed periodically for changes in flavor and dough functionality. VOACs were extracted from flour following a dynamic headspace purge and trap protocol and analyzed by gas chromatography-olfactory-mass spectrometry. VOACs were measured at the beginning, middle, and end of storage. A descriptive analysis was used to document the nature and extent of differences in sensory properties and was conducted with eight trained sensory panelists to describe differences in aroma, flavor, taste, and aftertaste in tortillas made from the stored flour. Dough functionality was measured every 3 months of storage. Rheological and mixing properties were measured using a Farinograph® and a texture analyzer equipped with a Kieffer rig. Gluten strength was measured using Brabender® GlutoPeak. Starch pasting profile was analyzed by Micro-Visco-Amylograph®.

IWG had significantly higher protein, insoluble fiber, total dietary fiber, and fat content than HRW. The steam treatment employed resulted in a significant decrease in lipase and LOX activity, without significantly reducing antioxidant content. Over storage, identified odor active VOACs included alkyl and enal aldehydes, alcohols, and furans, which are products of lipid oxidation. By the end of storage, whole IWG flour showed significantly greater intensity of nearly all identified VOACs, such as pentanal, hexanal, 1-octen-3-ol, and 2-pentylfuran, in comparison to HRW flour. However, due to IWG's higher antioxidant content, the induction period of VOACs in IWG was longer than that of HRW, indicating better short term storage stability. IWG was described as grassier and earthier compared to HRW, due to the presence of alkyl aldehydes, 2-pentylfuran, and 1-octen-3-ol. Steaming resulted in significantly lower intensities of VOACs, attributed to a reduction in enzyme activity. Partial refinement also resulted in a significant reduction in the intensity of VOACs. IWG had more earthy, grassy and Play-Doh® aromas, and higher intensities of peanut butter and beany flavor than HRW samples. IWG had greater intensity rating of flavor and the five basic taste (sweet, salty, bitter, sour, and umami) and aftertaste than HRW samples. Samples with

lower bran content had lower overall flavor, bitter and salty taste, and overall aftertaste. Steamed IWG samples had lower overall aroma, flavor and aftertaste compared to not-steamed IWG samples.

IWG flour had increases in dough development time, stability, resistance to extension, and gluten aggregation over short term storage, indicating an increase in dough strength. IWG also had improvement in starch pasting properties over storage, including peak, hold, and final viscosity. Partial bran refinement resulted in better dough functionality and starch pasting properties due to less interference of the fiber with the formation of the gluten network and a higher starch to non-starch ratio, respectively. Steaming resulted in higher dough development time and resistance to extension but had a slightly negative impact on starch pasting viscosity values.

Partial refinement of IWG resulted in lower intensities of off-odor flavor compounds, lower sensory attributes ratings, and improved functionality, while maintaining the nutritional benefits associated with the bran. The interruption of enzymatic activity by steam treatment helped off-set unfavorable flavor development, thus could be used to prolong the shelf-life of IWG flour. Together, these two processing practices make IWG viable for commercial use.

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1.1 Introduction

Since the beginning of agriculture, more than a fourth of the Earth's land has been converted for agricultural use (Cox *et al.*, 2006). With continuous climate change and a growing population that requires an unprecedented amount of food, a shift towards sustainable cropping systems that can both maintain our arable land and feed the world is needed. Grain production has dramatically increased in the past 50 years due to the increase in agricultural land, intensified land management, and new technologies (Neumann *et al.*, 2010). The top three grains that are produced and consumed worldwide are rice, wheat, and corn; however, they are all annual crops (Beta and Isaak, 2016). Annual crops currently comprise over two-thirds of global cropland and come with a multitude of sustainability issues. Farming of annuals has been associated with soil erosion, nitrogen leaching, and poor management of water and nutrients (Cox *et al.*, 2006; Glover *et al.*, 2010). A different approach would be developing perennial grain crops. Perennial grain crops have been shown to outperform annual crop systems in terms of many environmental benefits such as soil conservation, carbon sequestration, nitrogen cycling efficiency, and overall maintenance of nutrients in the soil (Culman *et al.*, 2013; Lewandowski, 2016).

In 1973, the Rodale Research Center started investigating perennial grain crops as a potential solution. Criteria for finding suitable perennial crops include the ability to compete with annual grain crops and be agronomically, nutritionally, and functionally viable. In 1988, roughly a hundred different perennial species were evaluated for the agronomical and nutritional traits. One of the perennial grain crops that showed a lot of promise is *Intermedium thinopyrum*, known as Intermediate wheatgrass (IWG) (Wagoner and Schaeffer, 1990). The potential for IWG as a solution for the deteriorating environment and agriculture has grown with

continual breeding efforts as well as efforts to enhance its marketability. As a new crop, determining its potential end-use and marketability is vital for its success. One important aspect that needs to be researched for future market use is its storage stability, which has been minimally addressed. Understanding the storage stability of food ingredients is necessary to recognize any changes that can affect quality and safety. Furthermore, storage stability will help determine the shelf-life of the ingredient and final product, which is necessary for commercial use.

When assessing the storage stability of grains, understanding the composition is vital to determine the potential threats to its quality and safety over time. The storage stability of grains can be determined in regards to microbial contamination, grain viability, or quality attributes including sensory acceptance, functionality, and nutritional properties (Pomeranz *et al.*, 1968). These attributes are all influenced by extrinsic factors of storage such as environmental conditions at time of harvest (i.e. weather and soil quality), relative humidity, temperature, and light (Doblado-Maldonado *et al.*, 2012), as well as intrinsic factors such as grain moisture content and composition (Galliard, 1994). IWG has many desirable nutritional traits such as higher protein, dietary fiber, and antioxidant content compared to annual wheat; however, it has a higher amount of fat and lipase (Rahardjo *et al.*, 2018; Tyl and Ismail, 2018). With high fat, the rancidity of IWG over storage is of concern, especially in the presence of higher lipase activity compared to wheat. Lipase causes hydrolytic rancidity due to the release of free fatty acids, which are more prone than intact fat to oxidative rancidity by lipoxygenase and autoxidation. The products from rancidity can reduce the overall quality, thus limiting the shelf-life (Galliard, 1994). However, IWG has a significantly higher amount of antioxidants compared to wheat, which can potentially counteract the oxidative rancidity (Tyl and Ismail, 2018). While hydrolytic and oxidative rancidity pose challenges to the storage stability of IWG, external methods can be used to help extend the storage stability.

Different methods can be used to extend the storage stability and the acceptance of grains. One way to extend shelf life is through the refinement of the milled grain, which results in the removal of the bran and germ fractions that contain most of the fat and enzymes. However, the use and consumption of refined grain are not recommended by the Dietary Guidelines for Americans, which encourage the consumption of whole-grain due to their superior nutritional content that can help prevent a variety of chronic diseases (Montonen *et al.*, 2003; O'neil *et al.*, 2010). Consumers drive the market and will be the key to the success of IWG. With increase desires for healthier and more sustainable foods, IWG may have a promising future. However, there is a concern for whether whole IWG grains and flour can maintain their functional use while still having acceptable sensory qualities over extended storage. Consumers and manufacturers will not use a product that is not functional or has undesirable flavor. Thus, investigating the different flavors that can form during storage and processing is essential. To address these challenges, determining ways to extend the storage stability of IWG, other than refinement, will be needed to establish proper industry practices.

One common method used to extend the shelf life of cereal grains is the use of thermal treatment. Oats and legumes are commonly thermally treated to deactivate enzymes that may reduce shelf life. Furthermore, thermal treatment has been found to also maintain antioxidant content that can help prolong shelf life (Rose *et al.*, 2008; Bergonio *et al.*, 2016; Hu *et al.*, 2018). Due to the high-fat content and enzyme activity that are of concern in IWG, the use of steam treatment could be a viable option in extending its shelf life. However, understanding the impact of steam on the functionality and its end-use acceptability is also important and needs to be addressed.

1.2 Hypothesis and Objectives

We hypothesize that the inactivation of enzymes through thermal treatment and a moderate reduction in bran and germ content will enhance the storage stability of IWG in terms of flavor and sensory attributes and will not have a significant impact on functionality. More specifically, thermally treated samples will have fewer off-flavors formed during storage compared to non-treated samples. Flour with lower bran content will have fewer oxidation products and off-flavors. Finally, flour from stored grains will not differ from that of freshly harvested grain in functionality and flavor development during storage.

Our specific objectives were:

- Evaluate the effects of prior grain storage, bran content, and steam treatment on the development of flavor in IWG flour over storage at 43% relative humidity
- Evaluate the effects of prior grain storage, bran content, and steam treatment on the functionality of IWG flour over storage at 43% and 65% relative humidity

1.3 Grain Use and Market

1.3.1 Cereal Grains

Cereal grains are ubiquitously consumed all over the world as an important source of energy and nutrition, providing approximately 30% of energy intake in adults (Truswell, 2002). Grains have high starch content that provides energy and contain a variety of dietary components such as dietary fiber, protein, lipids, as well as vitamins, minerals, and phytochemicals (Dewettinck *et al.*, 2008). Starch contributes to >50% of caloric intake in the Western world and up to 90% in the developing countries (Wang *et al.*, 2015). Around the world, a variety of different

cereals grains are grown. Rice, wheat, and corn are the most common cereals grown, followed by sorghum and millet. Environmental, cultural, and economic factors determine the type of grains grown in different parts of the world (Awika, 2011).

Rice, wheat, corn are the top three cereal crops, with over 90% of the global cereal production (Beta and Isaak, 2016). After rice, wheat is the second-largest cereal crop that is grown for human consumption. Wheat can grow in a wide range of conditions making it one of the most cultivated plants worldwide (Awika, 2011). As a cereal crop, it can be consumed as whole grain, but most of the wheat is milled into a flour to be further processed into a large variety of foods.

Consumption of cereal foods, preferably whole grain and without added fat, salt, or sugar can be found in several sets of national dietary guidelines (Truswell, 2002). Whole grains contain all the components of the intact seed, including bran, endosperm, and germ. Whole grains, therefore, include all the grain's vitamins, minerals, fibers, and phenolic compounds. Consumption of whole-grain foods has been linked to reduced coronary heart disease (CHD), type II diabetes, and all-cause mortality (Trumbo *et al.*, 2002; Montonen *et al.*, 2003; Slavin, 2004).

Regardless of all the nutritional benefits that whole grain has been associated with, the consumption of whole-grain is below the daily recommended value (O'Neil *et al.*, 2010). Wheat is typically milled into flour and then further refined to remove the bran and germ, removing most of the beneficial nutrients (Awika, 2011). The unsatisfactory consumption of whole grains could be potentially attributed to the flavor of whole-grain products, being described as more intense than those of refined flour. However, consumers are now becoming aware of the nutritional benefits of whole grains. In the 2019 Food and Health Report from the International Food Information Council Foundation, more than 80% of consumers perceive whole grain as healthy and try to incorporate more of it in their diet (*2019 Food and Health Survey*, 2019). From 2013-2016, whole grain intake as a

proportion of total grain intake among adults was 15.8% on average. The percentage of whole grain intake increased with age from 12.9% in adults ages 20-39 to 19.7% in adults 60 and over. In general, from 2015-2016, whole grain intake increased overall for adults. With increased consumption and demand, wheat production around the world will be expected to grow.

1.3.2 Wheat Production

The major wheat-producing countries by region are EU (France and Germany), FSU (the Russian Federation and Ukraine), North America (Canada and the United States of America), Oceania (Australia), and Asia (China, India, and Pakistan). While the top five major wheat exporters are France, the United States, Canada, the Russian Federation, and Australia (Beta and Isaak, 2016). The majority of the wheat produced is used for human consumption, with a limited amount being used directly for livestock feed. The wheat is milled into flour, where the primary use is for baking and extrusion to produce a variety of products such as bread and pasta (Beta and Isaak, 2016).

A decline of wheat production was seen in the 2018/19 season, but the current five-year projection is for production to increase by 39 million tons (mT) from 737 to 776 mT by 2023/24. Agricultural area for global wheat production is currently around 218 million hectares and is not projected to increase due to competition from other cereal crops. The projected increase in wheat production assumes that yields will rise. Consumption of wheat is projected to increase, on average 0.9% yearly from 2019-2024 due to a growth in food use, with production meeting those demands (InternationalGrainsCouncil, 2019).

With the increase in acceptability and consumers' interest in whole grain products that can deliver desirable health benefits, there has been a rise in the

development of new applications for new products. However, it is important to realize the limitation of new wheat products on the market.

1.3.3 Disease, Allergy, and Intolerance

A gluten-free diet is necessary for people who are diagnosed with celiac disease (CD), a permanent inflammatory autoimmune disease that occurs in genetically predisposed individuals, representing ~1% of the American population (Bao *et al.*, 2012; Rubio-Tapia *et al.*, 2012). CD is characterized as the damage caused to the jejunal and duodenal villi in the small intestine upon the ingestion of gluten proteins (Bao *et al.*, 2012). Gluten is formed during the mixing of flour and water. Symptoms include malabsorption of nutrients, gastrointestinal concerns, and potential psychological issues (Pynnönen *et al.*, 2004; Lebwohl *et al.*, 2015). Many people with CD, however, have limitations to gluten-free diets such as paying a premium for gluten-free alternative, social isolation, and running into hidden sources of gluten such as other non-wheat cereals (i.e. cross-contaminated oats), sauces, and various food ingredients (e.g. thickeners, fillers, seasonings) (Klyeisk, 2008; Lebwohl *et al.*, 2015).

Moreover, wheat is considered one of the big eight allergens in the United States and one of the most common food allergens in the world. The allergen is associated with immunoglobulin E (IgE)-mediated reaction that can be life-threatening, which is a distinct reaction in comparison to CD (Tatham, 2016). A response to wheat protein can manifest as baker's asthma, contact dermatitis, gastrointestinal and respiratory response, and anaphylaxis, the most severe reaction in which the whole body reacts, leading to death. Due to the similar homology of the prolamin proteins, those that are soluble in aqueous alcohol, found in wheat (gliadin), barley (hordein), rye (secalin), and oats (avenin), individuals who suffer from wheat allergens may have cross-reactivity to these grains (Tatham, 2016).

Other disorders that are related to wheat include non-celiac/non-allergy wheat sensitivity, which occurs in the absence of an IgE response or damage to the small intestinal villi. Associated diseases include dermatitis herpetiformis - a type of skin disorder and gluten ataxia – difficulty walking after the ingestion of gluten.

Wheat disease, allergy, and intolerance pose a challenge for consumers as well as difficulty for the wheat industry. At the other end of the spectrum from consumers, farmers who grow them face challenges in producing grains to feed the world.

1.3.4 Challenges

Farms around the world are currently producing more food than ever before to sustain the human population. The human population is now at 7.7 billion people and is projected to grow to 9.7 billion by 2050 (“World Population 2019,” 2019); thus, more food than ever will be needed. With limited natural resources and the need to nourish a growing population, the pressure that is placed into modern agriculture is immense. Agriculture is the “largest threat to biodiversity and ecosystem functions of any single human activity,” and the land available for farming is steadily decreasing (Clay, 2004). When it comes to sustainability, current agricultural practices use crops that tend to use more water and energy, exacerbate soil erosion, contribute to poorly managed water and nutrients, store less carbon below ground, and are less resilient to pests and diseases (Glover, 2005; Cox *et al.*, 2006).

Soil erosion is one of the most serious threats that modern agriculture faces as it decreases the amount of land available for food production. Farming of annual crops has especially exasperated this issue. Annual crops cover the soil for only a few months of the year, leaving the arable landscape uncovered and exposed to

potential erosion. One of the leading causes of soil erosion is from excess precipitation, especially during early spring, when crops have yet to emerge. The Upper Midwest of the United States has experienced an increase in rainfall leading to soil and nutrient loss due to changes in the climate (Morton *et al.*, 2015). It is estimated that roughly 80% of the world's agricultural land suffers moderate to severe erosion, while 10% experience slight erosion (Pimentel *et al.*, 2013).

Efforts to maintain arable land are mostly temporary and have other negative impacts. One solution has been deforestation to create land; however, if current agricultural practices continue, that new land will suffer the same fate. A second solution is utilizing crops that have been engineered to produce higher yields on the same amount of land. However, these engineered crops will not keep up with the growing population.

If grains continue to be grown and produced by current methods, Earth may not have the capacity and the resources to sustain adequate food production into the next century (Cox *et al.*, 2002). One potential solution, however, is the use of perennial crops. Perennial crops are of great interest as they can potentially solve the fundamental issue of land degradation. Perennial crops have been shown to provide several environmental benefits that could address agricultural concerns. With continued research and breeding efforts, perennial agriculture can be a promising alternative to annual crops.

1.4 Perennial Crops

1.4.1 Description and Environmental benefits

Our current agricultural landscape is dominated by annual crops. Approximately 70-80% of the world's cropland is used to produce annual crops (Cox *et al.*, 2002; Glover *et al.*, 2007). Current agricultural research on annual crop

production is geared towards making them more environmentally sustainable. However, there are several limitations, such as decreasing available farmlands and the large requirement of resources. One approach to managing environmental issues is through the breading of perennial crops (Glover *et al.*, 2010).

Growing perennial crops can result in many environmental benefits including preventing soil and water erosion of arable land, managing water and nutrients effectively, storing carbon below ground to combat climate change, and reducing the usage of harmful pesticides (Cox *et al.*, 2006; Glover *et al.*, 2010). Perennial crops can provide year-round soil protection due to their longer growing seasons and extensive root systems. Once planted, perennial crops can be harvested for several years, significantly reducing the need for mechanical operations on the soil that are required for annual crops, thus decreasing the labor and fuel cost (Monfreda *et al.*, 2008).

Differences between perennials and annuals stem back to how they allocate their carbohydrate stores. Annual crops allocate most of their carbohydrates to their surface biomass, prioritizing seed yield, whereas perennial crops direct their carbohydrates to their root system, prioritizing longevity (DeHaan *et al.*, 2005). The prioritized allocation of the carbohydrates to the ground creates an extensive root system. The more extended root system of perennial plants is of great interest due to their ability to increase the storage of carbon, known as carbon sequestration, above and below ground (Post and Kwon, 2000; Zan *et al.*, 2001; Boody *et al.*, 2006). The deeper root system and increased biomass in the soil can trap and utilize rainwater, reduce the risk of flooding and soil erosion, reduce nitrate leaching, and maintain essential plant nutrients, such as nitrogen (Culman *et al.*, 2013; Lewandowski, 2016). With fewer nutrients leaching, perennial crops can improve water quality below ground as well as reduce greenhouse gas emission related to the volatilization of nitrogen fertilizers (McIsaac *et al.*, 2010; Ruan *et al.*, 2016; Jungers *et al.*, 2017). Additionally, the higher retention of nutrients and

utilization of water will benefit farmers from a cost perspective as less input of fertilizer and labor would be needed.

Perennial crops, however beneficial they may be, still pose many challenges that must be overcome if they are to be successful. One challenge is increasing seed size as most perennial plants have a small seed size compared to annual plants, a trait that causes harvesting challenges and lower yields. (Lubofsky, 2016). Perennial crops are also known to grow tall and have weak stems, which makes them prone to falling over, known as lodging. Lodged crops are difficult to harvest, have abnormal seed growth, and can significantly reduce yields (Lovell, 2012; Lubofsky, 2016). Another issue with perennials is that they are prone to seed shattering, the natural shedding of their seeds as a means to propagate, resulting in further loss in yield (Cox *et al.*, 2006; Lubofsky, 2016).

Besides the inherent challenges of planting and growing perennial crops, there are management issues. With any new crop, one must determine the best practice in growing them, such as effective use of fertilization and row spacing. Over fertilization can exasperate the issue of lodging, but under fertilization can lead to low yields. Perennial plants can become too crowded and dense over years of growth, causing problems with harvest and dramatically decreasing yields. Therefore, proper row spacing will be needed. (Lubofsky, 2016).

Perennial crops offer many environmental benefits that can help combat climate change and ensure that food continues to grow sustainably to feed a growing population. With increasing consumer interest in sustainable agriculture, perennial crops are likely able to enter the commercial food markets. However, rigorous breeding efforts will be needed to address the challenges aforementioned, as well as continual research to develop end-use applications to incentivize farmers to plant perennial crops.

1.4.2 Perennial Crops' Breeding Efforts

Perennials crops would provide substantial benefits to farmers and humanity by providing solutions to restore farmlands and biodiversity while supporting the growing population as an additional source of food. With new advances in technology, successful breeding of perennial crops can be achieved. However, the question that is of concern is whether perennials crops can compete with annual crops. Yield and seed size are of concern, as these two traits will be vital for the inclusion of perennial crops in an agricultural world where annuals crops have been widely successful (Cox *et al.*, 2006). Perennial crops typically have lower yields and smaller seed size due to the previously mentioned allocation of its carbohydrates, which poses challenges for breeders and farmers (DeHaan *et al.*, 2005). Increasing yield while maintaining longevity is a targeted concern. Regardless of how environmentally beneficial perennial crops can be, farmers are only going to grow what is economically viable. Changing the agricultural landscape to include perennial crops that could be commercialized is the current focus (Cox *et al.*, 2002).

Research on new perennial grain crops began in 1983 at the Rodale Research Center in Pennsylvania. The concept of developing grains from perennial crops was first presented by Wes Jackson in his book *New Roots for Agriculture,* which reviewed the negative impact of U.S. agriculture and provided major solutions to restore those damaged lands (Jackson, 1980). Grain production from perennial grain crops can offer a second chance to the land that can no longer sustain annual crop production. The Rodale Research Center studied about 100 different perennial crops, where *Thinopyrum intermedium*, commonly known as intermediate wheatgrass (IWG), was chosen as the best candidate due to its agronomical, compositional, and nutritional traits (Wagoner, 1990).

1.4.3 Intermediate Wheatgrass

IWG is genetically related to common wheat, *Triticum aestivum L.*, and both are members of the grass family Poaceae (Ogle *et al.*, 2011). IWG is a Eurasian forage grass originating from Europe and Asia. It was brought over to the United States in 1932 and can now be found in the Great Plains and Intermountain West of the United States. The crop has been used as hay, pasture, and animal feeds for all classes of livestock and wildlife. IWG grows 1 to 1.2 meters tall, with seed spikes growing 10 to 20 centimeters long, and leaves that are 4 to 8 mm wide. Around 4 to 7 florets form on each spikelet florets. IWG is drought resistant, needing about 12 to 14 inches of total annual moisture or greater. Ideal conditions for growth are elevations between 3,500 and 9,000 feet in well-drained loamy to clay texture soils. It can be seeded in lower elevations, but moisture requirements are greater (Ogle *et al.*, 2011). Furthermore, IWG is tolerable to slightly acid to mildly saline conditions, cold weather, drought, moderate flooding, and fire (Ogle *et al.*, 2011)

IWG is very suitable at stabilizing disturbed soil due to its dense and deep root system (**Figure 1**). It can produce as much as 7000 pounds per acre of roots in the upper 20 centimeters of soil in five years. The large root system that IWG produces can revitalize damaged soil from erosion as well as maintain soil health. The extensive root system resulted in 86% decrease in soil nitrate leaching and a 13% increase in carbon sequestration compared to annual wheat (Culman *et al.*, 2013).

In comparison to other perennial crops, IWG is considered high yielding (Culman *et al.*, 2013) with relatively large seed size (Wagoner, 1990). IWG can produce seeds relatively easily and for several years if fields are maintained. Seed yields as measured by Culman et al. (2013), were as high as 1390-1662 kilograms per hectare. Yields peak in the second or third year of production (Wagoner and Schaeffer, 1990; Culman *et al.*, 2013) and drop significantly after the fourth year

(Ogle *et al.*, 2011). In comparison to other perennial crops that were screened, IWG has relatively higher threshability index, a more erect culm, synchronous maturation and flowering, and better resistance to seed shattering, all of which contribute to its relatively higher yield and ease of harvest (Wagoner and Schaeffer, 1990). However, IWG, as a superior and promising perennial crop, is not yet on par (with respect to harvestability and yield) with annual crops and further breeding efforts will be needed to improve IWG for commercial use.

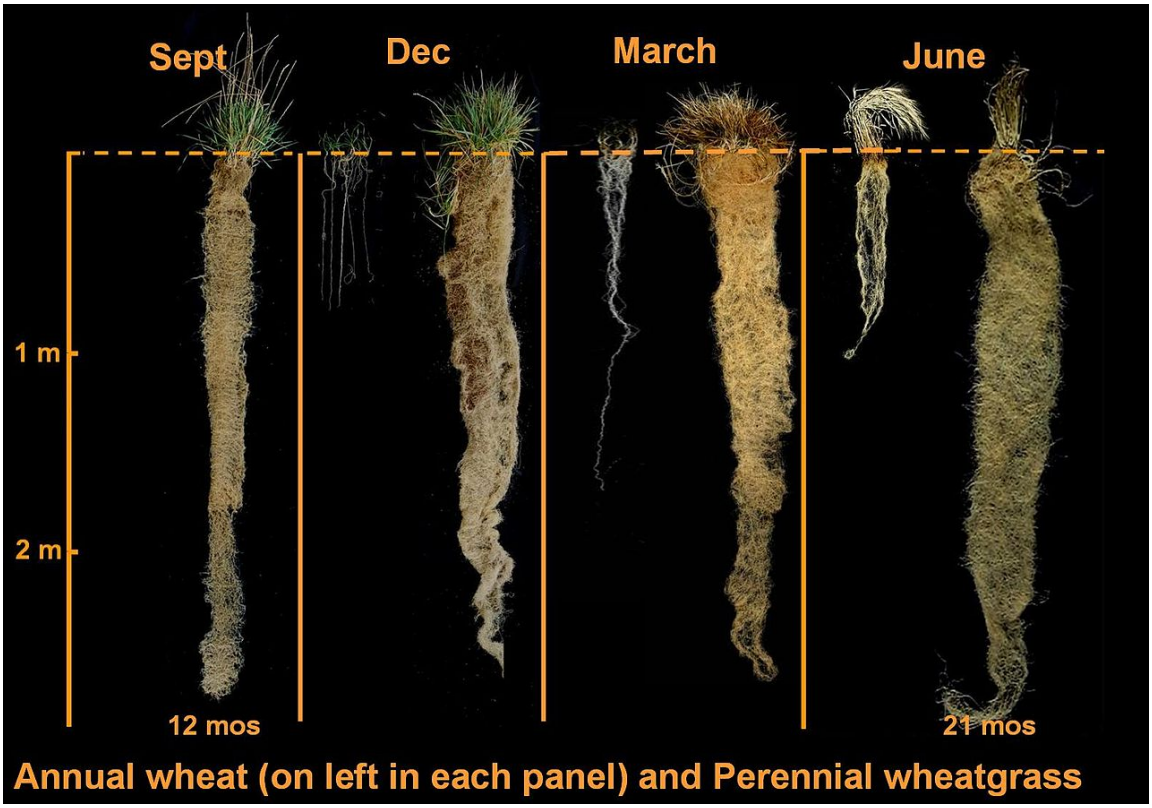


Figure 1. The root system of IWG compared to annual wheat over a growing cycle between annual wheat and perennial IWG (Cox *et al.*, 2006). Used with permission.

1.4.4 Intermediate Wheatgrass Breeding Efforts

As a perennial crop, breeding efforts for IWG focuses on selecting traits that are typical for annual crops. Breeding of IWG, from the perspective of a breeder,

has favorable potential. IWG has a high intraspecies genetic variation, which allows for the opportunity to improve traits and has high heritability for domestication traits (Cox *et al.*, 2006). For IWG, selection has been for increased yield, enlarged seed size, improved threshing ability, reduced plant height to prevent lodging, and early maturation (DeHaan *et al.*, 2013). After selection at the Rodale Research Center, IWG went through two cycles of selection beginning in 1988. The Land Institute (TLI) in Salina, Kansas began breeding IWG from those two cycles in 2003 (DeHaan *et al.*, 2013).

Two approaches are utilized in breeding IWG as a perennial grain with desirable traits: wide hybridization and direct domestication, both of which have unique strengths and challenges (DeHaan *et al.*, 2013). Wide hybridization involves crossing annual wheat with related perennial species. The goal of wide hybridization is to express in perennials the genes that control for yield, seed size, free-threshing ability, and quality. The main challenge with the wide hybridization of IWG with annual is maintaining perenniality and domestication traits as well as fertility (DeHaan *et al.*, 2013). Direct domestication involves cycles of selection of lines with the best traits, such as higher seed size, yield, and overall quality. However, in comparison to wide hybridization, the genetic variation can be lacking, which will then require a substantial amount of time to achieve desired traits (DeHaan *et al.*, 2013).

Through the efforts from TLI in 2003 using the first two cycles from the Rodale Institute, IWG yield was improved by 77%, and seed size increased by 23% (DeHaan *et al.*, 2013). In 2011, the University of Minnesota (St. Paul, MN) and the University of Manitoba (Winnipeg, Canada) joined the domestication effort using the germplasm from the third cycle TLI (Zhang *et al.*, 2016). With the breeding efforts through wide hybridization or direct domestication, and the growing support of using perennial crops, estimated complete commercial availability is in the next 20 years (Glover *et al.*, 2010). In 2019, the University of Minnesota released a limited amount of the first commercial variety of IWG named

MN-Clearwater, which is currently being grown on several hundred acres in Minnesota.

Breeding efforts for IWG has been focused on making it more desirable not only for farmers but to consumers as well. Efforts to make it comparable, if not better, to wheat are needed for IWG to become commercially successful. It is, therefore, important to consider the composition of IWG in comparison to wheat to determine applicability in various food products.

1.5 Intermediate Wheatgrass Grain Composition in Comparison to Wheat

With many environmental benefits and the potential for food use, understanding the composition of IWG is important to investigate how it may function in different food processes as well as how it may contribute to human nutrition. Thus, to know how IWG will perform, we must investigate its composition in comparison to wheat, the “gold standard.” Any grain that can potentially function as wheat will be compared to the “gold standard” in terms of its composition.

1.5.1 Kernel Anatomy

The wheat kernel consists of three primary parts: the bran, endosperm, and germ, representing 13-17%, 80-85%, and 2-3% on a mass basis, respectively (Sramkova *et al.*, 2009). The bran layer consists of multiple layers that protect the main part of the grain. Bran is rich in B vitamins and minerals, and more than half of the bran consists of fiber (~53%). The fiber composition of bran is complex but primarily consists of cellulose, pentosans, and arabinoxylans that are tightly bound to proteins. The endosperm consists of the outer aleurone layer, and the inner portion is referred to as the mealy or starchy endosperm (Sramkova *et al.*, 2009). The proteins found in the endosperm consist of albumins, globulins, and the gluten

forming proteins – gliadins and glutenins. Compared to bran, the endosperm contains low amounts of minerals and fiber. Lastly, the germ is the innermost part of the kernel that is rich in proteins and lipids, as well as minerals (Sramkova *et al.*, 2009).

In comparison to wheat, IWG has a smaller seed size, which gives it a higher bran to endosperm ratio (Rahardjo *et al.*, 2018). The majority of nutrients, namely phytochemicals, fiber, and micronutrients, are found in the bran and aleurone layer of the seed. Therefore, with higher bran to endosperm ratio, these nutrients comprise a more significant portion of the IWG seed as a whole (Marti *et al.*, 2015b; Rahardjo *et al.*, 2018; Tyl and Ismail, 2018). With a different compositional profile due to seed size, fundamental knowledge on the various components of wheat and their nutritional and functional use will be needed to understand the comparative end-use applications of IWG.

1.5.2 Composition of Wheat

1.5.2.1 Starch

Starch is a major storage carbohydrate and source of energy in wheat. Starch constitutes 60-75% of the wheat kernel's total dry weight (Sramkova *et al.*, 2009). It is comprised of amylopectin and amylose, which are insoluble polymers of glucose (Zeeman *et al.*, 2010). Amylose is a linear starch polymer that is composed of glucopyranose units linked via α-1,4 glycosidic bonds and forms the amorphous region in starch. Amylopectin is a highly branched starch polymer with α-1,4 glycosidic bonds forming the chain, while α-1,6 glycosidic linkages form the branches. Amylopectin is associated with the crystallinity of the starch due to the branches forming a double helix structure (Singh *et al.*, 2003a). In wheat, the amylose to amylopectin ratio ranges from 20:80 to 30:70 (Konik-Rose *et al.*, 2007).

The ratio of amylose to amylopectin is of importance as the interaction of the two polymers impacts starch pasting properties and overall functionality (Zeng *et al.*, 1997; Copeland *et al.*, 2009). The functional aspect of starch is based on the temperature-dependent interaction with water. As starch is heated in the presence of water, the starch granules begin to absorb water and swell to several times their initial size, losing their crystallinity and structure, and building viscosity. This process is irreversible and known as gelatinization (Zeng *et al.*, 1997; Copeland *et al.*, 2009). As the granules are heated with water, the water molecules form hydrogen bonds with the exposed hydroxyl groups of amylose and amylopectin, causing swelling and increased starch solubility, allowing for the leaching of amylose (Singh *et al.*, 2003a). Any presence of lipids in the system will affect the swelling capacity, as amylose can form amylose-lipid complexes, which are insoluble in water and will require higher temperatures to dissociate (Singh *et al.*, 2003a). The amylose to amylopectin ratio directly correlates with swelling capacity. Other factors that contribute to starch swelling capacity include the degree of polymerization, length and degree of branching of the amylopectin, and the molecular weight and distribution of the amylose and amylopectin (Singh *et al.*, 2003a).

As the gelatinization process continues with water absorption and heat, the swollen starch granules will burst and completely lose structure going from a semi-crystalline to an amorphous state (Copeland *et al.*, 2009). The process of gelatinization is dependent on available water, temperature gradient, shear force, and the composition of the mixture (Tang and Copeland, 2007; Copeland *et al.*, 2009).

As temperature decreases after gelatinization, the starch molecules form a gel and begin to re-associate via hydrogen bonding, turning into a semi-crystalline aggregate. This process is known as retrogradation (Tang and Copeland, 2007). Retrogradation involves the intermolecular interactions between amylose and amylopectin. During this process, amylose associates to form double helices, while

amylopectin molecules recrystallize. Amylose can complete its retrogradation within hours. However, amylopectin may take days to finish recrystallization, depending on the branched chain's ability to re-associate (Tang and Copeland, 2007). Retrogradation is an important property to understand as it can predict the functional properties of starch in different food applications. For example, in the case of wheat starch, retrogradation is important in predicting the staling behavior in baked bread or rigidity in pasta. Starch pasting, specifically, gelatinization, is an important property of starch that plays a large role in processes such as baking, extrusion, thickening, and gelling in foods (Biliaderis *et al.*, 1980).

1.5.2.2 Dietary Fiber

Dietary Fiber (DF) is defined as lignin and carbohydrates with three or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the human small intestine (i.e., escapes digestion in the small intestine) and passes into the large intestine where they go through complete or partial fermentation (Jones, 2014; Dreher, 2018). In wheat, DF represents ~13% of the wheat kernel's total dry weight (Fardet, 2010; Dhingra *et al.*, 2012). DF is found mostly in the bran (~45%) and some in the germ (~18% DF) (Fardet, 2010). Since DF is associated with the bran and germ, whole wheat has a higher amount of dietary fiber than refined wheat flour. With the increased consumer awareness of the health benefits, such as protection against the development of chronic diseases including obesity, type 2 diabetes, cardiovascular disease, and cancer, the consumption of whole-grain cereals has increased (Sramkova *et al.*, 2009; Fardet, 2010). Whole grain wheat is one of the best sources of DF in Western diets having 9 – 17 g total fiber per 100 g compared to <6 g/100 g in other plant sources, such as rice and legumes (Dhingra *et al.*, 2012).

DF is mainly composed of cellulose, non-cellulosic polysaccharides, such as hemicelluloses and pectic substances, lignin, arabinoxylans (AX), and β-

glucans (Kay, 1982; Maes and Delcour, 2002; Sramkova *et al.*, 2009). DF can be separated into two categories: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Of the fiber content in wheat, 9-12% is IDF, and 1-3% is SDF (Fardet, 2010). DF negatively impacts bread quality with high levels resulting in reduced loaf volume, increased crumb firmness, darker crump appearance, and undesirable flavor (Wang *et al.*, 2002).

AX, specifically, has been investigated significantly due to its role in dough development and bread-making performance. AX structure varies in different plant sources, but generally consist of β-(1,4)-linked xylopyranosyl backbone linked with arabinofuranose. (Lempereur *et al.*, 1997; Dervilly-Pinel *et al.*, 2001; Maes and Delcour, 2002). AX can be either water-extractable (WE-AX) or water-unextractable (WU-AX), both of which can affect the rheological properties and gluten formation of wheat. WE-AX are found loosely bounded to the cell wall, and WU-AX are attached to the cell wall via covalent and non-covalent linkages with other AX, proteins, lignin, and cellulose (Wang *et al.*, 2016). Both AX are capable of absorbing 15-20 times their weight in water, thus forming a highly viscous solution that may increase the gas holding capacity of wheat dough and potentially improve dough quality (Koehler and Wieser, 2013). WE-AX can bind up to 25% of added water in wheat dough, and WU-AX has high water-holding capacity and can assist in water binding during dough mixing.

WE-AX has a positive effect on dough development and bread baking performance. WE-AX can increase loaf volume due to its ability to increase the stability of liquid films and dough foam structures. WE-AX can form a network that can support the gluten matrix, due to their high molecular weight (Courtin *et al.*, 2001). WU-AX, on the other hand, has been shown to impact gluten formation negatively. WU-AX particles can compete for water, create barriers that destabilize gas bubbles, and prevent the development of gluten (Koehler and Wieser, 2013). However, this could be mitigated through enzyme activity (e.g., endoxylanase),

making WU-AX more soluble. (Dervilly-Pinel *et al.*, 2001; Autio, 2006; Koehler and Wieser, 2013).

Understanding how DF behaves physically and chemically is key to determining its effect on dough functionality and end-product quality. While DF has many health benefits, it can have a negative effect on functionality in different food systems (Wang *et al.*, 2002; Koehler and Wieser, 2013; Zhao *et al.*, 2017). Since DF has both negative and beneficial properties, a balance needs to be made to ensure that the final product can deliver nutritional benefits, while maintaining overall quality and acceptability in different food systems (Wang *et al.*, 2002; Hung *et al.*, 2007).

1.5.2.3 Protein

In wheat, protein content varies between 10 – 18% on a dry basis (Sramkova *et al.*, 2009). Wheat protein components are categorized based on the work of Thomas Burr Osborne. The main protein components found in wheat are albumins (soluble in water and dilute buffer), globulins (soluble in salt solutions), prolamins (soluble in 70-90% ethanol), and glutelins (soluble in dilute acid or alkali) (Osborne, 1907; Koehler and Wieser, 2013). Wheat proteins can be further classified as structural/metabolic (non-gluten forming) and storage proteins (gluten forming) (Shewry, 2003).

Structural and metabolic proteins include the albumins and globulins. Albumin and globulins comprise 15-20% of total wheat flour protein and are considered to be nutritionally better than the other proteins found in wheat due to their higher lysine and methionine content (Malik, 2009). Albumins and globulins are found in the bran, aleurone layer, germ, with trace amounts in the endosperm. Biologically, albumins and globulins serve to protect the seed embryo from insects and pathogens and are nutrient reserves for the germinating embryo (Dupont and

Altenbach, 2003; Malik, 2009). The majority of the active physiological proteins (enzymes) are found in the albumin and globulin fractions (Belderok *et al.*, 2000).

The storage proteins in wheat, gliadin and glutenin, contain high amounts of proline and glutamine amino acids and comprise 75-80% of the protein in wheat (Belderok *et al.*, 2000; Malik, 2009). Due to their high proline and glutamine amino acids, the storage proteins have been reclassified under a prolamin super family with three groups: sulphur-rich, sulphur-poor, and high molecular weight (HMW) prolamins (i.e. HMW glutenins) (Shewry and Tathamt, 1990; Shewry and Halford, 2002). Gliadins and glutenins are mainly located in the mealy endosperm, where they are in a continuous matrix around starch granules, and are not found in the bran or germ (Malik, 2009; Sramkova *et al.*, 2009).

Gliadins are monomeric proteins that consist of single-chain polypeptides. They form intra-chain cysteine disulfide bridges, which gives them a more globular monomeric nature. They are rich in proline and glutamine and have a low level of charged amino acids, hence their low solubility in water. Wieser (2007) grouped the gliadins into four different classes, α, β, γ and ω gliadins, based on their sequence, molecular weight (MW), and amino acid composition. α, β, γ-gliadins contain cysteine residues and can form inter-chain disulfide linkages and are sulfur rich prolamins, whereas ω-gliadins lack any cysteine residues and thus do not form disulfide bonds and therefore are sulfur poor prolamins (Shewry and Halford, 2002; Wieser, 2007). Gliadins, when hydrated, have little elasticity and are not as cohesive as their glutenin counterparts; they contribute to the viscosity and the extensibility of dough systems.

Glutenins are polymeric proteins categorized into two classes – HMW and LMW glutenin subunits. HMW glutenin constitutes ≤ 10% of the flour protein and plays a vital role in forming the glutenin polymer in comparison to LMW gluten. The glutenin proteins are responsible for the strength and elastic properties of dough systems (Dhaka and Khatkar, 2015).

The main function of gliadins and glutenins in a food system is the formation of the gluten matrix, which is formed when the two types of proteins are hydrated and mixed into a coherent mass. This coherent protein mass can have visco-elastic properties that can trap gas, which is suitable to create a large variety of food products such as bread, noodles, cookies, and cakes (Dhaka and Khatkar, 2015). Gluten is comprised of hundreds of protein components that are present in monomeric and polymeric forms that are connected by inter- and intrachain disulfide bonds, tyrosine crosslinks, and non-covalent bonds (hydrogen, ionic, and hydrophobic bonds) (Shewry and Tatham, 1997; Wieser, 2007). Cysteine residues found in gluten forming proteins play a crucial role in forming the inter-chain disulfide bonds between gliadin and glutenin. Furthermore, gluten forming proteins contain a high level of glutamine and proline, which facilitate the hydrogen and hydrophobic bonds, respectively, which aids in the formation of disulfide bonds and stabilizes the gluten matrix (Shewry and Tatham, 1997; Wieser, 2007).

1.5.2.4 Lipids

Lipids are a minor component in wheat grain. However, they have a significant effect on different qualities of foods, such as texture and flavor. In the whole grain, lipids make up 2-4% of the composition but are not evenly distributed in the wheat grain. In the grain, the germ fraction contains 30-35% of the total seed fat content; the endosperm, the largest proportion of the grain, contains the majority of the remaining 60-70% of total seed fat, which constitutes approximately 1.5-2.5% of the endosperm (Day, 2016).

In comparison to other cereal grains, wheat contains the highest amount of glycolipids (non-polar lipids) and phospholipids (polar lipids). Most of the non-polar lipids in wheat are in the form of triacylglycerol (~50%), and the remainder is di- and monoglycerides, free fatty acids (FFA), and sterol esters. In wheat, palmitic acid (16:0) is the major saturated FFA and linoleic acid (18:2, n-6) is the major

unsaturated acid. Oleic acid (18:1, n-9) and α-linolenic acid (18:3, n-3) are the other primary unsaturated FFAs that are present in wheat (Day, 2016).

Understanding the lipid composition is important as it has an impact on the physicochemical nature of dough systems (Morrison, 1963). One type of interaction that occurs is starch-lipid complexes. Amylose forms helices with a hydrophobic interior, which can interact with small non-polar molecules and hydrophobic moieties of amphiphilic molecules, such as FFAs and monoglycerides (Tang and Copeland, 2007). These complexes have an impact on dough functionality and starch pasting properties (Salman and Copeland, 2007). Furthermore, polyunsaturated FFAs can become oxidized, forming hydroperoxides that can further break-down into secondary oxidation products that are odor-active, which can impact acceptability (Heiniö *et al.*, 2002).

1.5.2.5 Antioxidants

Antioxidants are compounds that can delay or prevent the oxidation of substrates, such as lipids (Shahidi, 2000a). Antioxidants can prevent oxidation following three mechanisms: radical scavenging by hydrogen transfer (Toda *et al.*, 1991; Onyeneho and Hettiarachchy, 1992), reduction by electron transfer (Pan *et al.*, 1999), and metal chelation (Teixeira *et al.*, 2013). Cereals grains are rich in antioxidants. In wheat, antioxidants are mainly found in the bran, aleurone, and germ fraction (Smith and Hartley, 1983), and contribute to antioxidant activity significantly more than those found in the endosperm (Zielin´ski *et al.*, 2000). Plant-based antioxidants belong to the family of phenolic and polyphenolic compounds. For wheat specifically, the primary classes of antioxidants are phenolic acids (namely hydroxycinnamic acids), carotenoids, tocopherols, and lignans (Luthria *et al.*, 2015).

Hydroxycinnamic acids (HCAs) are derivatives of cinnamic acid, a type of phenolic acid, that are distinguished by the number of hydroxyl groups on the phenol ring (Kim *et al.*, 2006b; Yu and Cheng, 2007). The main reported types of HCAs in wheat are ferulic, *p-coumaric*, sinapic, and caffeic acid (El-Basyouni *et al.*, 1963). They are primarily found in the bran fraction of the grain in free, soluble conjugated, and insoluble bound forms. The bound phenolic acids are esterified to cell wall polysaccharides, often AX (Lineback and Rasper, 1988). Bound phenolic compounds are present in considerably higher amounts and thus contribute more to antioxidant activity than free phenolics. Therefore, accurate analysis requires either an alkaline or acid hydrolysis to release the bound phenolics, with the former being more efficient for extraction (Smith and Hartley, 1983). Of the esterified HCAs, ferulic acid is the most abundantly found. *p-Coumaric* and sinapic acid are also found in an appreciable amount and contribute to the overall antioxidant activity (Gallardo *et al.*, 2006; Tyl and Ismail, 2018).

Tocopherols and carotenoids are non-polar, lipid-soluble compounds. Tocopherols are classified as vitamin E and are present in four forms, α-, β-, γ-, and δ-tocopherols that differ by their side groups and vary in their vitamin E and antioxidant activity. In wheat, most tocopherols are present in the α and β forms, where α-tocopherols have the highest vitamin E activity (Becker, 2013). Overall, wheat tocopherols have good Vitamin E activity and antioxidant properties and serve to protect the wheat seeds from oxidative activity through hydrogen transfer (Morrison, 1988). Carotenoids, while present in a small amount, can play an essential part in the color of flour, pasta, and baked products (Day, 2016). The principal type of carotenoids found in wheat are xanthophylls, namely lutein and zeaxanthin (Morrison, 1988; Hussain *et al.*, 2015). Lutein and zeaxanthin play an important part in human health, such as promoting healthy eyes and skin, reducing the risk of cancer, and lowering the risk of cardiovascular disease (El-Sayed M. Abdel-Aal *et al.*, 2007; Hussain *et al.*, 2015). The antioxidant mechanism for

carotenoids involves the scavenging of singlet molecular oxygen and peroxyl radicals (Stahl and Sies, 2003).

1.5.2.6 Enzymes

Endogenous wheat enzymes mainly include carbohydrate-degrading enzymes, proteolytic enzymes, ester hydrolases, and oxidases (Kruger and Reed, 1988). Wheat enzymes are predominately found in the pericarp and germ with smaller amounts in the seed coat and aleurone layer. During germination, various types of enzymes are synthesized and secreted to the starchy endosperm, where they degrade seed material, such as starch and protein, to provide nutrients to the germ (Olaerts and Courtin, 2018).

α-Amylase, a carbohydrate-degrading enzyme, is considered the most important wheat enzyme due to its effect on bread-making quality and hence has been extensively researched. α-Amylase is an endohydrolase that cleaves α-(1,4)-D-glucosidic linkages of starch polymers into smaller polysaccharides and sugars, namely glucose. The sugars produced are used up by yeast during dough fermentation, resulting in improved rheological properties and end-product quality (Kim *et al.*, 2006a). However, elevated levels of endogenous α-amylase is considered a defect, since high activity can degrade the starch excessively, reducing its functionality, namely viscosity and gelatinization (Newberry *et al.*, 2018).

Proteolytic enzymes, namely endoproteolytic and acid carboxypeptidases, are important in the metabolism of storage proteins to supply nitrogenous compounds to the germinating seed (Dunaevsky *et al.*, 1989). Endoproteolytic enzymes target peptide bonds in the interior of peptide bonds, whereas acid carboxypeptidases target peptide bonds on the carboxy-terminal end of the chain. Like α-amylase, they are found and synthesized in the pericarp, seed coat, and

aleurone layer before secretion into the endosperm for proteolytic activity (Kruger and Reed, 1988).

Other important wheat enzymes include ester hydrolases and oxidases. The main ester hydrolase is lipase, an enzyme that hydrolyzes triaclyglycerols, resulting in free fatty acids, which are associated with rancid flavors (Scanlan *et al.*, 1965; Kruger and Reed, 1988). Lipases can be found in the bran and germ fraction and not in the endosperm. Oxidases in wheat include peroxidase and lipoxygenase (LOX). Peroxidase catalyzes the oxidation of aromatic amines and phenols using hydrogen peroxide and is involved in the discoloration of doughs (Kruger and Reed, 1988). Peroxidase is typically targeted in cereal processing, namely oats, due to their deleterious effects on quality and being more heat stable than other enzymes, thus denaturing any other potentially unwanted enzymes (Kruger and Reed, 1988; Ekstrand *et al.*, 1993; De Almeida *et al.*, 2014). LOX, which is mainly found in the germ, is another enzyme that has been extensively researched due to its bleaching effect on carotenoids and its impact on dough functionality (Galliard, 1986). LOX catalyzes the peroxidation of polyunsaturated fatty acids using molecular oxygen as a catalyst. LOX plays a major role in oxidative rancidity through the production of peroxides and subsequent secondary products, such as hexanal, which have negative impact on flavor (Shahidi, 2000b). Impact of lipase and LOX on development of flavor will be discussed in more detail in later sections.

1.5.3 Intermediate Wheatgrass Composition

Compared to wheat, IWG has a smaller seed size leading to higher bran to endosperm ratio, which directly impacts the chemical composition. As a result, fiber, micronutrients, and phytochemicals, which are concentrated in the bran and aleurone layer of the grain, are higher in IWG than in wheat (Rahardjo *et al.*, 2018).

IWG was reported to be higher in total DF (16-20%) compared to wheat (13.5-15%) (Rahardjo *et al.*, 2018; Tyl and Ismail, 2018). IWG had ~13-16% insoluble DF and ~3-4% soluble DF (Rahardjo, 2017; Tyl and Ismail, 2018). While higher DF is of nutritional significance, high levels of insoluble fiber can interfere with the dough development, specifically the formation of gluten. Of concern in IWG are the AX, namely the WU-AX, which has been shown to decrease the amount of water available for the establishment of the gluten network, thus negatively impacting the functionality (Autio, 2006).

Another promising traits of IWG from a nutritional standpoint is its high protein content of 18-21% on dry basis (Marti *et al.*, 2015b; Rahardjo *et al.*, 2018; Tyl and Ismail, 2018). However, the protein profile is different than that of wheat (Rahardjo *et al.*, 2018). The distribution of the protein components found in IWG does not support a strong gluten network that is desirable for bread. IWG is deficient in HMW glutenin (Rahardjo *et al.*, 2018; Tyl and Ismail, 2018), which is essential for the elasticity of the dough needed to hold gas in leavened baked goods. IWG had shorter dough development time and lower stability (Rahardjo *et al.*, 2018). Banjade *et al.* (2019) found that the addition of dough conditioners (i.e., transglutaminase and ascorbic acid) could improve dough properties of refined IWG flours by promoting gluten aggregation necessary for forming the gluten matrix. IWG has shown progress through breeding for improved characteristics, such as higher HMW glutenin content (Kantar *et al.*, 2016).

On the other hand, IWG has a significantly lower amount of total starch (46.7% dry basis) compared to wheat (74.9%) due to its smaller endosperm to bran ratio (Rahardjo *et al.*, 2018). Due to lower starch content, the functionality of IWG flour is markedly different than that of wheat (Marti *et al.*, 2015b; Rahardjo *et al.*, 2018). IWG flour had a higher gelatinization temperature, which was attributed solely to the starch content and not its DF content (Marti *et al.*, 2015b). The difference in starch content also results in IWG flour having lower pasting viscosities (i.e., peak, setback, and final viscosity) (Marti *et al.*, 2015b; Rahardjo *et al.*, 2018). With a

lower setback value, the rate of retrogradation and syneresis may be lower, which can be promising in the shelf-life quality of bread products, delaying staling (Marti *et al.*, 2015b).

In terms of fat, IWG has a greater amount (3.2% dry basis) than whole wheat (2.8%) (Tyl and Ismail, 2018), which can also be attributed to the smaller seed size as fat is located mostly in the germ. With a higher fat content, there is a concern regarding the storage stability of IWG due to potential hydrolytic and oxidative rancidity. Of interest for cereal grains is enzyme activity that catalyzes lipid hydrolysis and oxidation, namely lipase and LOX, respectively. Tyl and Ismail (2018) and Mathiowetz (2018) measured significantly lower lipoxygenase activity in IWG compared to HRW. However, lipase activity was found to be, in general, higher than HRW. The products of lipid hydrolysis and oxidation can impart off-flavors that decrease sensory acceptability, which will limit the storage life and quality.

Although IWG has a higher total fat content and lipase activity that can actuate rancidity over storage, IWG has a significantly higher amount of antioxidants than that of wheat (Tyl and Ismail, 2018). Tyl and Ismail (2018) measured the carotenoids lutein and zeaxanthin and hydroxycinnamic acids, namely trans-ferulic acid, trans-p-coumaric acid, and trans-sinapic acid and found that IWG has significantly higher levels than wheat. The storage stability of IWG compared to wheat and all the factors that contribute to or prevent rancidity over storage will be a focal point of discussion.

1.6 Storage of Wheat Grains and Flour

Preservation of food has always been a focal point in our food system to ensure safe and nutritional food is available for consumption. Many factors need to be known to ensure that food ingredients and final products are satisfactory to

consumers. With a substantial amount of wheat being grown and produced, optimal storage conditions will be needed to ensure highest nutritional and functional aspects as well as safety. After harvest, wheat can be stored as grain or immediately processed into flour. Grains have longer shelf life than flour, but the shelf life of either forms are dependent on several endogenous, environmental, and processing factors as will be discussed in the following sections.

1.6.1 Wheat Grain Storage

Upon harvest, wheat is often stored up to several years before milling and processing. Immediately after harvest, grains are dried to under 14% moisture content to maintain the quality of the grains (Pomeranz, 1992; Grundas and Wrigley, 2016). The grains are then stored in grain elevators or silos, where humidity and temperature are controlled (Wrigley, 2016). The storage conditions of the grains are essential in ensuring the quality of the grains and minimizing physical, chemical, and biological damage. Important factors for grain storage are temperature, moisture content, carbon dioxide, oxygen, grain characteristics, microorganisms, pests, and geographical location (Jayas *et al.*, 1995; Jayas and White, 2003).

The two most important factors for the shelf life of grains are temperature and moisture content. Under elevated temperature and moisture content, mycotoxins and microbial activity can render the grain unsafe for consumption. Therefore, proper temperature and humidity control are needed to protect the quality of the grains. Grain storage facilities are equipped with aeration systems that are used to maintain a homogenous temperature to prevent any large differentials that can occur. Wheat grains are kept below 14% moisture content at a temperature that does not exceed 35°C. Above 14% moisture content, microbial growth and insect infestation are of primary concern. Fumigation or the use of carbon dioxide in the storage silos can mitigate microbial growth and pest

infestation. On the other hand, at a moisture content below 8%, the rancidity of lipids can become an issue. Wheat bran lipase has exceptional activity under low moisture environments. At standard storage moisture content, around 12-14%, hydrolysis of lipids is slow, corresponding with a limited production of FFAs (Clayton and Morrison, 1972).

Another concern is grain respiration, which is defined as the oxidation of carbohydrates resulting in the production of carbon dioxide, water, and heat. If the moisture content is too high, an increase in grain respiration can allow for decrease stability, loss in mass, and loss in flour functionality (Grundas and Wrigley, 2016). Under proper storage conditions, stored intact grains can remain acceptable for several years with minimal degradation. After storage, whole grains are typically further processed into flour, which can be further stored or immediately used for food applications.

1.6.2 Wheat Flour Storage

In comparison to intact grains, wheat flour has a much shorter shelf-life. In the intact kernel, bran, endosperm, and germ are separated; thus, any enzymes and substrates are not in direct contact. However, once milled, enzymes and substrates can interact. The exposure of enzymes to substrates is of concern mostly in whole grain flour due to the presence of the germ and bran that contain the majority of the lipids and lipid degrading enzymes (Doblado-Maldonado *et al.*, 2012). The lipids in whole grain flour would be subject to lipolytic enzymes, namely lipase, and oxidative enzymes, namely LOX, resulting in hydrolytic and oxidative rancidity, respectively.

One way to extend the shelf-life of flour is through refinement, the removal of the bran and germ components. However, with the growing demand for whole-grain products, refinement may not be a viable solution. Therefore, it is

recommended that grains are stored as long as possible before being milled (Hansen and Rose, 1996). Storage of grains can be complicated, however, and immediate milling of wheat to flour may be necessary for the industry. Thus, understanding the different factors that affect flour shelf-life is important.

Similar to the grain, flour storage stability is impacted by storage temperature and relative humidity. Other factors that influence flour shelf-life include milling technique, degree of refinement, and the length of grain storage prior to milling (Doblado-Maldonado *et al.*, 2012). Prior to milling, wheat grains can be aged to improve characteristics for end-use due to changes in its composition over storage (Shelke *et al.*, 1992; Seguchi, 1993). Changes over the storage of wheat grains and flour and their impact on functionality will be discussed later.

The milling technique significantly impacts the flour's sensory quality and functionality. The two standard milling techniques are stone and roller milling. Stone milling is the older of the two processes that involve grinding wheat kernels between two stones (Kihlberg *et al.*, 2004; Doblado-Maldonado *et al.*, 2012). Stone milling can produce a considerable amount of heat due to friction, which can be problematic when lipid content is high. Heat can act as a catalyst for the autoxidation of lipids, leading to loss of flour quality through the formation of off-flavors (Shahidi, 2000b). Roller milling has the advantage of building less heat. Roller milling is the most common type of milling that involves the separation of the endosperm from the bran and germ, followed by the gradual size reduction of the endosperm. Different streams of the milled flour can then be blended to achieve different functionality as well as refinement (completely refined, partially refined, or whole flour). One important advantage of roller milling is that the separated bran and germ fraction can undergo further processing, such as thermal treatment or fine grinding, that can potentially deactivate problematic enzymes or improve functional properties, respectively (Doblado-Maldonado *et al.*, 2012).

As mentioned before, whole wheat flour has a shorter shelf-life compared to refined flour due to the higher level of lipids and enzymes. Thus, whole grain flours are typically stamped with a shorter use-by-date of three to nine months, whereas refined flour has a use-by-date of nine to fifteen months (Doblado-Maldonado *et al.*, 2012). For IWG, the focus will be on lipid degradation due to IWG's higher fat content and enzyme activity, and on functionality as well as the flavor changes over storage. Understanding the different factors that can affect shelf-life and their impact on acceptability and functionality for grain and flour is essential in the usability and marketability for future use.

1.6.3 Hydrolytic and Oxidative Rancidity over Storage

Over storage, the sensory acceptability and functionality of grains and flour can significantly be impacted by lipid rancidity, which is caused by enzymatic and non-enzymatic pathways. Specifically, two enzymes of concern are lipase and LOX, which cause hydrolytic and oxidative rancidity, respectively. Lipase hydrolyzes acylglycerols releasing free fatty acids. Free fatty acid (FFA), especially polyunsaturated fatty acids, are more prone to oxidation than the esterified counterpart. Galliard (1986) observed a linear increase of oxidative rancidity due to increased levels of FFAs in whole grain flour stored at 20°C, which correlated closely with the uptake of oxygen. FFAs can contribute to off-flavors, namely bitter and rancid, that can decrease sensory acceptability (Heiniö *et al.*, 2002).

LOX is an oxidative enzyme that catalyzes the oxidation of unsaturated fatty acids into hydroperoxides. The hydroperoxides formed are colorless and odorless; however, they are quite unstable. Hydroperoxides breakdown into alkoxy free radicals that can be substituted with aldehydes, alcohol, or hydrocarbon groups forming secondary oxidation products. These products are known to be volatile and contribute to off-flavors (Shahidi, 2000b). Hexanal is one of the first secondary oxidation products that form and typically used to determine shelf-life due to its

characteristics off-odor that has been correlated with bitter, rancid, and musty (Heiniö *et al.*, 2002). Formation of hexanal has good correlation with the formation of linoleates, the peroxide formed from linoleic acid, and with other off-flavors and overall sensory perceptions, making its detection a valid indicator of oxidative stability (Fritsch and Gale, 1977; Shahidi *et al.*, 1987; van Ruth *et al.*, 2000; Nissen *et al.*, 2004). Besides the enzymatic oxidation from LOX, lipids are also susceptible to non-enzymatic oxidation, such as autoxidation, which occurs spontaneously in the presence of oxygen and catalysts. However, oxidation through enzymatic and non-enzymatic pathways can be slowed down in the presence of antioxidants.

1.7 Changes in Functionality over Storage

Aging of wheat grains and flours short-term has resulted in improved functionality (Pomeranz, 1992). An enhancement in functionality was observed up to 2-4 months of storage of intact grains before a decrease in functionality was noted over continued storage (Pomeranz, 1992; Kibar, 2015). Stored grains and flour have improved baking qualities such as higher water absorption, higher hydration rate, better mixing tolerance, higher dough viscosity and stability, enhanced gas retention, and heightened loaf volumes (Wang and Flores, 1999; Kibar, 2015). The improvement in functionality was attributed to the slow oxidation of thiols, which aid in stabilizing the gluten network (Wang and Flores, 1999). However, over extended storage of the wheat flour or grain, loss in functionality was noted mostly due to the formation of oxidation products of unsaturated fatty acids (Pomeranz *et al.*, 1968).

Flour produced from previously-stored grains has favorable milling qualities and functionality in comparison to flour from freshly harvested grain. Improvements observed in milling and functionality due to storage are related to post-harvest maturation and physical changes (Wang & Flores, 1999). Often, flour from freshly

harvested grain is subjected to treatments or additives to improve functionality, such as addition of oxidizing agents to help promote gluten formation via disulfide bonds (e.g. ascorbic acid and potassium bromate) (Grosch and Wieser, 1999), enzymes to aid in dough fermentation (e.g. amylases, proteases, lipoxygenases) (Kim *et al.*, 2006a; Renzetti *et al.*, 2010), or bleaching agents to improve aesthetics (e.g. chlorination) (Tsen and Kulp, 1971). The storage of grain prior to milling may improve flour functionality without the use of additives, due to the formation of lipid catabolism products, which act as "natural" oxidizing agents and promote thiol interactions between gluten-forming proteins, thus strengthening the gluten network (Doblado-Maldonado, 2012; Every, Simmons, & Ross, 2006; Kanner, 2007; Malekian et al., 2000).

Changes in the lipid content and starch can also alter dough functionality and baking qualities. Over storage, changes in the lipid profile through enzymatic lipolysis and subsequent interactions with starch components can have an impact on functionality. The release of FFAs over storage from endogenous lipase is painted as mostly negative due to their unfavorable organoleptic changes, but lipid hydrolysis can positively influence flour functionality (Tait and Galliard, 1988; Zhang and Hamaker, 2005). Low concentrations of non-esterified polyunsaturated fatty acids positively correlated with high loaf volumes due to the oxidation of FFA and the formation of lipid-starch complexes over storage (Miller and Kummerow, 1948; Carr *et al.*, 1992). However, at high levels, upon prolonged storage, polyunsaturated FFA can interfere with the formation of gluten, thus negatively impact loaf volumes.

Lipid-starch complexes, specifically amylose-lipid complexes (AM-L), can form over storage. AM-L are formed when the starch and lipids, namely mono-diacylglycerols and FFA, are heated together (Kugimiya *et al.*, 1980; Gerits *et al.*, 2015), and can influence the starch pasting profile of the wheat flour. AM-L are formed when amylose takes on a helix conformation in the presence of aliphatic and amphiphilic regions of lipids, which interact with the hydrophobic interior of the

helix (Conde-Petit and Escher, 1992; Putseys *et al.*, 2010). The AM-L complexes are insoluble and aggregate in an aqueous system to form an inter-granular network that results in increased viscosity (Conde-Petit and Escher, 1992; Putseys *et al.*, 2010). AM-L can increase the gelatinization peak viscosity, holding viscosity, and final viscosity, along with setback and breakdown values (Zhang and Hamaker, 2005; Fierens *et al.*, 2015; Gerits *et al.*, 2015). Over storage, AM-L complexes can form in unmilled wheat groats or form after milling. The degree of AM-L complex formation is influenced by the degree of unsaturation and length of the fatty acid chains (Tufvesson *et al.*, 2003; Fierens *et al.*, 2015), with a lower degree of unsaturation and longer chain lengths favoring formation of the complexes due to the hydrophobic nature of the interior of amylose helices.

IWG has a high fat content and can have high FFA content over storage as a result of its high lipase content. The chemical composition of IWG predisposes it to various chemical changes that have not been thoroughly documented. Therefore, it is necessary to observe changes in IWG's starch pasting profile and dough functionality over storage in order to understand its storage stability in terms of functional performance. Mathiowetz (2018) found that stored IWG groats had improved dough development time, resistance to extension, and dough stability time compared to fresh groats. Furthermore, Mathiowetz (2018) found that IWG had improved starch pasting viscosities over storage. However, studies on the mechanisms of functionality changes over the storage of IWG flour have not been conducted. Due to the nature of flour compared to groats, the mechanisms of change over storage, namely lipid rancidity, need to be explored in IWG. Understanding these changes in stored IWG flour will help with choosing the proper processing strategies, such as refinement or heat treatment, to elongate shelf-life.

1.8 Changes in Flavor over Storage

1.8.1 Flavor Perception

Consumers drive the food industry. One of the most critical aspects that influences the consumer's decision to buy and eat a food product is its flavor. Flavor is defined as the sensory perception of taste (non-volatile compounds) and aroma (volatile compounds). The nature of the human perception of flavors is overwhelmingly complex and multimodal. Perception of food involves taste, mouthfeel, vision, olfaction, trigeminal stimulation, and auditory signals that all contribute to the experience. The interactions between these perceptive modalities are complex and non-linear (Bult *et al.*, 2007). As such, due to the immensity of the human olfactory system, the dogma surrounding the relationship between the senses of smell and taste perception is not entirely understood.

Aromas stem from small volatile odor active compounds (VOACs). Over 7,000 VOACs have been identified and described in food and beverages, all of which have unique odor qualities (Goff and Klee, 2006). In humans, studies have shown that we have a very keen sense of smell (Shepherd, 2004; Weiss *et al.*, 2014). The act of smelling odorant is done through two routes; sniffed through the nose (orthonasal) or through the mouth followed by propagation up the throat into the nose (retronasal) (Shepherd, 2004; Weiss *et al.*, 2014). Odors then travel from the back of the nose to the olfactory epithelium containing millions of sensory neurons. Odors then bind to olfactory receptor cells where they are detected. However, the difference between individuals can be immense. For instance, a compound perceived as offensive by one can be pleasant or odorless to another (Keller *et al.*, 2007; Keller and Vosshall, 2008). Therefore, it is important when studying flavors that multiple panelists are involved in order to characterize what is perceived considering human variability.

For the successful commercialization of IWG, sensory evaluation and flavor analysis need to be conducted to fully characterize its flavor profile. Research on the flavor properties of IWG is minimal, especially on the changes in flavor over storage. Sneddon and Peterson (2016) analyzed the comparative aromas found in IWG bread crust. However, for IWG to be a successful crop, flavor development during the storage of the grain and flour needs to be evaluated. Research on the development of flavor in IWG flour over storage will help devise a plan to maintain acceptable sensory quality for end-use applications. To mitigate flavor development over storage it is imperative to understand the pathways that lead to flavor development.

1.8.2 Flavor Pathways in Flour

In flour, the main pathway for flavor development is the degradation of lipids via enzymatic and non-enzymatic activity. Since flour typically does not go through any heat processes, types of flavor compounds formed are limited. The primary source of volatiles in flour is the oxidation of unsaturated fatty acids resulting in the formation of secondary metabolites that can be odor active. The volatiles produced upon oxidation includes pentanal, hexanal, 1-octen-3-ol, and other types of alcohols and aldehydes (**Figure 2**) (Maeda *et al.*, 2008). In stored oat flour, the primary volatile compounds were hexanal and 2-pentylfuran (Molteberg *et al.*, 1996). These volatile compounds are typically associated with green and earthy aromas. There is currently limited research done on the volatile compounds from wheat flour, as it is not an end-product. Most research on wheat flavors is primarily on end-products focusing on the flavors formed during dough fermentation or flavors in breads and other baked goods. However, odor active volatiles found in breads have also been found in flours, thus odor active compounds in flour can potentially impact the flavor profile of end-products (Czerny and Schieberle, 2002).

IWG has a similar composition to wheat and other cereals, albeit in different proportions. No research has been done on the flavor profile of IWG flour, but previous research on flavor profiles from other cereal flours, such as annual wheat and oats, can be relatable due to their similar composition. The aroma pathways involved in widely known cereal flours will most likely be similar to that of IWG flour.

There are multiple pathways in which aroma compounds are formed in foods. The compounds that give foods their flavor depends on the diversity of the raw material and the processing conditions. In wheat the most important pathways include caramelization, the Maillard reaction, yeast fermentation, and lipid oxidation.

1.8.2.1 Flavor Development through Caramelization, Maillard Reaction, and Fermentation

Caramelization and the Maillard reaction are important in the formation of flavors in baked products (e.g., bread, cakes, and cookies). Caramelization is a non-enzymatic browning reaction that occurs with the decomposition of sugars occurring at temperatures greater than 120°C, forming aromatic volatiles and brown-colored compounds such as furans, furanones, pyrones, and carbocyclic compounds (Kroh, 1994). The Maillard reaction, which can form a variety of flavor compounds, is another non-enzymatic reaction that occurs between free amino groups of amino acids, peptides, proteins, and carbonyl moiety of reducing sugars (Ruan *et al.*, 2018). Types of flavor compounds from the Maillard reaction include furans, pyrones, cyclopentenes, carbonyls, acids, pyridines, pyrazines, imidazoles, oxazoles, and thiazoles (Nursten, 2019).

Bread dough fermentation by yeast and bacteria also plays a huge role in the flavor profiles of wheat bread. During dough fermentation, the addition of yeast (e.g., *Saccharomyces cerevisiae*) or bacteria (e.g., *Lactobacillus)* starter cultures

are used to generate flavor compounds. Lactic acid bacteria contribute by producing lactic acid, acetic acid, ethanol, and CO_2, as well as other minor compounds. Flavor compounds formed by yeast fermentation include alcohols, aldehydes, 2-3-butanedione (diacetyl), and esters (Birch *et al.*, 2013).

The previously mentioned flavor pathways do not readily occur over the storage of flour. The primary mechanism of flavor generation over the storage of flour is lipid catabolism, which begins with the hydrolysis of triacylglycerols releasing FFAs via lipase, the oxidation of FFA through LOX, and further enzymatic or non-enzymatic reactions to form secondary products. In comparison to the Maillard reaction, lipid oxidation does not produce as wide of a diverse set of flavor compounds but still plays a significant role in flavor development.

1.8.2.2 Flavor Development through Lipid Catabolism

Flour composition will influence the level of lipid catabolism. Whole grain flour contains more fat than refined flour; therefore, it is expected that there will be higher levels of lipid-derived flavor compounds in whole grain flour. In comparison to wheat, IWG has significantly higher fat. With higher fat content, the two enzymes of concern for the storage stability of IWG flour are lipase and LOX that contribute to hydrolytic and oxidative rancidity, respectively.

Hydrolytic rancidity of lipids is the first step of lipid catabolism. Hydrolysis of lipids, namely acylglycerols, occurs in the presence of lipase and adequate moisture to break the ester bond between the glycerol and fatty acid, producing FFAs (Shahidi, 2000b). Oats contain relatively high lipid content with a large proportion of unsaturated fatty acids and a significantly more active lipase activity compared to other cereals, such as barley and wheat (O'Connor *et al.*, 1992). Over the storage of oats, levels of FFAs increased due to hydrolytic rancidity by lipase (Lein Molteberg *et al.*, 1995). Mathiowetz (2018) investigated the change in total

FFAs of stored IWG groats and found a steady increase over storage, indicating lipase activity. However, changes in FFA content in stored IWG flour have yet to be conducted, as well as research on changes in flavor over storage. FFAs are a major contributor to aroma and taste for certain foods; however, elevated levels can have undesirable sensory qualities (Scanlan *et al.*, 1965). FFAs have been described as having sour, astringent, pungent, and burning bitter flavors (Chale-Rush *et al.*, 2007). With the hydrolytic release of FFAs from triacylglycerols, the next concern is the subsequent oxidation of the FFAs, leading to further rancidity and undesirable flavor formation. Free and unsaturated FFAs are of concern as they are more prone to lipid oxidation than their esterified counterparts through a variety of pathways leading to different FFA derived aroma compounds (O'Connor *et al.*, 1992; Shahidi, 2000b).

Lipid oxidation, which includes autoxidation, photo-oxidation, and enzyme-derived oxidation, can result in a diverse set of metabolites (Shahidi, 2001). Unsaturated FFAs released upon lipase hydrolysis of wheat lipids serve as the main substrate for lipid oxidation. Mathiowetz (2018) found that the predominant unsaturated fatty acids in both wheat and IWG were oleic acid, linoleic, and linolenic fatty acids. Lipid oxidation pathways are influenced by the type of FFAs released upon hydrolysis of triacylglycerols, presence of antioxidants, and metal ions (Shahidi, 2000b).

The primary oxidation products (i.e., hydroperoxides) formed from lipid oxidation are tasteless and odorless; however, they are highly unstable, due to a weak oxygen-oxygen bond. Hydroperoxides readily breakdown to secondary oxidation products, such as hydrocarbons, aldehydes, and alcohols, that are often volatile and contribute to off-flavors (Shahidi, 2000b; Heiniö *et al.*, 2002). The mechanism for lipid oxidation is illustrated in **Figure 2,** which shows the formation of hydroperoxides (ROOH) to the alkoxy radical (RO˙) and subsequent breakdown into secondary oxidation products (Shahidi, 2000b).

Autoxidation and photooxidation are two non-enzymatic forms of lipid oxidation, forming very similar end products via different mechanisms. Both require an unsaturated fatty acid; however, autoxidation uses a triple oxygen species with an initiator (e.g. heat, light, transition metal ions, etc.), while photooxidation uses a singlet oxygen and photosensitizer (e.g. wheat chloroplast). The resulting hydroperoxides formed from these two oxidation pathways are unique. For example, autoxidation of linoleic acid forms a 1:1 ratio of 9-OOH and 13-OOH hydroperoxides, whereas photooxidation forms a 2:1:1:2 ratio of 9-OOH, 10-OOH, 12-OOH, and 13-OOH, respectively. The breakdown of these hydroperoxides will then form different carbonyl compounds that are odor active (Shahidi, 2000b).

LOX, as previously discussed, is an enzyme found endogenously in wheat that oxidizes fatty acids to form hydroperoxides. Hydroperoxides are formed through LOX by adding molecular oxygen to the fatty acid specifically at the cis, cis 1,4-pentadiene (**Figure 3**). The resulting hydroperoxides are available for further degradation by other enzymatic or non-enzymatic reactions (Shahidi, 2000b; Peterson and Reineccius, 2002). The resulting compounds from further degradation of peroxides generated from LOX are known to cause the formation of undesirable off-flavor compounds (Shahidi, 2000b).

IWG, in comparison to wheat, has a very similar fatty acid profile; however, due to the higher fat content and higher lipase activity, IWG may have more FFAs produced over storage, of which unsaturated FFAs are prone to LOX. **Figure 4** shows the potential cleavage sites of oleic acid and linoleic acid, and the possible resulting odor active compounds that can form upon their oxidation. The unstable hydroperoxides and alkoxy radicals are further broken down by enzymes, such as peroxidases and isomerases, lyases, alcohol dehydrogenases, or by non-enzymatic reactions, resulting in various non-volatile and volatile flavor active compounds (**Figure 2)** (Shahidi, 2000b; Än Salas *et al.*, 2005).

As time progresses and rancidity occurs, certain VOACs from lipid oxidation can be used as markers. For example, hexanal is a major odor active oxidation compound that has been proposed as an indicator of the extent of oxidation in cereals and other products prone to lipid oxidation (Shahidi, 2000b; van Ruth *et al.*, 2000). **Figure 5** shows the formation of hexanal through the oxidation of linoleic acid and its hydroperoxides. IWG flour will most likely behave similarly to other stored cereal flours, and thus lipid hydrolysis and oxidation will most likely be the primary pathway of developing flavor compounds.

As of yet, no research has been conducted on the development of flavors via lipid catabolism of IWG flour. Investigating the different flavor compounds that can form over storage will be a tedious task as the possibility of having many different compounds formed through these various pathways is high. Evaluating the flavor compounds and changes in sensory attributes of IWG flour will require methods that allow for the extraction of the various compounds and the determination of the compounds that contribute the most to the overall flavor profile.

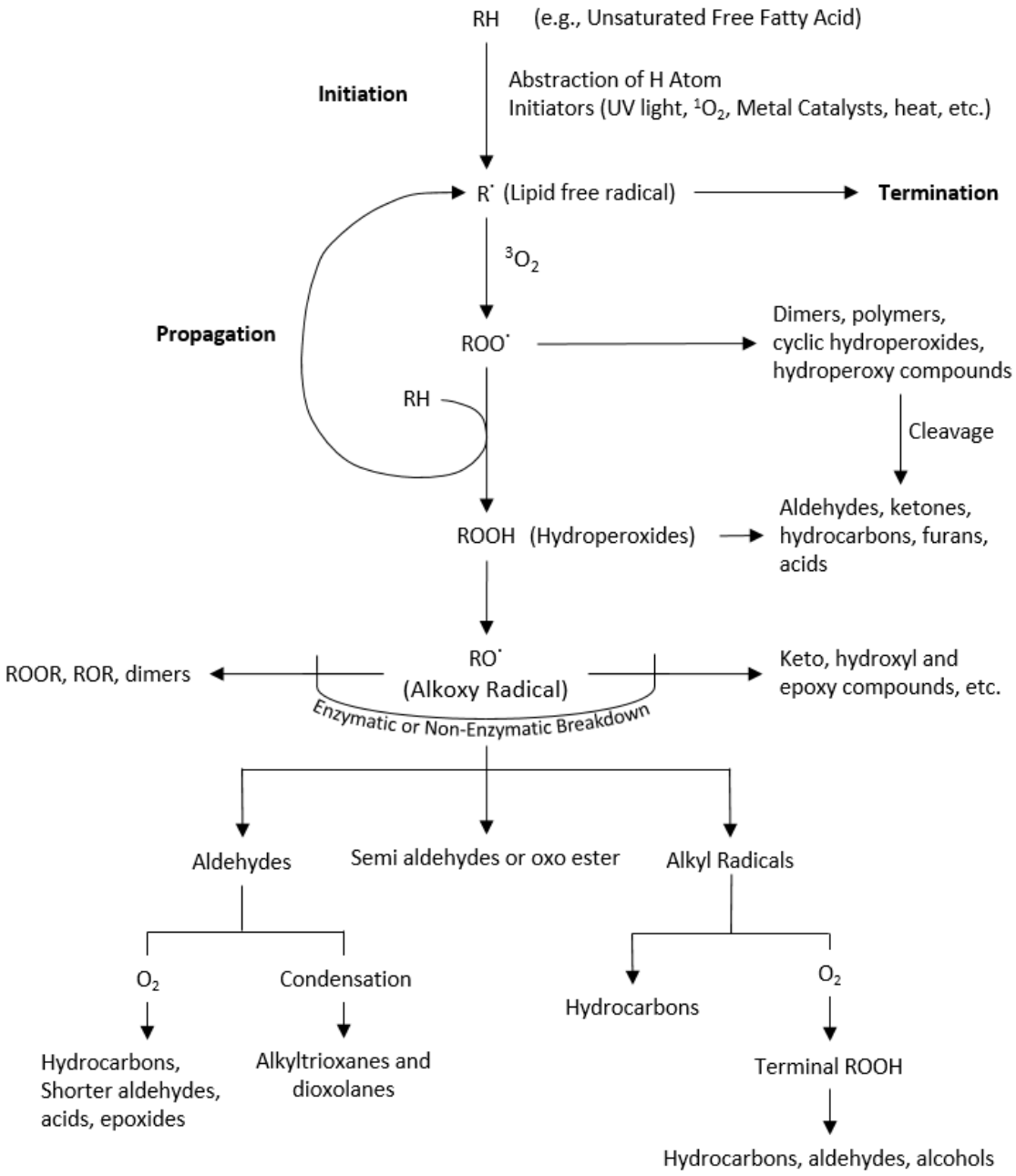


Figure 2. Mechanism of lipid oxidation and formation of primary and secondary degradation products (Shahidi, 2000b)

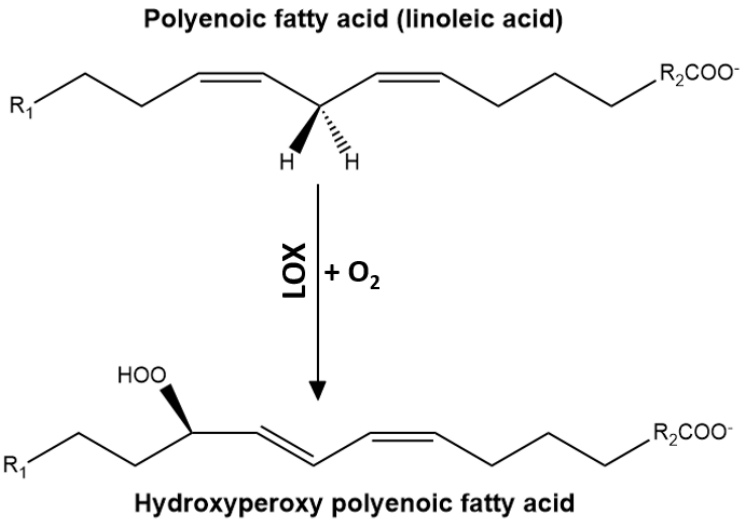


Figure 3. Simplified scheme of the lipoxygenase reaction. LOXs convert polyenoic fatty acids containing at least one 1, 4-pentadiene moiety to their corresponding hydroperoxyl derivatives. Atmospheric oxygen serves as a second substrate.

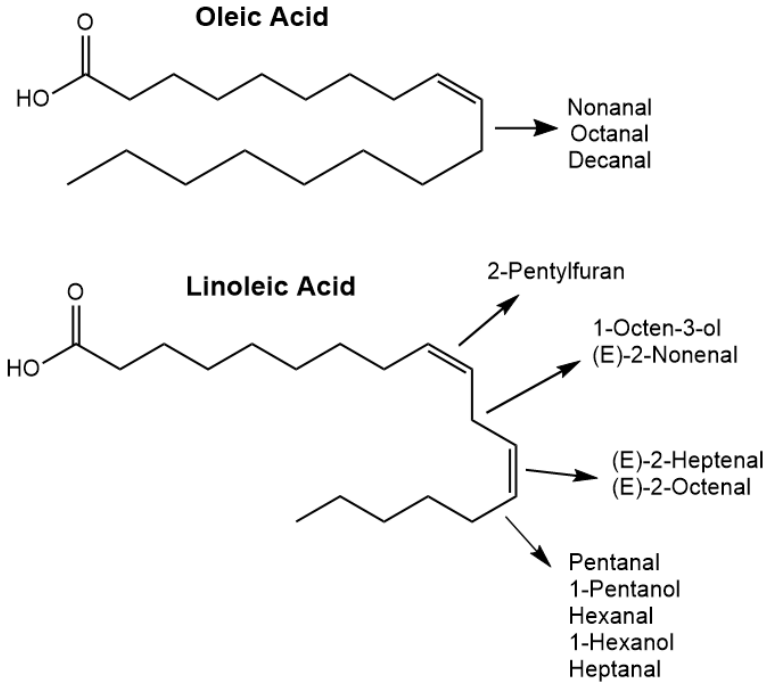


Figure 4. Example aroma compounds and their cleave potential cleavage point from the oxidative degradation of oleic and linoleic acid

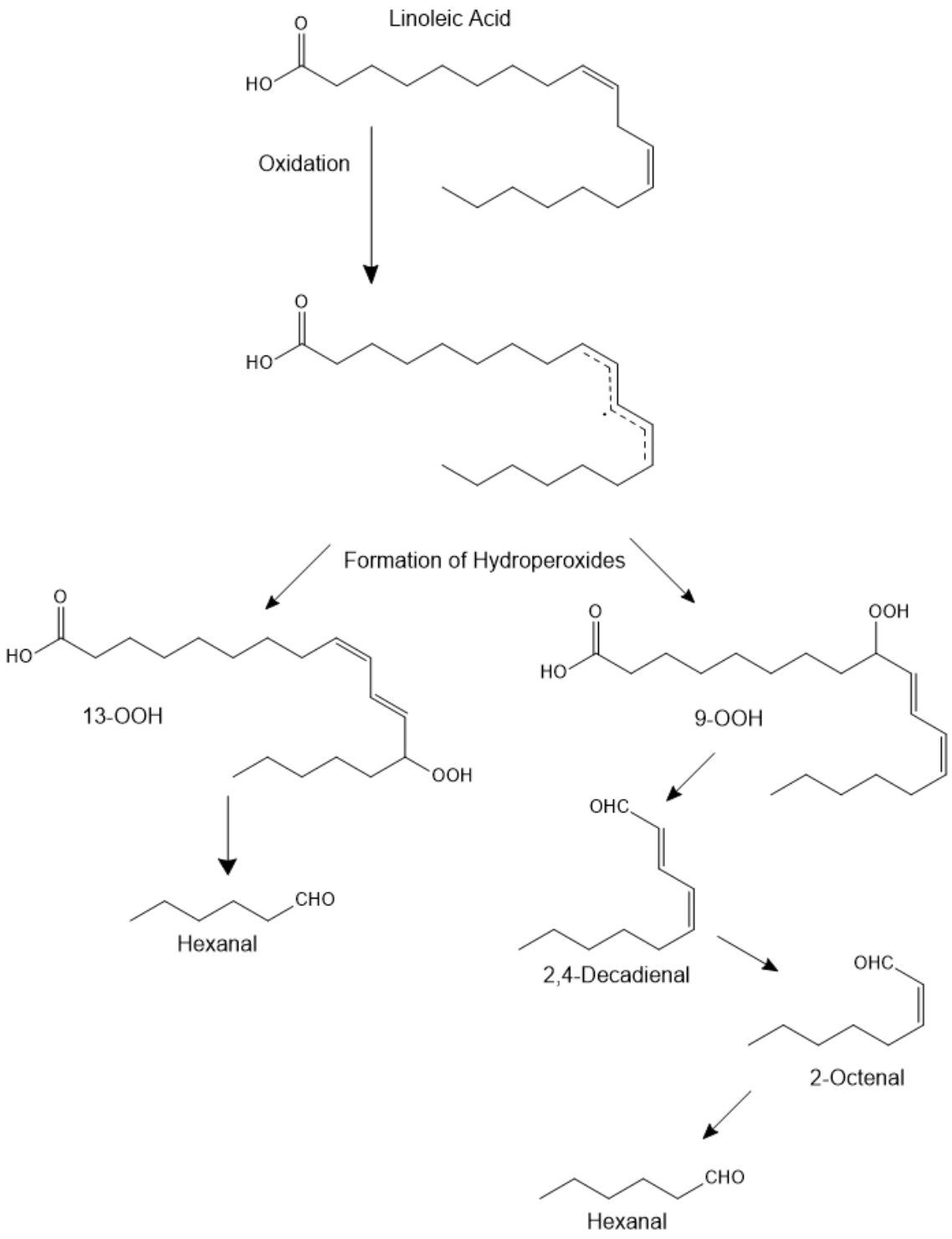


Figure 5. Oxidation of linoleic acid and formation of hexanal and 2-octenal from 2,4-decadienal.

1.8.3 Methods of Detecting Change in Flavor

1.8.3.1 Instrumental Analysis of Odor-Active Compounds

Gas chromatography (GC) is the most widely used analytical technique for evaluating aromatic compounds (i.e., volatile) in foods. The separation of aroma compounds by GC is often coupled with mass spectrometric detection (GC-MS). However, volatile compounds detected by GC-MS may not be odor active. Olfactometry is often coupled with GC analysis to identify odor-active compounds (van Ruth, 2001). GC-olfactometry (GC-O) is performed by splitting the effluent coming through the GC column and directing it to a capillary located inside a heated arm with a nose “sniff” cone. An analyst will then be sniffing the eluting volatile compounds as they get separated and leave the column. This technique allows for the simultaneous MS identification of the volatile compounds along with its odor, if active (van Ruth, 2001; Delahunty *et al.*, 2006).

There are limitations, however, to using GC-O. Differentiation between odor compounds that contribute intensely to a flavor profile and those that are background is a difficult task. Another issue is that in comparison to the human nose, the sensitivity of the instrument could fail to detect an odor active compound that was detected by a panelist. Chang et al. (1995) measured VOACs from a wheat sample where odor active compounds were above the human threshold but below the instrument detector’s threshold. This could be due to having a too low concentration of these VOACs, thus concentrating the compounds could potentially solve this problem using different extraction methods. While the human nose is a lot more sensitive than instrumental detectors, the sensitivity differs among individuals, and it changes throughout the day, resulting in large variability in the data (Rabin and Cain, 1986). Another limitation is that the evaluation of the odors through GC-O is not representative of the usual delivery of the sample. Therefore, it is important to employ an extraction method that will extract most if

not all odor active compounds that may contribute to the flavor profile as well as complementing the analytical data with sensory evaluation to understand the flavor profile.

A common flavor extraction technique is the headspace method — concentrating volatile compounds in the headspace of the food sample followed by trapping the compounds onto an absorbent. This technique can be performed by purge-and-trap (PnT), also referred to as dynamic headspace. PnT can trap both volatile and semi-volatile compounds. PnT involves using an inert gas, such as helium (He) or nitrogen (N_2), a sample holder, and a trap at the exhaust of the system. The purge gas strips the VOACs from the food matrix and carries them to the headspace and through a trap, located at the exhaust, that is packed with an absorbent material, such as Tenax-TA (Sucan *et al.*, 1998). The traps with the absorbed VOACs can then be put through an injector system that will thermally desorb the compounds to be analyzed by GC (Chang *et al.*, 1995; Sucan *et al.*, 1998). The use of PnT can help identify the main odor active compounds that are most involved with the overall profile of a food product. Results from instrumental analysis can then be further correlated with sensory analysis to determine what aroma compounds contribute to organoleptic importance.

1.8.3.2 Sensory Analysis of Flavor

With the limitations of instrumental analysis, sensory analysis can be used to complement analytical data. Descriptive analysis (DA) is a technique that utilizes human senses to evaluate sensory properties based on the basic sense of taste, smell, touch, and sight. DA allows for a better understanding of product acceptance and preference (Gacula, 1997). Panelists, typically 8-15 participants, are trained on specific attributes and descriptors formulated by the group for a particular food. Once trained, each panelist separately evaluates the samples, and judge them

quantitatively using an interval scale (Gacula, 1997). The end goal of DA is profiling all the perceived sensory characteristics.

Sensory evaluation is a reliable method in determining if a product has acceptable qualities to consumers. Thus, sensory evaluation can be coupled with analytical measurements to relate off-flavor development to lipid oxidation over storage (Bett and Grim, 1994). Utilizing DA to analyze the sensory attributes of stored IWG flour and seeing the changes in the intensity of the different attributes over storage can help determine at which point of storage these changes become significant. Producing both analytical and sensory data allows for a better conclusion of a product's acceptability over storage.

1.9 Thermal Treatment to Enhance Storage Stability

The degradation of lipids is a common mode of failure for cereal grains that impacts the final product's sensory and functional properties over storage. Efforts to minimize lipid degradation in grains occur throughout the processing chain, starting with controlling environmental conditions immediately after harvest. Besides controlling environmental conditions, as discussed previously, other processes can be employed to enhance grain and flour stability over storage. Heat treatment has been used to extend the shelf-life of grains, such as oats, by inactivating enzymes that are responsible for hydrolytic and oxidative rancidity (Ekstrand *et al.*, 1993). Thermal inactivation of peroxidase, a thermally stable enzyme found in grains, has been used as an indicator of general enzyme inactivation (Bookwalter *et al.*, 1991). There are multiple ways to thermally treat cereal grains utilizing different types of technology. The various types of heat treatment delivered can have different impacts on end-product functionality and quality. Therefore, it is important to select a thermal treatment that provides the expected outcome, while maintaining food quality and acceptability.

1.9.1 Types of Heat Treatments

1.9.1.1 Dry Heat Treatment

Traditionally, dry heat methods are used to bring down the moisture content of grains, typically to 12-14%, to maintain quality and safety during storage (Bala, 2017). Dry heat treatment can be applied through conventional ovens, vacuum ovens, blast ovens, kilns, and microwave. Temperatures (100° - 230°C) and duration of treatment can vary depending on the process. Typically, whole grains (e.g. whole wheat or oats) are subjected to heat treatment, but bran and germ fractions are sometimes treated separately during the milling process to extend shelf life (Rose *et al.*, 2008; Bala, 2017).

Forced draft oven at 175°C and microwave (1,000W) dry heating methods at various lengths of time have been shown to significantly decrease lipase activity in whole wheat flour while maintaining antioxidant content (Rose *et al.*, 2008). However, Hu *et al.* (2018) found that dry heating of wheat bran at 170°C from 1 – 20 minutes, in comparison to a wet heat treatment, resulted in lower antioxidant activity. The difference in dry heat treatment, in terms of temperature and time, as well as the difference in starting material can have varied effectiveness.

Although dry heat can reduce enzyme activity, the process requires a relatively long time at high temperatures, which can cause auto-oxidation of lipids (Hu *et al.*, 2018). Furthermore, dry heating has been shown to negatively impact functionality. Microwave heating of whole wheat flour caused denaturation of gluten proteins, thus resulting in a weaker gluten network (Qu *et al.*, 2017). Milder dry heat methods with lower temperatures and longer time have been successful in lowering enzyme activity and preserving quality, but treatment was deemed not feasible for the industry due to the longer time involved and the sheer amount of grains being harvested and stored. Dry heat methods are thus not practical for commercial use due to the time involved and the cost for the higher energy input.

1.9.1.2 Micronization

Micronization is a form of dry heat process that utilizes infrared electromagnetic radiation to penetrate the grains, causing the water to vibrate. The vibrations cause increased internal heating and water vapor pressure that can inactivate enzymes and reduce microbial count (Fasina *et al.*, 1999; McAllister and Sultana, 2011). Micronization is a simple heat treatment that can be utilized in the food industry due to its simplicity of equipment and operation in comparison to other dry-heat methods (Fasina *et al.*, 1999). In industry, it has been used on cereals and legumes to dry, shorten cooking time, reduce bacteria, mold, and pests, and inactivate anti-nutritional components such as trypsin inhibitors (Sun *et al.*, 2006). Deepa and Umesh Hebbar (2017) found that the peroxidase enzyme was inactivated in cornflour treated by micronization, and lipase activity reduced by 84%, resulting in increased shelf-life. However, micronization was found to have detrimental effects on wheat gluten functionality by decreasing protein solubility and impairing rheological properties (Sun *et al.*, 2006).

1.9.1.3 Wet Heat Treatment

The most common form of thermal treatment to inactivate unwanted enzyme activity is wet heat treatment. In comparison to other types of heat treatment, wet heat treatment, specifically steam treatment, outperformed in terms of lowering enzyme activity (Bookwalter *et al.*, 1991; Ekstrand *et al.*, 1993; Rose *et al.*, 2008; De Almeida *et al.*, 2014). Steam treatment operates on the principle that steam has a higher enthalpy than water; thus latent heat can be transferred when vapor condenses, allowing for a quick and penetrative treatment that can more effectively inactivate enzymes compared to dry heat treatment (Stapley *et al.*, 1999; Chungcharoen *et al.*, 2015; Hu *et al.*, 2018). In regards to the steam treatment of wheat, the heat is able to penetrate the kernel of cereal grains.

Furthermore, enzymes are more susceptible to denaturing in a wet environment (Bergonio *et al.*, 2016). De Almeida et al. (2014) reported a decrease in lipase activity (84.4%), peroxidase activity (98.9%), endoxylanase activity (99.1%), and α-amylase (85.4%) in whole grain flour. Ekstrand *et al.* (1993) measured lipase activity in oats after wet and dry thermal treatment. After steam treatment, lipase activity was completely inactivated, while dry heat treatment resulted in a negligible reduction. Steam treatment, therefore, is commonly performed industrially for whole grain oats to inactivate enzymes and extend their shelf life (Webster, 1983; Ekstrand *et al.*, 1993).

Three different heat treatments were performed on whole wheat flour, where the reduction of lipase activity was monitored (Rose *et al.*, 2008). Heating by force draft oven, microwave, and steam achieved a significant decrease in lipase reduction, but the steam treatment had the highest reduction of 96%. With multiple techniques available for heat treatment, choosing the best method that reduces enzyme activity, while simultaneously maintaining antioxidants and functionality will be an important consideration. While steam treatment has been the superior choice in deactivating enzymes and maintaining antioxidant contents, it can affect the functionality and flavor of cereal grains.

1.9.2 Thermal Treatment: Impact on Functionality

As mentioned earlier, the production of FFAs can have a negative effect on functionality. Therefore, inactivation of lipase can maintain or improve the functional properties of flour over storage. Poudel and Rose (2018) found that steam treatment of whole wheat kernels for up to 90 seconds resulted in reduced lipase activity over storage, while starch and gluten properties were not impacted. De Kock *et al.* (1999) found significant increases in loaf volume and height of bread made from flour that had autoclaved bran. It was concluded that the positive effects of the heat-treated bran were partially due to the reduced enzymatic activity (Chen

and Schofield, 1996; de Kock *et al.*, 1999). While some research shows an improvement of functionality from thermal treatment, thermal treatment can also have a negative impact on rheological properties.

Gluten-forming proteins play a vital role in the functionality of dough. Protein content is generally not affected by steam treatment (Bookwalter *et al.*, 1991; de Kock *et al.*, 1999); however, the protein profile can be altered. In wheat samples that were subjected to micronization, a significant reduction of monomeric proteins and soluble glutenins was observed. This reduction had detrimental effects on gluten functionality by decreasing protein solubility, water absorption, as well as dough development, stability, and strength, attributed to the formation of hydrophobic and disulfide bonds during heat treatment (Sun *et al.*, 2006). Alterations of the protein profile and disruption of gluten-forming proteins can have irreversible effects. Schofield *et al.* (1983) measured the breadmaking quality of heated gluten proteins and found that overall gluten functionality progressively decreased as temperature increased, with complete loss when heated at temperatures over 75°C. The decline in gluten functionality was attributed to the unfolding of the tertiary structure of glutenin proteins followed by disulfide rearrangement upon cooling. These changes in the gluten forming proteins will reduce the extensibility of the dough and increase elasticity beyond what is desired for use.

Steaming of grains leads to the absorption of water by the starch granules resulting in swelling and pregelatinization (Arntfield *et al.*, 1997; Stapley *et al.*, 1999). Pregelatinization is often found in thermally treated oats (Runyon *et al.*, 2015). Pregelatinized starch, once rehydrated, can have reduced viscosity and have a grainier paste due to re-association of the starch during processing (Jane, 1995). Yadav *et al.* (2012) measured the pasting properties of pearl millet flour that was subjected to steam treatment. With the increased duration of steam treatment, pasting properties were negatively impacted, which is not desirable for products that require a viscous texture such as sauces and fillings (Rojas *et al.*, 1999). On

the other hand, a lower setback viscosity can indicate a lower tendency for retrogradation and syneresis, which can be desirable in baked pastries and bread (Yadav *et al.*, 2012).

Changes in IWG functionality over storage of steamed and not-steamed groats were researched by Mathiowetz (2018). Steam treatment resulted in a significant decrease in peak, hold, and breakdown, however final viscosity and setback values were not significantly impacted. Yadav *et al.* (2012) saw a decrease in all the pasting properties upon steaming. The conflicting results could be attributed to the difference in steam treatment and grain composition. Yadav *et al.* (2012) had the millet grains soaked to higher moisture content and then autoclaved, whereas Mathiowetz (2018) steamed the IWG groats in a proofing oven. Changes in functionality of stored IWG flour, however, have not been researched. Understanding the changes in functionality of IWG flour as impacted by thermal treatment of the grains will be essential in determining the optimal processing conditions and end-product use.

1.9.3 Thermal Treatment: Impact on Flavor Development

With lower enzyme activity through thermal treatment, the development of FFAs and oxidation products will be reduced, thus limiting the number of volatile compounds with undesirable flavor attributes. Molteberg *et al.*, (1996) found that stored flour from oats that were thermally treated had reduced levels of FFAs and several off-odor volatile compounds, such as hexanal, 1-,hexanol, 1-octen-3-ol, 2-pentylfuran, and other aldehydes compared to non-treated samples. Heat-treated samples had less perceived levels of bitterness and astringency and overall correlated less with undesirable flavors due to reduced levels of FFAs. During short term storage of oat flour, the chemical analysis indicated increased levels of volatile compounds, but sensory testing did not detect a consistent difference in aroma between untreated and treated samples. However, after prolonged storage,

strong odors were detected, indicating only short term stability (Molteberg *et al.*, 1996). It is expected that there will be changes in the levels of volatile compounds over storage in IWG flour, however sensory analysis will be needed to determine if changes in the level of volatile compounds will have a noticeable difference between storage time points and to identify the onset at which those levels become significant.

Roti, a round flatbread, made with thermally treated cereal (rice and sorghum) and millet flours had better aroma and taste than roti made from raw flour (Vidya *et al.*, 2013). With the inactivation of enzymes through thermal treatment, the production of undesirable flavor compounds is reduced over storage, thus extending the overall shelf-life of grains and flour and maintaining acceptability of end-products. Research is currently limited in the development of volatile compounds in wheat flours over storage, and none exists for IWG flour. Researching flavor changes in IWG flour will be key in determining the storage stability and acceptability for market use.

1.10 Conclusion

IWG is a promising perennial crop that has witnessed successful strides in its development as a grain crop. Since its selection in 1988, breeding efforts and research to develop, characterize, and improve IWG have been promising. The combined efforts from agronomist, geneticists, and food scientists have led to the improvement in yield, seed size, and ease of harvest (DeHaan *et al.*, 2013; Zhang *et al.*, 2015), as well as an extensive characterization for food use (Rahardjo *et al.*, 2018; Tyl and Ismail, 2018). For IWG to be ready for market use, its storage stability will need to be addressed. An investigation into IWG storage stability was done by Mathiowetz (2018) to understand the changes that can occur in IWG groats due to IWG's unique properties. However, further understanding is needed for the storage stability of the flour. Determining storage stability is important as it

will inform manufacturers and food processors of the mode of failure during storage and guide them in their choice of post-harvest handling and processing (Galliard 1986, 1994). With its high-fat content and enzyme activity, the progress of hydrolytic and oxidative rancidity is of concern due to the impact on the functionality and production of off-flavor compounds. Mathiowetz (2018) found that the IWG groats are stable due to their high antioxidant contents and the compartmentalization of the fat and enzymes. However, once milled into flour the enzymes of concern have access to their substrates. The refinement of milled grains has been used to separate the fat and enzymes from endosperm to enhance storage stability. However, with the growing interest of consuming whole grain products, it is important to explore other approaches to extend shelf life. Steam treatment is a promising strategy that is already utilized in the industry to inactivate enzymes and reduce rancidity over storage (Rose *et al.*, 2008; Bergonio *et al.*, 2016b; Hu *et al.*, 2018). Although steam treatment is utilized in the industry due to its success in extending storage stability in oats, other grains such as wheat are not typically thermally treated. Thus, it is important to consider how thermal treatment of IWG may impact the flour's functionality, flavor, and sensory acceptability over storage.

Although at a small scale, IWG has been used to make various bread products, beer, and cereals, but its continual use and success will depend on its acceptance by consumers. Research on the functionality of IWG flour is needed to understand how it performs over storage and in various end products. As a new crop, it has to have acceptable and desirable sensory attributes and be functional in various food applications. Determining changes in functionality, flavor, and sensory properties will guide the industry in choosing viable handling and processing conditions for optimal use.

Chapter 2: Effect of Bran Content and Thermal Treatment on Flavor Development during Storage of Intermediate Wheatgrass Flour

2.1 Overview

Intermediate wheatgrass (IWG), *Thinopryum intermedium*, is a perennial grain with superior environmental benefits and has the potential to be commercialized as a food ingredient. Evaluating the storage stability of IWG and identifying ways to improve its stability will help incentivize farmers to plant IWG for commercial use. The aim of this work was to evaluate the effects of refinement and thermal treatment on changes of flavor over storage of IWG flour compared to hard red wheat (HRW) flour. Fifteen odor active volatile odor active compounds (VOAC) were identified through gas chromatography-olfactometry-mass spectrometry (GC-O-MS), with the majority formed through lipid rancidity. A descriptive analysis (DA) was also performed on flour tortillas made from stored flours. Over storage, the intensity of the VOACs increased. IWG flour had higher VOAC intensities at the end of storage in comparison to HRW flour, attributed mostly to higher fat content. Partial refinement of IWG resulted in a significant reduction in VOAC intensities. Refined IWG flour had lower VOAC intensities, but at the end of storage was comparable to whole and partially refined samples, likely due to a lack of antioxidants. Steam treatment resulted in a significant reduction in the intensity of key VOACs, such as hexanal, in whole and partially refined IWG samples. DA results showed that tortillas made with stored IWG flour had a more intense flavor profile compared to HRW. Panelists detected an increase in the intensity of earthy and Play-doh® aromas and raw dough flavor with the storage of IWG flour. Refinement significantly lowered salty and bitter taste and overall flavor and aftertaste. Tortillas made from steamed IWG flour compared to those made from not-steamed flour had a significantly lower overall aroma, flavor, and

aftertaste associated with lipid oxidation. Correlation and PCA analysis confirmed that identified lipid oxidation VOACs in the flour sampled contributed to several detected flavor and aromas in the tortilla samples. Overall, results confirmed that refinement and steam treatment can be used in tandem to increase the storage stability of IWG for commercial use.

2.2 Introduction

Current agricultural practices are dominated by annual crops that have been associated with soil erosion, water run-off, and nutrient loss (Cox *et al.*, 2006). Perennial crops have been under the spotlight as a potential solution to restore agriculture and ecosystems. Perennial crops can restore farmland due to their longevity. Once planted, perennial crops can grow every year without replanting, resulting in lower cost and energy use for farmers. The longevity is attributed to the allocation of nutrients to the underground roots (Zan *et al.*, 2001; Monfreda *et al.*, 2008). *Thinopyrum intermedium*, commonly known as intermediate wheatgrass (IWG) is one promising perennial crop chosen by the Rodale Research Institute in 1983 due to its potential agronomic benefits attributed to its extensive root system (Wagoner, 1990; DeHaan *et al.*, 2005). The extensive root system can sequester carbon, prevent nitrogen leaching, utilize water and nutrients efficiently, and combat soil and water erosion (Culman *et al.*, 2013; Lewandowski, 2016). In order for farmers to adopt this crop for its environmental benefits there should be a market pull. IWG grains, in comparison to wheat, have desirable nutritional traits such as higher protein, fiber, and antioxidant content (Tyl and Ismail, 2018). However, no matter how environmentally and nutritionally beneficial IWG may be, consumer acceptance will ultimately drive the adoption of this grain into the marketplace.

Understanding the flavor characteristics of IWG is necessary to advance the prospects for its commercialization. Flavor characterization provides the

information needed for companies to incorporate IWG as an ingredient in end products that garner consumer acceptability. Flavor analysis and sensory evaluation of IWG as an ingredient or as incorporated in a product are currently limited. Flavor and sensory properties of IWG are impacted by several factors including handling, processing, and storage. With storage, several factors may contribute to the development of undesirable off-flavors leading to consumer disinterest. Understanding how the flavor and sensory attributes are impacted by storage is critical to its success.

IWG grains are higher in fat and lipase compared to wheat (Tyl and Ismail, 2018), thus are more susceptible to rancidity over storage, affecting quality and acceptability. Lipase and lipoxygenase (LOX) in IWG can cause hydrolytic and oxidative rancidity, respectively, and are the primary sources of off-flavors over storage. Given the relatively high fat and lipase content and the presence of LOX, the storage stability of IWG needs to be investigated in terms of off-flavor production. Mathiowetz (2018) measured rancidity changes of stored IWG groats and found them to be stable for an extended period of storage. However, the development of rancidity over the storage of IWG flour has not been monitored. In the kernel, enzymes and their substrates are separated, but once the kernels are milled into flour, enzymes and substrates become accessible to each other, making the flour more prone to rancidity. In comparison to intact kernels, flour has a noticeably shorter shelf-life due to the accessibility of lipase and LOX enzymes to the fat in the milled flour (Doblado-Maldonado *et al.*, 2012). Therefore, investigating ways to extend the storage stability and prolong the quality and acceptability of IWG flour is needed.

Different methods have been utilized to increase the storage stability of grains and flour. Steam treatment is one method that is used in the cereal industry to prolong shelf life by inactivating enzymes that catalyze lipid hydrolysis and oxidation in grains high in lipids, such as oats (Rose *et al.*, 2008). In a study by Molteberg *et al.* (1996), heat-treated oats had lower levels of free fatty acids due

to the inactivation of lipase. Molteberg et al. (1996) observed a corresponding reduction of off-flavor compounds, particularly those that contribute bitterness and astringency. Due to the relatively high lipid content and enzymes in IWG (Tyl and Ismail, 2018), steam treatment can potentially be used to extend its shelf-life.

Another method that is used to extend the shelf life of grains such as wheat is refinement. By removing the bran and germ fractions, most of the unwanted enzymes are removed, and the lipid content is lowered. Refined flour has been desired by consumers due to its light color and pleasant sensory attributes. However, with a growing interest in whole-grain foods, monitoring flavor development over the storage of whole IWG flour will be essential to determine its usability and marketability.

Overall, the objective of this study was to evaluate the effects of prior grain storage, bran content, and steam treatment on the development of flavor in IWG flour over storage at 43% relative humidity. Aroma compounds will be extracted from the flour and analyzed to monitor changes over storage in compounds that are odor active. The aroma of foods is considered one of the most influential factors of the overall flavor profile and thus affects consumer acceptability. A descriptive analysis will also be performed in tandem on tortillas made from the stored IWG flour to further understand the flavor profile and relate analytical data to sensory evaluation. Tortillas were chosen due to its simplicity (water and flour) to distinguish changes in IWG flour over storage. Continued research on the flavor profile of IWG over storage is a step forward towards commercialization. The development of a new sustainable crop will lead to a healthy alternative for consumers.

2.3 Materials

2.3.1 Intermediate Wheatgrass and Hard Red Wheat Grains

Intermediate wheatgrass (IWG) grains were harvested from the University of Minnesota (UMN) Rosemount Fields, MN in August 2016 and represented an improved breeding population originating from The Land Institute (TLI) Cycle 2 (bred from 2006-2008), obtained from The Land Institute in Salina, KS (Zhang *et al.*, 2016). A blend of 24 cultivars of hard red wheat (HRW) from July 2016 harvests from St. Paul, MN and Lamberton, MN was also obtained.

Freshly harvested IWG bulk sample from the UMN Rosemount Fields was obtained in August 2017. This sample represented improved breeding populations originating from TLI Cycle 5 (bred in 2010-2015), selected for improved grain yield and seed size (DeHaan *et al.*, 2013; Zhang *et al.*, 2016). Freshly harvested bulk HRW sample of 26 cultivars (mostly the same as those in 2016) consisting of harvests from St. Paul, MN, and Lamberton, MN was also obtained in July 2017. All grains were kindly supplied by the Agronomy and Plant Genomics Department at the University of Minnesota – Twin Cities.

2.3.2 Chemicals, Reagents, and Standards

n-Alkanes, pentane, hexane, heptane, octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, and heptadecane were purchased from Millipore Sigma (Burlington, MA). The internal standard (ISTD), 2-methyl-3-heptanol, was also obtained from Millipore Sigma.

2.4 Experimental Design

The experiment was a factorial design with grain type (IWG and HRW), thermal treatment (steamed and not-steamed), and bran content (100%, 75%, 0%) as factors. Freshly harvested and one-year-old IWG and HRW grains were used to produce the flour samples.

IWG and HRW grains harvested in 2016 were cleaned, dehulled, and stored at refrigeration temperature (ca. 4°C) for one year. Half of the IWG grains were steamed. HRW grains were not-steamed as this is not a common practice in the wheat industry. One-year-old IWG grains were milled and had two refinement levels, whole (100% bran) and partial (75% bran), whereas HRW had only one refinement level (whole). All flour samples were stored at 43% RH for 0, 3, and 6 months. Flour from one-year-old grains was not used for sensory analysis due to the grain being deemed unfit for human consumption.

IWG and HRW grains harvested in 2017 were immediately milled into flour after cleaning and dehulling. Prior to milling, half of the IWG grains were steamed. IWG had three levels of refinement, whole (100% bran), partially refined (75% bran), and refined (0% bran). HRW had two levels of refinement, whole (100%) and refined (0%). All flour samples were stored at 43% RH at ambient temperature for 0, 4.5, and 9 months. “Time 0” samples from each treatment condition were analyzed to establish a baseline for storage measurements.

2.5 Steam Treatment, Milling, and Sample Preparation

A portion of IWG grains was subjected to direct steam treatment. Grains were steamed by placing a single layer of grains in a ~1mm mesh sieve approximately 1 inch above a boiling water bath for 120 seconds. The optimal time for steam treatment was previously determined. Lipase, lipoxygenase, and peroxidase activities were significantly reduced by about 50%, 17%, and 20%,

respectively, after 120 seconds of steam treatment in comparison to samples that were not-steamed (0 seconds) (**Figure 24, Appendix A**). There was no significant reduction in the content of antioxidants, namely hydroxycinnamic acids (p-coumaric acid, ferulic acid, and sinapic acid) and carotenoids (lutein and zeaxanthin) post steam treatment (**Table 21, Appendix A)**. After steam treatment, grains were subsequently equilibrated at ambient conditions for 24 hours before milling.

All grain samples (steamed and not-steamed) were milled using a Brabender Quadrumat Junior mill (C.W. Brabender Instruments, Inc., Hackensack, HJ). The endosperm was passed through a 0.025 mm screen, separating the bran. The bran portion was further milled using a hammer mill (Howell Electric Motors, Howell, MI), and passed through a 0.04 mm screen. Milled bran was then added back to the endosperm at 100, 75, and 0% of their original bran content according to their original bran-endosperm ratio (**Table 22, Appendix B**). 75% bran content was chosen based on preliminary work that showed optimal protein secondary structure at this bran level. Resulting flour was then stored as described below.

2.6 Flour Storage

Flour samples from one-year-old and from freshly harvested grains were stored in uncovered polystyrene bottles containing ~50 g of flour in sealed fish tank desiccators with saturated potassium carbonate solution. Saturated potassium carbonate has a water activity of ~0.43 (43% RH) at ambient temperatures (Labuza *et al.*, 2008), which corresponds to ~10% moisture content in wheat at equilibrium (Pixton and Warburton, 1971). After storage, bottles were flushed with nitrogen, capped with a polystyrene cap, and kept under freezer conditions (-40 to -20°C) until analysis. One-year-old grain flour samples were removed at 0, 3, and 6 months of storage. Freshly harvest grain flour samples were removed at 0. 4.5, and 9 months of storage.

For descriptive analysis, flour from freshly harvested grains was stored in reverse order. At the beginning of storage, 9 months samples were stored in desiccators, and 0 and 4.5 months flour samples were kept frozen. The 4.5-month samples were removed from the freezer and placed in desiccators at 4.5 months from initial storage. The 0-month flour sample was removed from the freezer immediately before sensory evaluation.

2.7 Methods

2.7.1 Proximate Analysis

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

2.7.2 Analytical Flavor Analysis of IWG and HRW Flour

2.7.2.1 Purge and Trap

In triplicate, 10 grams of flour sample was placed in a 250 mL cylindrical round bottom flask and mixed with 99 mL of double-distilled water (DDW) and 1 mL of 1 ppm 2-methyl-3-heptanol (internal standard, ISTD) to have 10 ppb of ISTD in 100 mL of solution. The sample flask was then sealed with a rubber stopper and

placed in a water bath at 40°C and stirred for 1 hour to allow the ISTD to equilibrate with the flour sample. After ISTD equilibration, the sample flask was capped with a dual-port, glass adapter. The first port had an #8 Ace-thread joint with a FETFE O-ring (Ace Glass Inc., Vineland, NJ) and the second port had a thermometer inlet adapter (ChemGlass Life Sciences, Vineland, NJ) with a Thermogreen™ LB-2 septa (Supelco Inc., Bellefonte, PA) and was used to hold the gas purge needle (**Figure 6)**. Forty mL/min flow of nitrogen (N_2) was used to purge the sample for 15 minutes at 40°C, controlled by a water bath, while being continuously stirred. Purged volatile odor active compounds (VOAC) were trapped using a Tenax® TA absorbent in a glass thermo-desorption (TD) tube (Supelco Inc, Bellefonte, PA) that is compatible with the Gerstel thermodesorption system placed inside the #8 Ace-thread joint and held by the FETFE O-ring (**Figure 6)**. After purging, the Tenax® TA glass TD tube was removed and dry-purged with N_2 for 30 minutes to remove excess moisture from the Tenax™ TA absorbent using a custom-made flow controller.

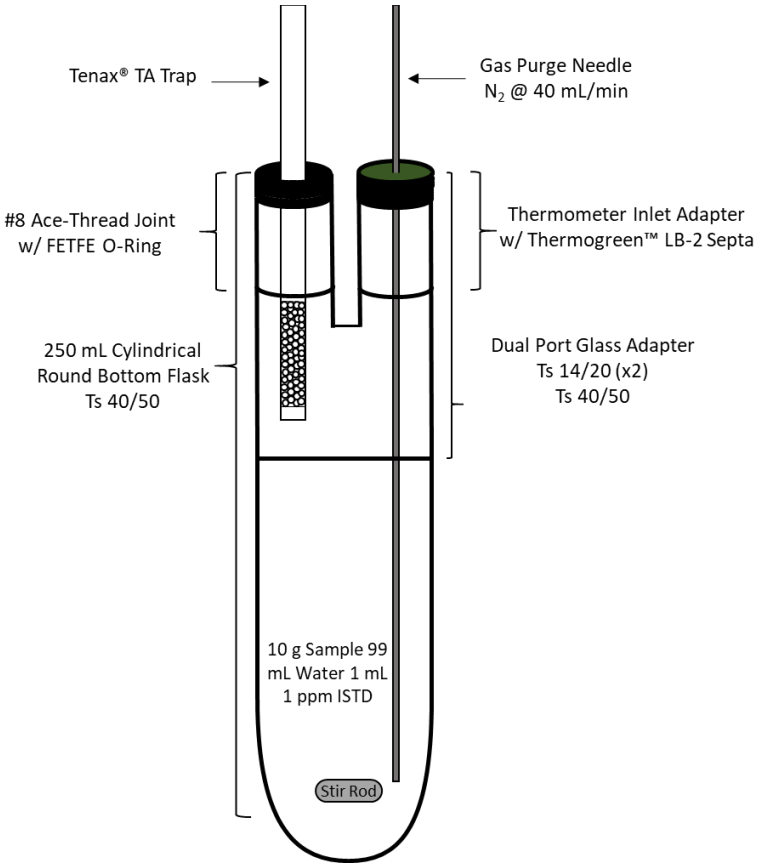


Figure 6: Purge and Trap Set-up. Ts denotes a taper-ground joint size.

2.7.2.2 Gas Chromatography-Olfatometry-Mass Spectrometry

VOACs trapped on the Tenax® TA trap were thermally desorbed using a Gerstel Thermodesorption System (TDS) (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany) equipped with a Gerstel Cooled Injection System (CIS) (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany). Gas chromatography-olfactometry-mass spectrometry (GC-O-MS) was performed using an HP/Agilent G1530A (Agilent Technologies, Santa Clara, CA). The GC was equipped with a GC capillary column splitter (SGE Analytical Science, Ringwood, Australia) that directed the eluent from the column to a Hewlett Packard 6890 quadrupole mass spectrometer detector (MSD) (Hewlett Packard, Palo Alto, CA) and olfactometry

port (Hillesheim, heated transfer line with nose cone, Waghäusel, Germany). The eluent was divided 1:1 split ratio between the MS and the olfactometry port. The TDS temperature was programmed to increase from 40°C to 240°C (held for 4 min) at a rate of 50°C min^{-1}. The desorbed VOACs were cryofocused in the CIS at -60°C using liquid nitrogen. After desorption and cryofocusing, the CIS temperature was programmed to increase from -60 to 240°C (held for 1 minute) at 10°C sec^{-1} to inject the compounds onto the analytical column. Separation of VOACs was performed on an HP-5 column (30 m x 0.25 mm ID x 0.25 µm, film thickness; Agilent Technologies, Santa Clara, CA). The carrier gas, helium, was set to a constant flow of 1.3 mL min^{-1}. The GC oven temperature gradient program was as follows: 40°C initial temperature and ramped at a rate of 7°C min^{-1} until 230°C for a total of 27.14 minutes. A panelist sniffed the eluent at the sniff port until no further aromas were detected (~20 min). Three panelists (two females, one male; ages 23-26 years) recorded the aroma of the samples with one panelist per replicate for each sample. Panelists were trained on different aromas using aqueous solutions of different compounds to become familiar with an aroma lexicon; such as ‘hexanal’ for ‘green/grassy.’ The ionization source for the MSD was electron impact. MSD conditions were as follows: ionization capillary transfer line was set at 250°C, the ion source was held at 230°C and the quadrupole was held at 150°C. The MSD was operated in scan mode (total ion chromatogram) with an m/z range from 29 to 300 amu with 4.37 scans sec^{-1} following a two-minute solvent vent delay. MSD ChemStation E.02.02.1431 with a NIST Standard Reference Database was used to analyze MS data and identify compounds based on their mass spectra. Compounds of interest were identified by mass spectra, odor descriptors, and calculating Kovats Retention Index (KRI) of each odor active compound based on their retention time and their relationship with respect to n-alkanes during a ramp (**Table 1**). Alkanes used for determining the KRI can be seen in **Tables 24** and **25**, **Appendix C.** A gas chromatogram temperature ramp will elute n-alkanes at regular intervals. Each window between two n-alkanes is

labeled by the n of the previous alkane. For example, if a compound elutes following nonane (n = 9) it will have a KRI of 900 to 999, and a compound that elutes following decane (n = 10) will have a KRI of 1000 to 1099. Once a compound of interest has been allocated a KRI, using **Equation 1**, it can be compared to databases of aroma compounds and previous reports (along with aroma, GC retention time, and mass spectrometry) for identification. The total ion chromatogram from mass spectrometry was integrated for peak areas for each identified VOAC. The area of each identified VOAC was then normalized using the area of the ISTD, as shown in **Equation 2**, resulting in the final reported intensity values.

Equation 1:

$$KRI = 100(n) + 100 \times \frac{tr_i - tr_n}{tr_N - tr_n}$$

Where:

- KRI = Retention Index of compound
- i = compound being analyzed
- n = carbon number of alkane which elutes before “i”
- N = carbon number of alkane which elutes after “i”
- tr_i = Retention time of “i”
- tr_n = Retention time of the alkane which elutes before “i"
- tr_N = Retention time of the alkane which elutes after “i"

Equation 2:

$$Reported\ \ VOAC\ Intensity = \frac{(Area\ of\ VOAC_{sn}) \times (Average\ Area\ of\ ISTD_A)}{Area\ of\ ISTD_{sn}}$$

- sn = individual sample
- A = all samples

Table 1: GC-O-MS selected aroma compounds and corresponding molecular weights.

Compound*	Molecular Weight
3-Methyl Butanal	88
Pentanal	86
1-Pentanol	88
Hexanal	100
1-Hexanol	102
Heptanal	114
2-Methyl-3-heptanone (ISTD)	128
[E]-2-Heptanal	112
1-Octen-3-ol	128
2-Pentyl Furan	138
Octanal	128
Limonene	136
[E]-2-Octenal	126
Nonanal	142
[E]-2-Nonenal	140
Decanal	156

*Compounds identified by mass spectra and Kovats Index; ISTD (internal standard)

2.7.3 Flour Tortilla for Quantitative Descriptive Analysis

Flour from freshly harvested grains at each storage time point was used to make a simple flour tortilla adapted from a recipe given by General Mills Inc. A 1:1 ratio of flour to water was mixed to form a paste. The flour paste was then evenly added to 2.54 cm^3 silicone ice cube tray, filling each well halfway, and spreading the paste evenly. The silicone tray was then placed in an Instant Pot IP-Duo60 (InstaPot, Los Angeles, CA) and cooked on high pressure for 30 minutes. After cooking, paste cubes were removed, placed between two square sheets of non-stick aluminum foil, flattened using a tortilla press with 1mm thick guides, and heated in a panini press for 1 minute. The finished tortilla was then cut into four equal pieces, ~2 g per piece, and served immediately to the panelists.

2.7.4 Descriptive Analysis of IWG and HRW Tortilla

Descriptive analysis (DA) was used to document the nature and extent of differences in sensory properties of tortillas made from IWG and HRW flour that differed by storage time, steaming, and extent of refinement. Flour from freshly harvested grains stored at 43% RH was used to make the tortilla for DA testing. The panelists consisted of eight individuals (2 males and 6 females) that had received training in DA through the Sensory Center at the University of Minnesota (Saint Paul, MN). All members were 6-n-propylthiouracil (PROP) tasters or supertasters. Development of a lexicon describing the nature of the samples, rating the samples, and finding suitable references was done over the course of seven, one-hour training sessions. The descriptive terms and references developed by the panelist for aroma and flavor attributes can be found in **Tables 2** and **3**, respectively. Panelists also analyzed for the five basic tastes (sweet, salty, bitter, sour, and umami) and aftertaste (overall, sweet, salty, sour, bitter, umami, and astringent). The University of Minnesota's Institutional Review Board approved all recruiting and experimental procedures.

Table 2: Flavor descriptor lexicon, definition, and references for IWG and HRW flour tortilla as developed by DA panelists for sensory evaluation.

Descriptor	Definition	Reference Sample
Vanilla	Flavor blend of sweet, vanillin, woody, brown notes, sometimes having chocolate, tobacco, floral or spicy components	Essential Everyday, Imitation Vanilla Flavor
Green pear	Flavor associated with fresh pears	Anjou, green pear
Peanut butter	Roasted nut flavor	Essential Everyday, unsalted, dry roasted peanuts
Raw dough	Flavor defined as raw, doughy, and yeasty	Pillsbury™ crescents original
Beany	Flavor defined as an astringent and starchy	Essential Everyday, black beans

Table 3: Aroma descriptor lexicon, definition, and references for IWG and HRW flour tortilla as developed by DA panelists for sensory evaluation.

Descriptor	Definition	Reference Sample
Brown sugar	A rich full-bodied brown sweet aromatic	Dark brown sugar (Essential Everyday)
Nutty	Slightly sweet, brown, woody, oily, musty, astringent, and bitter	Wheat germ (Kretschmer)
Floury	Aromatic of intermediate wheatgrass flour	Whole IWG Flour
Corn	Aromatic associated with a starchy, earthy, slightly sour notes	Mini corn on the cob (Essential Everyday)
Popcorn	Popcorn	Yellow popcorn kernels (Market Pantry)
Earthy	An earthy/dirty aroma that refers to the outside skin of a potato	Russet potato (Green Giant Fresh)
Green Grassy	Aromatic associated with fresh vegetation, green vegetables, and grass	Cis-3-Hexen-1-ol (Sigma-Aldrich)
Beef	Aromatic associated with cooked animal fat	Lean ground beef (Cargill)
Play-Doh	A sweet, solvent-liked aroma characteristic sometimes associated with vanilla flavored products	Modeling compound (Play-Doh)
Burnt toast	A burnt aroma notes like charred toast	White bread (Cub Foods)
Cardboard	Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging	Pieces of wet cardboard
Rancid oil	Aromatic associated with oxidized fats, oils, and fishy	Pure vegetable oil (Essential Everyday)
Sulfur	Flavor associated with egg white	Large fresh grade A eggs (Cub Foods)

For evaluation, tortilla samples were served at room temperature in lidded 2 oz. plastic soufflé cups coded with random 3-digit numbers. Each sample was served in duplicate, with different codes for the duplicates and served on two separate testing days (**Table 4**). Panelists participated in four test sessions. Half of the samples were evaluated during the first session and the other half during

the second session. Sessions three and four served as sensory replicates. During testing sessions, each panelist evaluated each sample by rating the intensity of the attributes on a 20-point line-scale labeled ‘none’ (0) to ‘intense’ (20) and recorded their scores using a computerized data collection system, SIMS 2000. Intensity ratings of flavor were made on the standard citric acid scale (Karalus *et al.*, 2010), while the evaluation of aromas was made on the standard butanol scale (Conshohocken, 2013). The panelists were instructed to wear nose clips when evaluating taste attributes. Nose clips were not worn for aftertaste attributes.

Table 4: List of sample descriptions and IDs used for descriptive analysis testing.

Sample description (Grain, Steam Treatment, Refinement, Storage time [months])	Sample Codes	
	Rep 1*	Rep 2
HRW Not Steamed Refined 0	759	423
HRW Not Steamed Refined 4.5	571	314
HRW Not Steamed Refined 9	192	481
HRW Not Steamed Whole 0	967	834
HRW Not Steamed Whole 4.5	478	614
HRW Not Steamed Whole 9	255	472
IWG Not Steamed Partial 0	324	951
IWG Not Steamed Partial 4.5	497	392
IWG Not Steamed Partial 9	946	947
IWG Not Steamed Refined 0	632	552
IWG Not Steamed Refined 4.5	834	310
IWG Not Steamed Refined 9	208	146
IWG Not Steamed Whole 0	202	628
IWG Not Steamed Whole 4.5	889	492
IWG Not Steamed Whole 9	371	122
IWG Steamed Partial 0	914	409
IWG Steamed Partial 4.5	356	202
IWG Steamed Partial 9	591	296
IWG Steamed Refined 0	475	526
IWG Steamed Refined 4.5	191	759
IWG Steamed Refined 9	636	936
IWG Steamed Whole 0	506	667
IWG Steamed Whole 4.5	753	935
IWG Steamed Whole 9	120	191

*Each rep was split into 2 separate testing days

2.8 Statistical Analysis

2.8.1 Flavor Analysis

Analysis of variance (ANOVA) was done using SPSS Software version 25 (IBM Corporation, Armonk, NY) to determine the difference among samples at a single time point or within one sample at all-time points of storage. Differences among the means were determined using Tukey-Kramer Honest Significant Difference (HSD) test ($P < 0.05$). Two-way ANOVA was carried out to asses for interaction effects among treatment variables, including storage time, thermal treatment, and bran content based on various dependent variables. An independent two-sample t-test was performed to analyze differences between steamed and not-steamed samples. ANOVA tables can be found in **Appendix D, Tables 26-40**.

2.8.2 Descriptive Analysis

Statistical analysis of DA samples was separated into two sets: comparing HRW samples with IWG samples and comparing only IWG samples. To examine differences due to grain type (HRW vs. IWG), storage time (0, 4.5, and 9 months), steam treatment (steam and not-steamed), and refinement (refined vs. whole) ANOVA (SAS® PROC GLM) was used. Dependent variables were the intensities of the sensory attributes. Predictors were panelists, taste position, replicate, grain type, storage time, steam treatment, and refinement. Interactions on grain type, storage time, steam treatment, and refinement were evaluated using ANOVA. ANOVA tables can be found in **Appendix D, Tables 41-72.** Differences among the means were determined using HSD ($P \leq 0.05$).

2.8.3 Correlation and Principal Component Analysis

Correlation between the variables was analyzed with R Version 1.1.463 (Rstudio Inc, Boston, MA). Due to the experimental design, repeated measures (mixed-effects) model was used to adjust for changes over time. Each combination of treatments (storage time, refinement level, thermal treatment, grain type) was treated as a unique factor to account for the fact that some samples were not subjected to specific treatments (i.e. HRW samples were not-steamed). Additionally, data were adjusted to account for changes in storage. Baseline measurements (Time 0) were subtracted from later storage time (4.5 months and 9 months) to compare how measurements from later storage time changed from previous measurements (i.e. assess whether values increased or decreased from the original measurement). Furthermore, response variables between analytical measurements and DA were standardized to be on the same scale (analytical flavor analysis in the millions vs. sensory evaluation in the tens). Standardization was performed by subtracting the mean from each response and dividing by the standard deviation of that response. The data was then fitted into a mixed-effects model and residuals were then calculated and modeled to account for variability caused by different treatments and times. Relationships among flavor compounds and sensory attributes were evaluated by Principal Component Analysis (PCA) using Pearson's product-moment correlation coefficient ($P < 0.05$), using a total of 47 flavor compounds and sensory attributes (**Table 5**).

Table 5: Flavor compounds and sensory attributes of IWG and HRW flour and tortilla used for correlation and PCA.

Flavor Compound	Sensory Attributes			
	Aroma	**Taste**	**Flavor**	**Aftertaste**
3-Methylbutanal	Overall	Sweet	Overall	Overall
Pentanal	Brown Sugar	Saltiness	Vanilla	Sweet
1-Pentanol	Nutty	Sourness	Green Pear	Salty
Hexanal	Floury	Bitterness	Peanut Butter	Sour
1-Hexanol	Corn	Umami	Raw Dough	Bitter
Heptenal	Popcorn		Beany	Umami
(E) 2-Heptanal	Earthy			Astringent
1-Octen-3-ol	Grassy			
2-Pentylfuran	Beef			
Octanal	Play-Doh			
Limonene	Burnt Toast			
(E) 2-Octenal	Cardboard			
Nonanal	Rancid Oil			
(E) 2-Nonanal	Sulfur			
Decanal				

2.9 Results and Discussion

2.9.1 Nutrient Composition as Affected by Seed Size and Refinement

Whole IWG was significantly higher in fat, ash, and protein and was significantly lower in carbohydrate content compared to whole HRW (**Tables 6** and **7**), an observation attributed to its relatively smaller seed size (**Table 23, Appendix B**) and consequently higher bran to endosperm ratio (DeHaan and

Ismail, 2017; Rahardjo *et al.*, 2018). Similar observations were reported by Becker *et al.* (1991), Marti *et al*. (2015), and Rahardjo *et al*. (2018). However, the protein content of whole IWG was lower than that determined by Becker et al. (1991) and Rahardjo et al. (2018), which may be a result of breeding efforts to increase seed size (**Table 23, Appendix B**). Albumins and globulin proteins are primarily found in the germ and aleurone layers of the seed (Sramkova *et al.*, 2009). Therefore, reduced bran to endosperm ratio (**Table 22, Appendix B**) due to an increase in seed size would result in lower overall protein content. The higher fat content of whole IWG flour, on the other hand, puts it at a higher risk for hydrolytic and oxidative rancidity compared to whole wheat flour.

With refinement, IWG had a significant decrease in fat and ash, which is an expected observation since fat and ash are mainly located in the bran and germ (Day, 2016). Reduction in fat content through refinement can potentially prolong IWG's storage stability.

Table 6: Nutrient composition (g/100 g flour, on a dry basis) of hard red wheat and intermediate wheatgrass from freshly harvested grains at different levels of refinement.

Sample	Fat	Ash	Protein	Total Carbohydrate†
Whole^ HRW	2.96^{c*}	1.64^{d}	12.6^{b}	81.3^{a}
Refined HRW	2.34^{d}	0.65^{e}	11.8^{b}	83.8^{a}
Whole IWG	4.56^{a}	3.10^{a}	18.0^{a}	73.2^{c}
Partial IWG	3.78^{b}	2.87^{b}	17.1^{a}	74.3^{c}
Refined IWG	3.18^{c}	1.90^{c}	16.6^{a}	76.6^{b}

*Lowercase superscripts represent significant differences (P ≤0.05) among samples according to the Tukey's HSD means comparison test.

† Calculated by difference.

^ Whole = 100% bran; Partial = 75% bran; Refine = 0% bran

Table 7: Nutrient composition (g/100 g flour, on a dry basis) of hard red wheat and intermediate wheatgrass from one-year-old stored grains at different levels of refinement.

Sample	Fat	Ash	Protein	Total Carbohydrates†
Whole^ HRW	3.33[b]	1.94[b]	14.0[c]	80.7[a]
Whole IWG	4.26[a]	2.79[a]	16.7[a]	76.3[c]
Partial IWG	3.43[b]	1.83[b]	15.3[b]	79.4[b]

*Lowercase superscripts represent significant differences (P ≤0.05) among samples according to the Tukey's HSD means comparison test.

† Calculated by difference.

^ Whole = 100% bran; Partial = 75% bran

2.9.2 Identified Aroma Compounds in Stored IWG and HRW Flour

Fifteen aroma compounds including alkyl aldehydes (pentanal, hexanal, heptanal, octanal, nonanal, and decanal), enal aldehydes ([E]-2-heptenal, [E]-2-octenal, and [E]-2-nonenal), alcohols (1-pentanol, 1-hexanol, and 1-octen-3-ol), 3-methylbutanal, 2-pentylfuran, and limonene were found in both HRW and IWG flours **(Figure 7)**. All identified compounds have been previously observed in cereal flours and are mostly formed through lipid oxidation (Molteberg *et al.*, 1996; Heiniö *et al.*, 2002; Cramer *et al.*, 2005; Maeda *et al.*, 2008). Lipid oxidation of unsaturated fatty acids into hydroperoxides, via autoxidation or LOX, has been researched extensively (Frankel, 1983; Shahidi, 2000b). Hydroperoxides formed from lipid oxidation are tasteless and odorless; however, they are unstable due to a week oxygen-oxygen bond. Hydroperoxides are further broken down by enzymes, such as peroxidases, isomerases, lyases, alcohol dehydrogenase, or by non-enzymatic reactions producing secondary metabolites that are odor active (Shahidi, 2000b; Än Salas *et al.*, 2005). Lipid oxidation of fatty acids is comprised of pathways with a propensity to form specific types of hydroperoxides, thus lipid oxidation has a higher likelihood of producing specific compounds.

The alkyl aldehydes pentanal, hexanal, and heptanal are formed mainly from the oxidative breakdown of linoleic and linolenic acid, whereas octanal, nonanal, and decanal are formed from the oxidative breakdown of oleic acid (Shahidi, 2000b). The formation of octanal, nonanal, and decanal is most likely due to oleic acid autoxidation, as LOX acts on a pentadiene moiety, which oleic acid lacks (Buśko *et al.*, 2010). The volatile aldehydes formed from lipid oxidation can be very potent, with concentrations lower than 1 ppm being odor active (Frankel, 1983). Panelists, sniffing odor active compounds at the GC-O-MS sniffing port, described pentanal and decanal as having a musty odor, hexanal, heptanal, and nonanal as having a green and grassy odor, and octanal as having a citrusy odor.

Formation of enal aldehydes, [E]-2-heptenal, [E]-2-octenal, and [E]-2-nonenal, can be attributed to enzyme activity, such as lyases and isomerases, on the hydroperoxides from oxidative rancidity (Än Salas *et al.*, 2005). [E]-2-heptenal was described as having a pungent odor, [E]-2-octenal having a grassy and vanilla odor, and [E]-2-nonenal having fresh/cucumber odor.

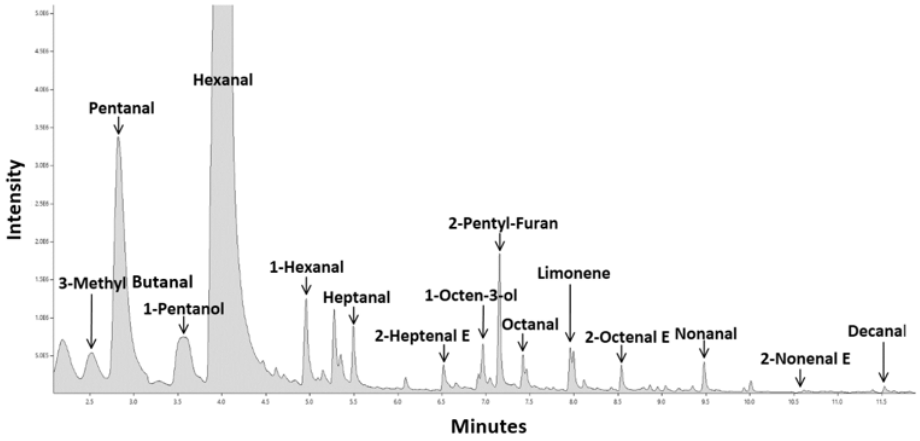
1-Pentanol and 1-hexanol were likely formed through the enzymatic action of alcohol dehydrogenase on pentanal and hexanal, respectively (Lehto *et al.*, 2003). 1-pentanol was described as musty and pungent, where 1-hexanol was described as herbal and sweet. 1-octen-3-ol, which is known as the mushroom aroma compound, and likely formed through a lyase enzyme that is specific to the 10-hydroperoxide of linoleate (Matsui *et al.*, 2003; Buśko *et al.*, 2010). 1-octen-3-ol was described as having an earthy and mushroom odor.

3-Methylbutanal is known to contribute to malty odors and is another common lipid oxidation product (Cramer *et al.*, 2005; Kaseleht *et al.*, 2011). 3-methylbutanal was described as having a caramel and alcohol-like odor. 2-Pentylfuran is another important marker of lipid oxidation of fats and oils due to its contribution to beany odor and taste. The formation of 2-pentylfuran was likely from

the oxidation of linoleic acid (Krishnamurthy *et al.*, 1967). 2-pentylfuran was described by panelists as having a green and fruity odor.

Limonene in cereals, such as durum wheat, is not formed through lipid oxidation but is rather a fungal metabolite (Buśko *et al.*, 2010). Limonene was detected by panelists in all stored flour samples and was described as having a citrusy odor. It should be noted that the extraction of VOACs from the flour samples was done in a lab that heavily utilized limonene, thus its presence in the flour samples could potentially be an artifact.

The formation of the identified VOACs through lipid rancidity can decrease the overall acceptability, thus looking at different processing methods to slow down or prevent their formation is desirable. IWG is a new crop and has not been extensively researched in terms of its flavor profile. HRW, therefore, was used for comparison purposes. Refinement, which results in reduced fat and enzyme content, may limit the formation of off-odors. Thermal treatment to denature enzymes involved in lipid rancidity, namely lipase and LOX, may also limit the formation of off-flavors.



Predicted Odorants [a]	Average Retention Time (min)	Odor Description [b]	Calculated Kovats Retention Index	Literature Kovats Retention Index Range [c]
3-Methylbutanal	2.20	Caramel/Alcohol	627	664-700
Pentanal	2.53	Musty/Pungent	673	671-677
1-Pentanol	3.55	Green/Yeasty	789	744-801
Hexanal	3.93	Grassy	819	769-823
1-Hexanol	4.96	Herbal/Sweet	889	839-889
Heptanal	5.49	Grassy	919	855-921
[E]-2-Heptanal	6.54	Pungent	974	927-978
1-Octen-3-ol	7.00	Earthy/Mushroom	998	958-999
2-Pentylfuran	7.15	Fruity/Green	1006	977-1012
Octanal	7.43	Citrusy/Lime	1020	973-1021
Limonene	7.96	Sweet/Lemon	1046	1010-1060
[E]-2-Octenal	8.55	Grassy/Vanilla	1075	1034-1076
Nonanal	9.50	Banana/Yeast/Grassy	1121	1073-1130
[E]-2-Nonenal	10.63	Cucumber	1177	1129-1180
Decanal	11.57	Old Musty	1224	1183-1231

Figure 7: Example chromatogram, predicted odorants, descriptions, and calculated Kovats of VOAC extracts from IWG and HRW flour samples determined by gas chromatography-olfactometry-mass spectrometry analysis. [a] Compounds identified through mass spec, aroma, and Kovats Retention Index; [b] Odor described by panelist using gas chromatography sniffing port; [c] Range from the National Institute of Standards and Technology Mass Spectrometry Data Center.

2.9.3 Changes in VOACs over Storage of Flour from Freshly Harvested Grains

2.9.3.1 Development of VOACs in IWG Flour from Freshly Harvested Grains compared to HRW Flour

At 0 months of storage, whole IWG flour had a significantly higher intensity of heptanal in comparison to whole HRW. At 4.5 months of storage, whole IWG flour had a significantly higher intensity of most alkyl aldehydes (**Figure 8 A-E**), [E]-2-octenal (**Figure 9 C**), and 2-pentylfuran (**Figure 9 E**). At 9 months of storage, whole IWG flour had a significantly higher intensity of most identified compounds, compared to whole HRW flour, except for octanal, decanal, limonene, and [E]-2-nonenal (**Figure 8** and **9**).

3-Methylbutanal was not detected in whole HRW flour at any of the storage time points; however, it was detected in whole IWG flour at 4.5 to 9 months of storage (**Figure 9 A**). According to Cramer et al. (2005), 3-methylbutanal is not commonly present in wheat flour but was present in barley flour as a prominent odor-active compound with malty characteristics. As a product of lipid oxidation, 3-methylbutanal could be produced upon the action of different types of enzymes in IWG that may form specific secondary metabolites. 3-methylbutanal intensity could have been below the threshold for instrumental detection in most HRW samples as it was detected at the end of storage for refined HRW flour (**Table 9 A**).

Octanal, nonanal, and decanal (**Figure 8 D, E,** and **F**) were lower in intensity compared to pentanal, hexanal, and heptanal (**Figure 8 A, B,** and **C**) at 9 months of storage for whole IWG flour, which can be attributed to the lower oleic acid content compared to linoleic acid of IWG (Mathiowetz 2018). Formation of hexanal correlated well with the formation of linoleates, the peroxide formed from linoleic acid, production of other off-flavors, and overall sensory perceptions, making its detection a valid indicator of oxidative stability. Hexanal was positively correlated

with most VOACs (r = 0.5-0.9) except for pentanal and [E]-2-nonenal, which did not correlate with any other VOACs (**Table 73**, **Appendix E**). Hexanal in whole HRW and IWG flour increased in intensity by 23 and 22 folds, respectively from 0 to 4.5 months of storage (**Figure 8 B**). From 4.5 months to 9 months, hexanal did not significantly increase in whole HRW flour. Whole IWG flour, on the other hand, had a significant 3-fold increase in hexanal intensity from 4.5 to 9 months of storage. Whole HRW flour did not have significant changes in the other alkyl aldehydes over storage and had only significant increases in 1-hexanol and 1-octen-3-ol from 0 to 4.5 months of storage followed by a decrease from 4.5 months to 9 months of storage (**Figure 10 C** and **D**). These observations suggest that whole HRW reached peak rancidity by mid storage. These results are further supported by Hayek (2020), who found that stored HRW flour had a peak in hydroperoxides at 1.5 months of storage, whereas IWG flour had a peak around 4.5 months of storage. Whole IWG flour had significant changes in intensity at each stage of storage for most of the VOACs, indicating continual rancidity.

The observed differences in the progression of rancidity between HRW and IWG flour can be attributed to differences in fat, enzymes, and antioxidant content. While HRW has a lower amount of fat in comparison to IWG (**Table 6**), IWG has significantly lower LOX activity compared to HRW (Tyl and Ismail, 2018). HRW's higher LOX activity could have accelerated the rancidity of polyunsaturated FFAs leading to complete rancidity. At the end of storage, intensity for most of the VOACs for HRW was significantly lower than that of IWGs. HRW also contains a significantly lower amount of antioxidants in comparison to IWG (Tyl and Ismail, 2018). Antioxidants, such as carotenoids and hydroxycinnamic acid, can inhibit the decomposition of hydroperoxides into undesirable VOACs by terminating lipid propagation and interfering with decomposition reactions (Frankel, 1996; Masisi *et al.*, 2016). Complete rancidity of HRW, however, does not imply acceptability is lower than IWG. HRW has a lower fat content than IWG; therefore, there is less fat available for lipid rancidity, resulting in an overall lower intensity of VOACs.

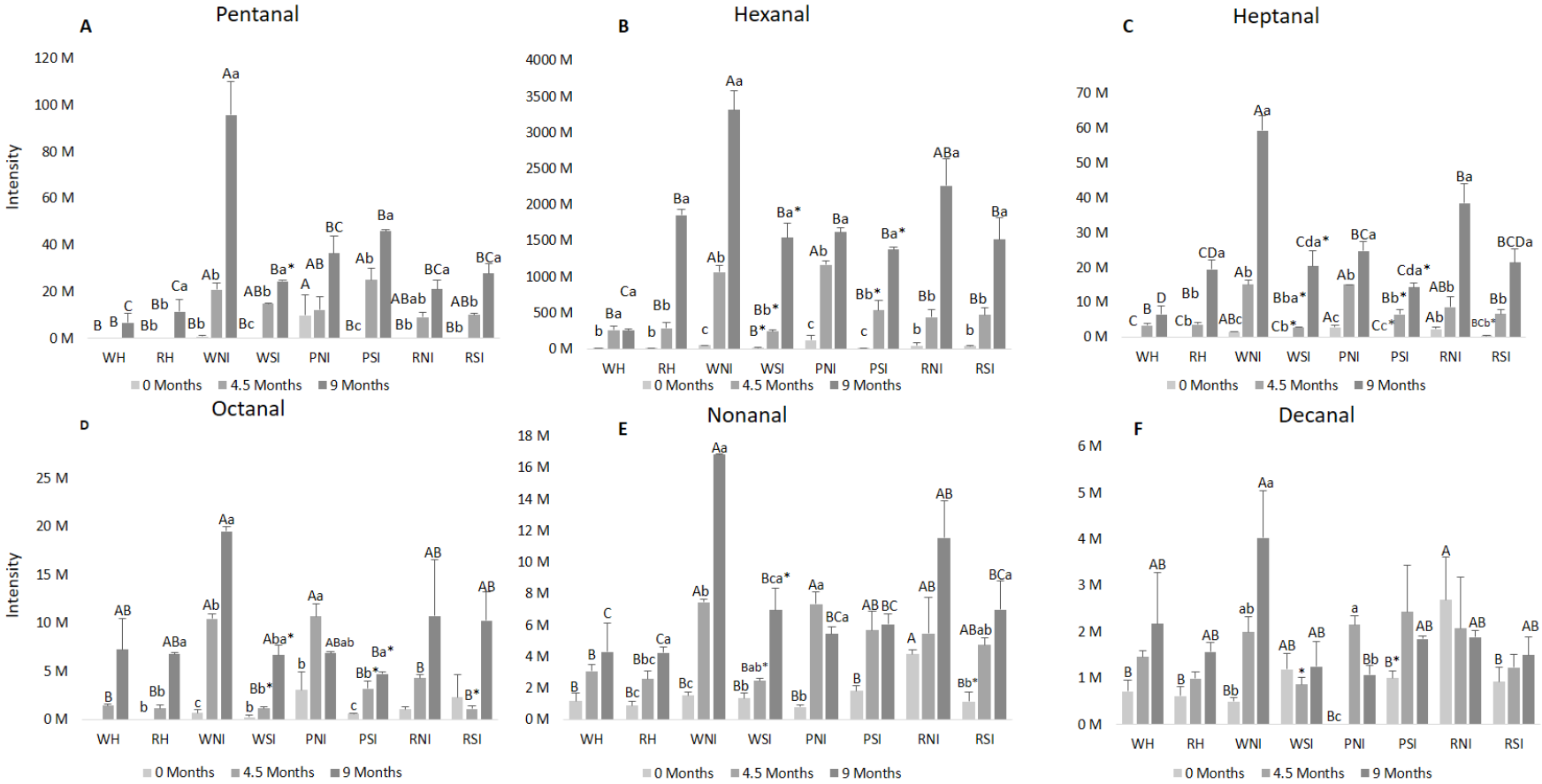


Figure 8: Alkyl aldehyde aroma compounds (A-F) intensity over the storage of HRW and IWG flour from freshly harvested grains. W = Whole, P = Partially Refined, R = Refined, N = No Steam, S = Steam, H = HRW, I = IWG. Upper case letters indicate significant differences across samples within a single time point, and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$). Samples without letters indicate no significant differences. An asterisk denotes a significant difference between not-steamed and steamed samples of the same refinement level according to a two-means comparison test ($P \leq 0.05$). Error bars represent standard error; n = 3.

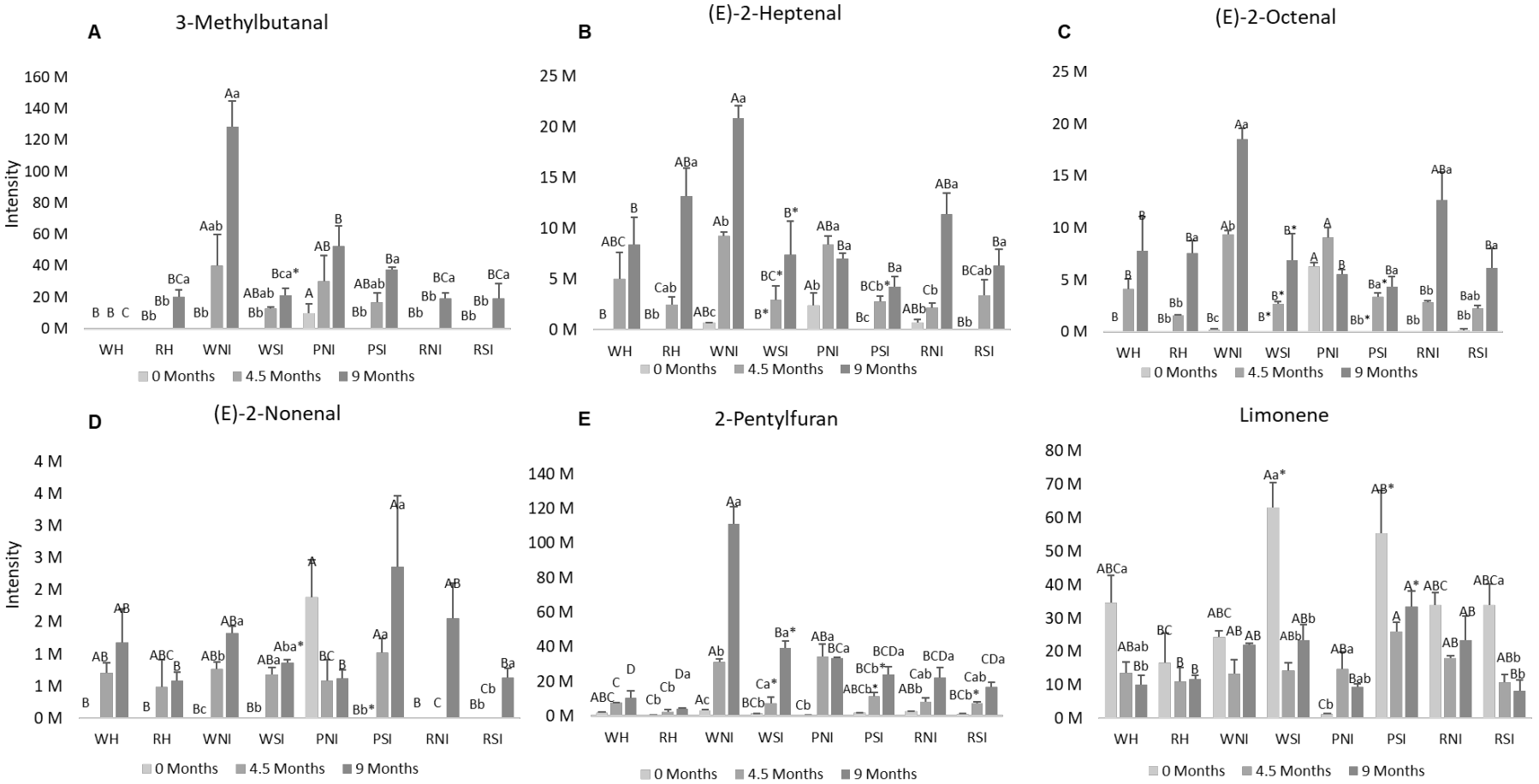


Figure 9: 3-Methylbutanal (A), enal aldehydes (B-D), 2-pentylfuran (E), and limonene (F) aroma compound intensities over the storage of HRW and IWG flour from freshly harvested grains. W = Whole, P = Partially Refined, R = Refined, N = No Steam, S = Steam, H = HRW, I = IWG. Upper case letters indicate significant differences across samples within a single time point, and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$). Samples without letters indicate no significant differences. An asterisk denotes a significant difference between not-steamed and steamed samples of the same refinement level according to a two-means comparison test ($P \leq 0.05$). Error bars represent standard error; n = 3.

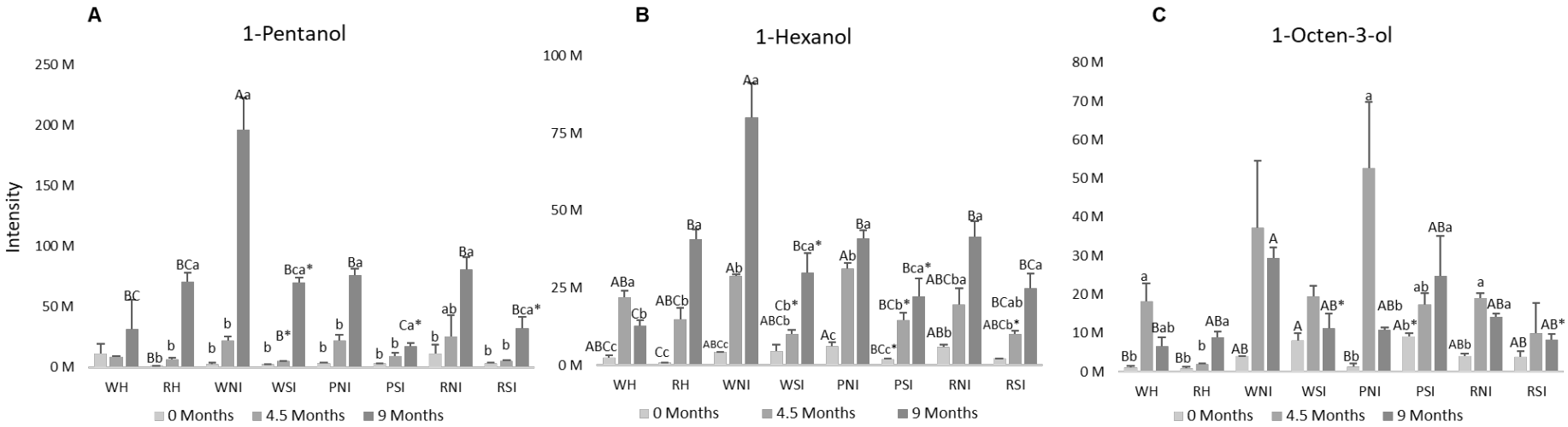


Figure 10: Alcohol aroma compounds (A-D) intensity over the storage of HRW and IWG flour from freshly harvested grains. W = Whole, P = Partially Refined, R = Refined, N = No Steam, S = Steam, H = HRW, I = IWG. Upper case letters indicate significant differences across samples within a single time point, and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$). Samples without letters indicate no significant differences. An asterisk denotes a significant difference between not-steamed and steamed samples of the same refinement level according to a two-means comparison test ($P \leq 0.05$). Error bars represent standard error; n = 3.

2.9.3.2 Development of VOACs in Stored HRW and IWG as Affected by Refinement Levels

Intensities of VOACs increased over storage in flour samples at all refinement levels. Partially refined IWG flour had significantly higher intensities of pentanal, 3-methylbutanal, (E)-2-octenal, and (E)-2-nonenal in comparison to all flour samples at 0 months of storage (**Figure 8 A** and **Figure 9 A**, **C**, and **D)**. Similarly, partially refined IWG flour was significantly higher in 1-hexanol intensity in comparison to refined HRW flour, and higher in (E)-2-heptenal intensity than both whole and refined HRW (**Figure 10 B** and **Figure 9 B**). Significant differences in VOAC intensities across the different samples at 0 months of storage could be attributed to lipid oxidation caused during sample preparation of the flour.

At 4.5 months of storage, whole and partially refined IWG flour had significantly higher intensities of hexanal, heptanal, octanal, nonanal, (E)-2-octenal, and 2-pentylfuran than both whole and refined HRW and refined IWG flour samples (**Figure 8 B, C, D,** and **E,** and **Figure 9 C** and **E**). Whole IWG flour was significantly higher in 3-methylbutanal intensities (**Figure 9 A**) than whole and refined HRW and refined IWG flour samples. Refined HRW and IWG flours were lower in (E)-2-heptenal and (E)-2-nonenal intensities compared to whole HRW and IWG and partially refined IWG flours (**Figure 9 B** and **D**).

At the end of storage, whole IWG flour was significantly higher in pentanal, heptanal, 3-methylbutanal, 2-pentylfuran, 1-pentanol, and 1-hexanol intensities than all the other samples (**Figure 8 A** and **C**, **Figure 9 A** and **E**, and **Figure 10 A** and **B**). Whole IWG flour was significantly higher in hexanal than partially refined IWG flour (**Figure 8 B**). By the end of storage, refined IWG flour was not significantly different than whole IWG flour in hexanal, nonanal, decanal, (E)-2-heptenal, (E)-2-octenal, (E)-2-nonenal, and 1-octen-3-ol (**Figure 8 B, E,** and **F, Figure 9 B, C,** and **D**, and **Figure 10 C**). Partially refined and refined IWG flour did not have any significant differences in intensities in any of the VOACs. Refined

HRW flour was also significantly higher in hexanal (**Figure 8 B**) than whole HRW flour at the end of storage and was not significantly different in the intensity of other VOACs. This observation is in contrast to what was expected as with refinement the bran and germ are removed from the flour, lowering the total amount of fat that can be oxidized. Morrison and Hargin (1981) found that approximately 50% of the triacylglycerols found in the germ of the kernels are removed and can end up in the endosperm during the milling process. The polyunsaturated fatty acids hydrolyzed by lipase from triacylglycerols are particularly prone to enzymatic and autoxidation (Galliard, 1986). Although refined flour samples had a lower fat content (**Table 6)**, the types of fat found in the endosperm fraction, such as triacylglycerols transferred during milling, could have potentially contributed to greater intensities of unwanted VOACs at the end of storage in refined flour samples. One other possible reason is that refined flour is deficient in antioxidants, typically found in the removed bran and germ fractions. Thus, at the end of storage, deterioration of lipids can continue unabated. Hayek (2020) found that refined HRW and IWG flour had significantly lower antioxidant activity, having 2-4 times less activity in comparison to whole and partially refined IWG samples. All flour samples were stored in clear sealed fish tanks that would allow light, which can catalyze the oxidation of lipids (Shahidi, 2000b). Since refined flour lacks antioxidants, these storage conditions could have enhanced the formation of VOACs through lipid oxidation.

Whole and partial IWG were not significantly different in intensities of many of the VOACs, from 0 to 4.5 months of storage, suggesting that the rate of lipid oxidation is similar between the two samples. These results corroborate the observations of Hayek (2020), which showed partially refined IWG flour was not significantly different in LOX activity in comparison to whole IWG flour throughout storage. However, at 9 months of storage, whole IWG flour had the highest intensities of VOACs, attributed to the higher fat (**Table 6**) and enzyme content. The 25% bran reduction in partially refined IWG flour resulted in a significant

decrease in hexanal (and other VOACs) intensity at the end of storage. Since hexanal has been used as an indicator of rancidity (Shahidi *et al.*, 1987; van Ruth *et al.*, 2000), partial refinement of IWG may be a suitable option for increased storage stability.

2.9.3.3 Development of VOACs over Storage as Affected by Steam Treatment

In general, steam treatment did not have a significant impact on VOAC intensities in flour samples at 0 months of storage. However, steamed whole IWG flour did have a significant reduction in hexanal, heptanal, and [E]-2-octenal intensities (**Figure 8 B** and **C** and **Figure 9 C**). Additionally, steamed partially refined IWG flour had a significant reduction in heptanal, [E]-2-nonenal, and 1-hexanol intensities (**Figure 8 C**, **Figure 9 D**, and **Figure 10 B**). Production of these VOACs could have begun during sample preparation before storage.

The effects of stream treatment were more noticeable at 4.5 months and 9 months of storage. In whole IWG flour at 4.5 months of storage, steam treatment significantly reduced the intensities of hexanal, heptanal, octanal, nonanal, [E]-2-heptenal, [E]-2-octenal, 2-pentylfuran, 1-pentanol, and 1-hexanol (**Figures 8 B, C, D,** and **E, Figure 9 B, C,** and **E,** and **Figure 10 A** and **B**). At 9 months of storage, steam-treated whole IWG flour had a significant reduction in all VOACs except for decanal. For partially refined IWG flour at 4.5 months of storage, significant reduction in intensities of hexanal, heptanal, octanal, [E]-3-octenal, and 1-hexanol was observed (**Figure 8 B, C, D, Figure 9 C,** and **Figure 10 B**). At 9 months of storage, hexanal, heptanal, octanal, [E]-2-heptenal, [E]-2-octenal, and 1-pentanol, and 1-hexanol intensities were significantly reduced in steamed partially refined IWG flour (**Figure 8 B, C, D, Figure 9 B** and **C,** and **Figure 10 A** and **B**). The effects of steam treatment were more pronounced in whole and partially refined IWG flour. Refined IWG flour does not contain bran and germ, thus is lacking the enzymes of concern. Therefore, steam refined IWG flour did not see many

significant reductions of VOACs over storage in comparison to its not-steamed counterpart.

Most of the VOACs are formed from the decomposition of hydroperoxides generated from LOX activity (Krishnamurthy *et al.*, 1967; Shahidi, 2000b; Lehto *et al.*, 2003; Matsui *et al.*, 2003; Än Salas *et al.*, 2005; Buśko *et al.*, 2010). In whole IWG flour, the reduction in intensities of most of the VOACs could indicate that steam treatment significantly lowered the rate of enzymatic rancidity by partially inactivating lipase and LOX. Lower VOACs formation corroborates the results from Hayek (2020), who reported that steam treatment significantly decreased lipase and LOX activity at 0 and 4.5 months of storage in whole and partially refined IWG flour. The use of thermal treatment, specifically steam treatment, has been used in oats and tested in other whole-grain cereals to decrease lipolytic enzymes to improve shelf-life, without risk of lowering consumer acceptance (Rose *et al.*, 2008). These results confirm that the steam treatment of IWG grains can be used to prevent and slow down lipid rancidity in whole and partially refined IWG flour.

2.9.4 Changes in VOAC over Storage of Flour from One-Year-Old Grain

Similar VOACs were found and identified in stored flour samples from one-year-old grains (**Figures 11, 12, and 13**). Flour from one-year-old grains had similar trends as those observed in the flour from freshly harvested grain. Over storage, intensities of VOACs formed through lipid oxidation increased in all the flour samples, with 6 months (end of storage) having the highest intensities.

Whole HRW flour samples had similar intensities of VOACs to those of whole IWG flour sample from beginning to end of storage. These results are different from those of the flour from freshly harvested samples, where by the end of storage the intensities of VOACs in whole HRW were significantly lower than those of whole IWG. However, the storage time was shorter (6 months vs. 9 months) for the flour from one-year-old grains. One noticeable trend was that the

intensities of VOACs in HRW had a linear increase throughout storage, whereas IWG flour samples had a non-linear trend. This observation indicated that the development of VOACs in IWG had a slower induction period, likely due to the higher antioxidant content compared to HRW (Tyl and Ismail 2018; Mathiowetz 2018; Hayek 2020). IWG's higher antioxidant content could have slowed down the development of off-odor compounds and prolonged the rancidity process of IWG, which suggests that IWG has superior short-term storage stability in comparison to wheat. With further storage of flour from one-year-old grains, continual lipid rancidity could potentially be observed.

Whole flour samples at 6 months of storage had higher intensities in general of alkyl aldehydes pentanal to nonanal (**Figure 11 A-E**), enal aldehydes (**Figure 12 B-D**), 2-pentylfuran (**Figure 12 E**), and alcohols (**Figure 13**) compared to partially refined flour samples. Lower VOAC intensities in partially refined flour match the trend found in the flour from freshly harvested grains.

Steam treated samples had overall lower intensities of VOACs compared to not-steam samples; however, by the end of storage, most VOACs were not significantly different. Hayek (2020) measured LOX of steam-treated samples from one-year-old grains and found that there was no significant reduction in LOX activity, which could explain the similar intensities of VOACs between steamed and not-steamed flour samples.

Compared to flour from freshly harvested grains, there were less noticeable changes in intensities of VOACs over storage, although trends are similar, they were not always significant. Changes in VOACs over storage had high variability resulting in less distinguished trends compared to results of flour from freshly harvested grains. Variation of the data could be attributed to the fact that the grains have been stored for a year prior to milling and had likely undergone chemical changes that could have impacted the development of VOACs. Hayek (2020) measured the chemical changes in one-year-old grains compared to freshly harvested IWG grains and observed similar variable trends.

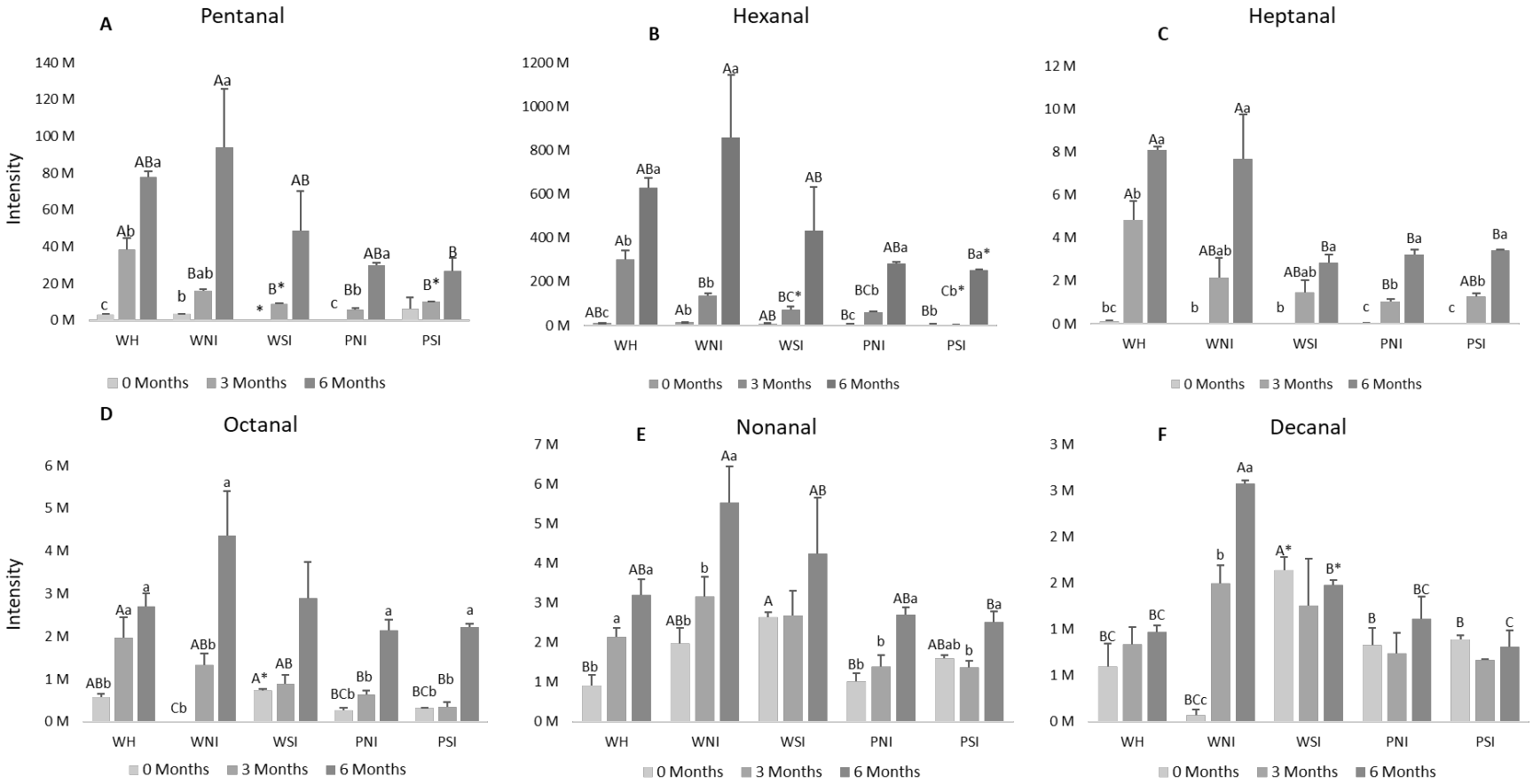


Figure 11: Alkyl aldehyde aroma compounds (A-F) intensity over the storage of HRW and IWG flour. W = Whole, P = Partially Refined, N = No Steam, S = Steam, H = HRW, I = IWG. Upper case letters indicate significant differences across samples within a single time point, and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$). Samples without letters indicate no significant differences. An asterisk denotes a significant difference between not-steamed and steamed samples of the same refinement level according to a two-means comparison test ($P \leq 0.05$). Error bars represent standard error; n = 3.

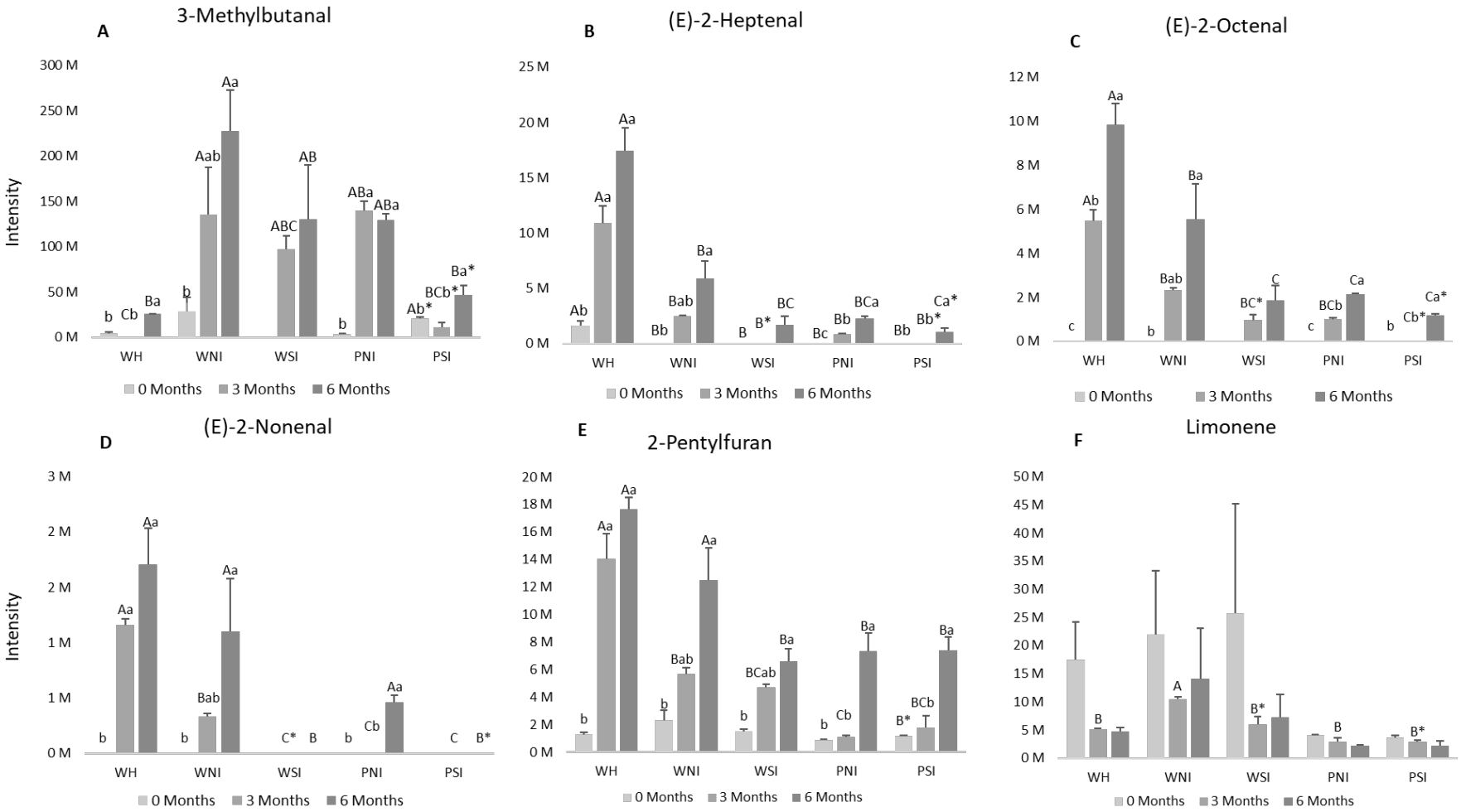


Figure 12: 3-Methylbutanal (A), enals (B-D), 2-pentylfuran (E), and limonene (F) aroma compound intensities over the storage of HRW and IWG flour from one-year-old grains. W = Whole, P = Partially Refined, N = No Steam, S = Steam, H = HRW, I = IWG. Upper case letters indicate significant differences among samples within a single time point, and lowercase letters indicate significant differences among a single within among time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$). Samples without letters indicate no significant differences. An asterisk denotes a significant difference between not-steamed and steamed samples of the same refinement level according to a two-means comparison test ($P \leq 0.05$). Error bars represent standard error; n = 3.

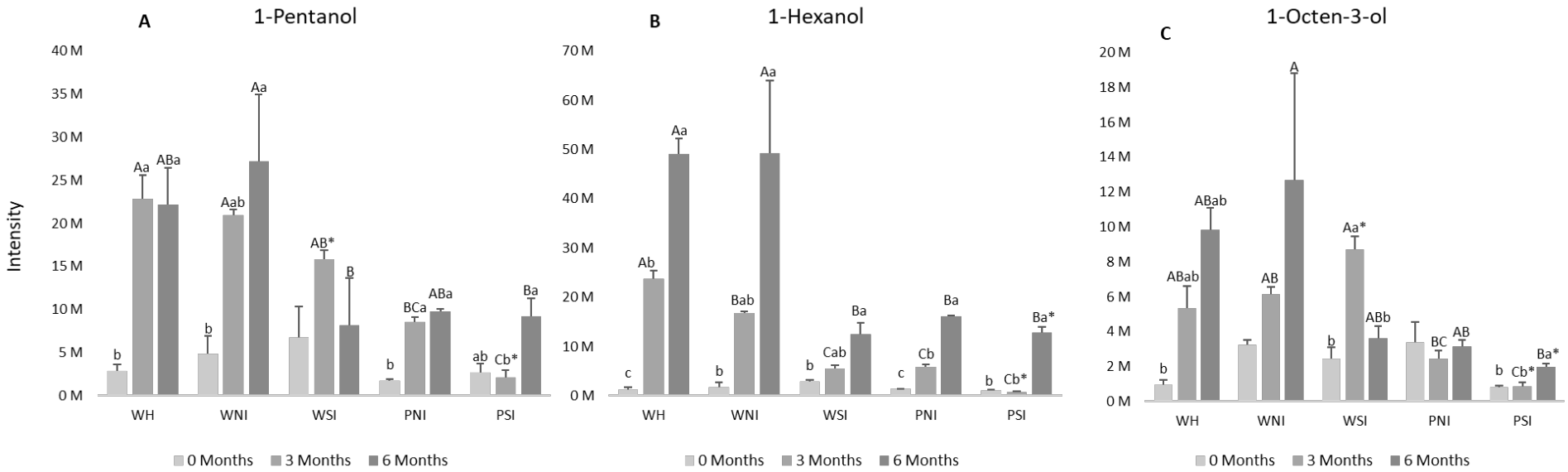


Figure 13: Alcohol aroma compounds (A-D) intensity over the storage of HRW and IWG flour from one-year-old grain. W = Whole, P = Partially Refined, N = No Steam, S = Steam, H = HRW, I = IWG. Upper case letters indicate significant differences among samples within a single time point, and lowercase letters indicate significant differences within a single sample among time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$). Samples without letters indicate no significant differences. An asterisk denotes a significant difference between not-steamed and steamed samples of the same refinement level according to a two-means comparison test ($P \leq 0.05$). Error bars represent standard error; n = 3.

2.9.5 Descriptive Analysis of Flour Tortillas

2.9.5.1 Impact of Grain Type, Refinement Level, and Storage Time of the Flour on Sensory Attributes

Comparison between HRW and IWG flour tortillas, collapsed over refinement level and storage time, showed that IWG flour tortilla samples had significantly higher intensity ratings of earthy, grassy, and Play-Doh® aromas (**Table 8**). Play-Doh is described as "a sweet, slightly musky, vanilla-like fragrance, with slight overtones of cherry, and the natural smell of a salted, wheat-based dough" (Hasbro, 2018). Earthy aroma had a significant positive correlation with hexanal, heptanal, 3-methylbutanal, 1-pentanol, and 1-hexanol (r = 0.70, 0.55, 0.55, 0.52, and 0.54, respectively) (**Figure 14** and **Table 74**, **Appendix E**), which were described as having green and grassy odors and had higher intensities in IWG flour samples compared to HRW samples over storage (**Figure 7, Figure 8 B** and **C, Figure 9 A,** and **Figure 10 A** and **B**)

IWG samples had significantly higher ratings of overall, vanilla, green pear, peanut butter, and beany flavor than HRW samples. IWG has an appreciable content of ferulic acid, which can be degraded to vanillic acid (Lesage-Meessen *et al.*, 1996). Vanillic acid has been identified in wheat and thus could be found in IWG (Hernández *et al.*, 2011). Vanillin is the reduced form of vanillic acid and could be the source of the vanilla flavor that contributed to the Play-Doh aroma described by the panelists. For taste, IWG samples were rated significantly higher for sweet, sour, bitter, and umami taste and had a significantly higher overall, salty, sour, bitter, umami, and astringent aftertaste than HRW samples (**Table 8**). Due to the bran to endosperm ratio, IWG contains more phenolic compounds in comparison to HRW (Tyl and Ismail, 2018). Phenolic compounds, especially those found in the bran layer, have been found to contribute to intense bitter and astringent qualities in cereal-based products (Heinioä *et al.*, 2008), which could be the cause of the

higher bitter and astringent taste and aftertaste ratings of IWG samples, but this does not necessarily explain higher salt, sour, or umami.

The difference between whole and refined flour, collapsed over grain type and storage time, showed that whole flour samples had significantly higher ratings of brown sugar aroma, overall flavor, green pear flavor, peanut butter flavor, and beany flavor, and overall aftertaste than refined grain samples. Whole flour contains more fat and higher intensities of flavor compounds, as discussed previously. The higher bran content in whole flour also contributed to more intense flavor and aftertaste than in refined flour samples, mostly due to phenolic compounds. Flour tortillas made with refined flour had more Play-doh® aroma and a more raw dough flavor than whole grain samples. Most flour purchased by consumers is refined; thus, the attribute of raw dough flavor in refined flour is likely more familiar to the panelists.

The effects of storage time, collapsed over grain type and refinement level, showed that 0 months of storage had a significantly higher nutty aroma than 4.5 and 9 months of storage. The tortillas had mild heat treatment that could have produced flavor compounds with nutty like flavors, which are common aroma characteristic of compounds formed from the Maillard reaction (Bredie *et al.*, 1997). Nutty aroma had a negative correlation with rancid oil aroma (r = -0.65) (**Table 75**, **Appendix E**). As lipid oxidative rancidity progressed with the production of VOACs, the nutty aroma was likely masked and became less intense over storage. The rancid-oil aroma attribute did not significantly change in intensity over storage; however, the average rating increased over time (**Table 8**), which would be expected due to the development of rancidity. With storage, the Play-Doh and cardboard aroma attributes were rated higher and were significant at 9 months of storage. Play-doh aroma, which increased significantly by the end of storage, did not have any significant correlations with any flavor compounds but did correlate with grassy aroma (r = 0.77, **Table 75, Appendix E**), which was an odor descriptor

used for hexanal and heptanal VOACs and had significant increases over storage in IWG flour samples (**Figure 7** and **Figure 8 B** and **C**).

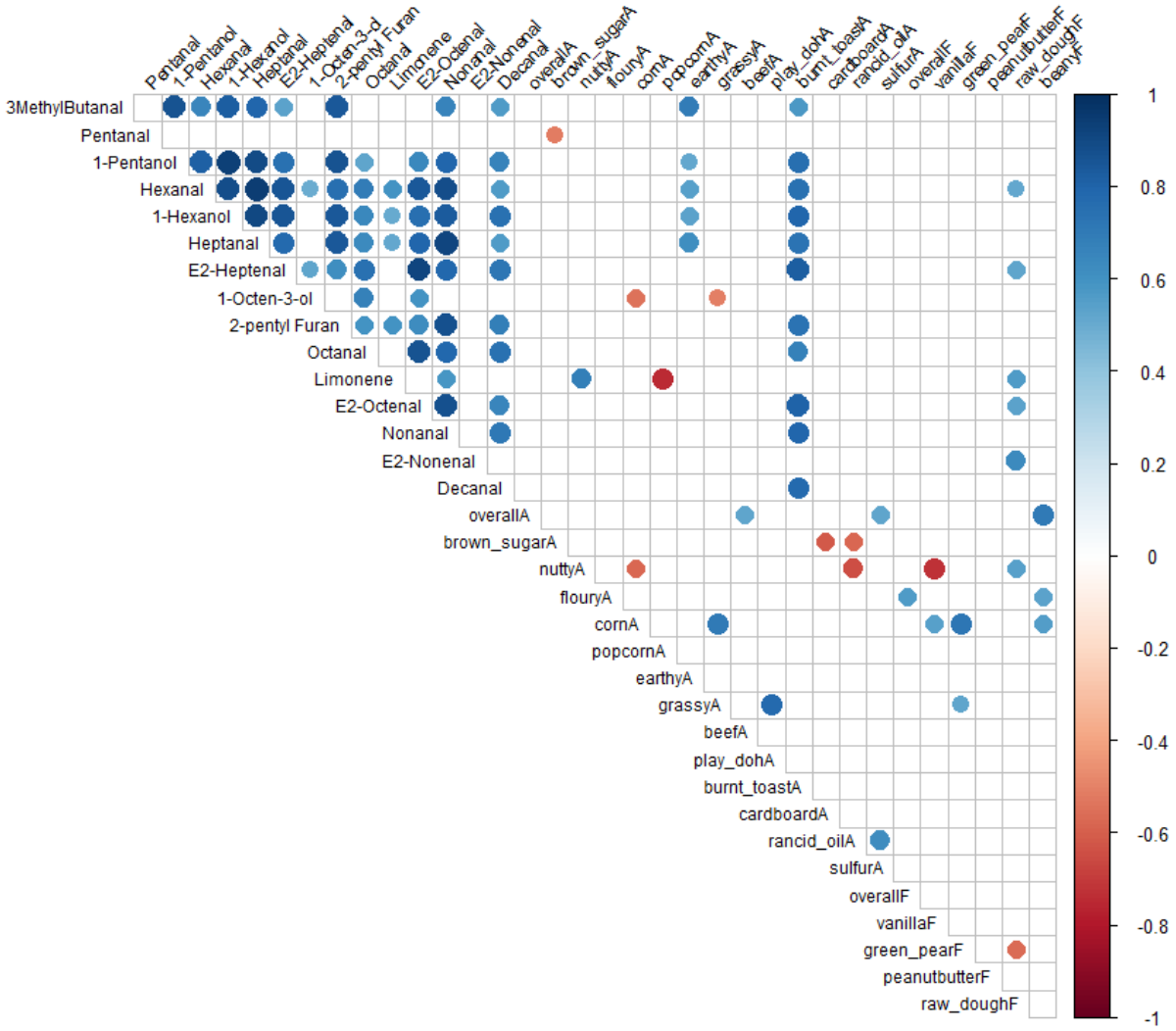


Figure 14: Visual representation of correlation data depicting relationships among flavor compounds and sensory attributes (aroma and flavor). The presence of a color dot between two attributes indicates a significant correlation ($P \leq 0.05$) according to the color scale, where blue is a positive correlation, and red is a negative correlation.

Table 8: Mean values (over all panelists [n = 6 - 8] and sensory replicates) by grain type, refinement level, and storage time of tortillas made from stored HRW and IWG flour. Intensity ratings were made on 20-point line scales (0 = none; 20 = intense).

Sensory attributes	Factors						
	Grain		Refining		Storage time (months)		
	HRW	IWG	Refined	Whole	0	4.5	9
Aroma							
Overall	5.6	6.2	5.9	5.9	5.7	6.2	5.8
Brown sugar	2.9	2.9	2.7[b*]	3.1[a]	2.9	3	2.8
Nutty	3	2.8	2.8	2.9	3.2[a]	2.8[b]	2.6[b]
Floury	2.8	3.1	3	2.9	2.9	3	3
Corn	3.5	3.4	3.5	3.4	3.6	3.7	3.1
Popcorn	3.6[a]	3.1[b]	3.3	3.3	3.4	3.3	3.2
Earthy	2.1[b]	2.6[a]	2.4	2.3	2.1	2.4	2.5
Grassy	3.6[b]	4.1[a]	3.9	3.9	3.7	3.9	4
Beef	1.8	1.6	1.6	1.7	1.7	1.8	1.6
Play-Doh	2.9[b]	3.4[a]	3.4[a]	2.9[b]	2.9[b]	3.0[b]	3.5[a]
Burnt toast	2.3	2.1	2.2	2.2	2	2.2	2.4
Cardboard	1.9	2.1	2.1	1.8	1.7[b]	1.8[b]	2.4[a]
Rancid oil	3.1	3.3	3.4	3	2.9	3.2	3.5
Sulfur	2	1.7	2	1.7	1.7	1.9	1.9
Taste							
Sweetness	1.9[b]	2.6[a]	2.4	2.2	2.2	2.4	2.2
Saltiness	1.7	1.7	1.6	1.8	1.5	1.7	1.9
Sourness	1.3[b]	2.0[a]	1.5	1.8	1.6	1.7	1.6
Bitterness	1.9[b]	2.8[a]	2.1	2.7	2.3	2.3	2.5
Umami	1.3[b]	1.8[a]	1.5	1.6	1.5	1.6	1.7
Flavor							
Overall	3.3[b]	5.3[a]	3.7[b]	4.9[a]	4.2	4.3	4.4
Vanilla	2.1[b]	2.5[a]	2.2	2.4	2.2	2.4	2.3
Green pear	2.3[b]	2.8[a]	2.2[b]	2.8[a]	2.3	2.7	2.6
Peanut butter	2.2[b]	2.4[a]	2.1[b]	2.4[a]	2.2	2.3	2.3
Raw dough	3.1	3.3	3.5[a]	2.9[b]	3.2	3.1	3.3
Beany	1.7[b]	2.6[a]	1.8[b]	2.5[a]	2.1	2.3	2.1
Aftertaste							
Overall	4.0[b]	6.0[a]	4.6[b]	5.5[a]	4.7	5.2	5.2
Sweet	2.5	2.4	2.3	2.5	2.7	2.4	2.2
Salty	1.7[b]	2.1[a]	1.8	2	1.9	1.9	1.8
Sour	1.6[b]	2.6[a]	2	2.2	2.1	2.4	1.9
Bitter	2.2[b]	3.9[a]	2.9	3.2	2.6	3.4	3.1
Umami	1.6[b]	2.1[a]	1.8	1.9	1.9	1.9	1.8
Astringent	2.3[b]	2.9[a]	2.5	2.7	2.3	2.8	2.7

[*] Lowercase superscripts represent significant differences ($P \leq 0.05$) between samples according to the Tukey's HSD means test. The absence of letters indicates no significant differences.

2.9.5.2 Impact of Steam Treatment, Refinement, and Storage time of IWG flour on Sensory Attributes

Comparison of sensory attributes of IWG samples over storage, collapsed over refinement level and steam treatment, showed that samples stored for 4.5 months and 9 months saw an increase in floury aroma compared to 0-month samples (**Table 9**). At 9 months of storage, earthy and Play-Doh aromas of IWG samples were rated significantly higher than 0 and 4.5 months of storage. As previously mentioned, earthy aromas had a significant positive correlation with 3-methylbutanal, hexanal, heptanal, 1-pentanol, and 1-hexanol (r = 0.70, 0.55, 0.55, 0.52, and 0.54, respectively) (**Figure 14** and **Table 74**, **Appendix E**). Over storage, lipid rancidity resulted in the formation of characteristic VOACs that likely contributed to these aroma attributes.

Refinement of IWG flour, collapsed over storage time and steam treatment, resulted in a significant decrease in overall and beany flavors. Whole IWG samples were more salty and bitter in taste compared to refined IWG samples. Whole IWG flour had a higher overall, salty, and bitter aftertaste in comparison to refined IWG samples. The stronger flavor, taste, and aftertaste in whole IWG flour is most likely due to the higher phenolic content associated with higher fiber content (**Table 7**). As previously mentioned, phenolic compounds are known to contribute to bitter and astringent characteristics in whole grain cereals (Heinioä *et al.*, 2008). With the decrease in bran and germ content, panelists were able to perceive significant differences between samples with bran (whole and partially refined) and refined samples (**Table 9**).

Steamed IWG flour tortilla samples had significantly lower intensity ratings of overall aroma, overall flavor, salty taste, overall aftertaste, and bitter aftertaste than not-steamed samples. Although not significant, steam-treated samples did see numerically lower ratings in Play-doh and rancid oil aroma attributes (**Table 9**). There were no significant differences noted for specific aroma attributes between not-steamed and steamed samples. The decrease in overall aroma in

steamed samples complements the results of VOAC analysis. Compared to not-steamed samples, steamed samples had significantly lower intensity of most of the identified aroma compounds formed from lipid oxidation, as previously discussed in **Section 2.9.3.3**. With significantly lower intensities of odor active VOACs, a lower overall aroma was expected.

Table 9: Mean values (over all panelists [n = 6-8] and sensory replicates) by refinement level, storage time, and steaming of tortillas made from stored IWG flour. Intensity ratings were made on 20-point line scales (0 = none; 20 = intense).

Sensory attributes	Factors							
	Refinement Level			Storage Time			Steam Treatment	
	Refined	Partial	Whole	0	4.5	9	No	Yes
Aroma								
Overall	5.8	6.1	6	5.8	6.1	6	6.3^{a*}	5.7^{b}
Brown sugar	2.9	3.1	3	2.9	3.1	2.9	3	3
Nutty	2.8	2.9	3	3	2.9	2.8	2.9	3
Floury	3.2	3.4	3.2	3.0^{b}	3.4^{a}	3.3^{a}	3.2	3.3
Corn	3.4	3.6	3.3	3.5	3.6	3.2	3.4	3.5
Popcorn	3	3.3	3.2	3	3.3	3.1	3.2	3.1
Earthy	2.6	2.4	2.4	2.1^{b}	2.5^{ab}	2.6^{a}	2.5	2.3
Grassy	3.9	3.8	3.9	3.6	4	4	3.9	3.8
Beef	1.6	1.6	1.6	1.7	1.6	1.5	1.6	1.6
Play-Doh	3.2	3.3	3.1	2.9^{b}	3.1^{b}	3.6^{a}	3.4	3
Burnt toast	2	2.3	2.1	1.9	2.2	2.2	2.2	2.1
Cardboard	2.1	2	2.1	1.8	2	2.3	2.1	2
Rancid oil	3.2	3.4	3.1	2.9	3.3	3.5	3.4	3.1
Sulfur	1.7	2	1.7	1.6	2	1.9	1.8	1.8
Taste								
Sweetness	2.8	2.7	2.5	2.7	2.8	2.4	2.6	2.7
Saltiness	1.5^{b}	2.1^{a}	2.0^{a}	2	1.7	2	1.8	1.9
Sourness	1.6	1.8	1.9	1.7	1.8	1.8	1.9	1.7
Bitterness	2.1^{b}	2.6^{ab}	3.0^{a}	2.5	2.3	2.8	2.9^{a}	2.2^{b}
Umami	1.8	1.7	1.9	1.8	1.6	1.9	1.7	1.8
Flavor								
Overall	4.3^{b}	5.1^{a}	5.5^{a}	4.8	4.9	5.3	5.4^{a}	4.6^{b}
Vanilla	2.4	2.5	2.5	2.5	2.6	2.4	2.5	2.5
Green pear	2.7	3	2.9	2.9	2.8	2.9	2.8	2.9
Peanut butter	2.3	2.7	2.6	2.6	2.4	2.5	2.5	2.5
Raw dough	3.4^{a}	2.9^{b}	3.1^{ab}	3.1	3	3.1	3.2	3
Beany	2.3^{b}	3.1^{a}	3.3^{a}	2.8	2.9	3	2.9	2.9
Aftertaste								
Overall	5.1^{b}	5.7^{ab}	6.2^{a}	5.4	5.6	5.9	6.1^{a}	5.2^{b}
Sweet	2.7	2.7	2.4	2.6	2.7	2.4	2.6	2.6
Salty	1.9	2	2.3	2.1	2	2.1	2.1	2.1
Sour	2.2	2.2	2.5	2.3	2.2	2.5	2.4	2.2
Bitter	3.3	3.2	3.8	3.3	3.5	3.5	3.7^{a}	3.1^{b}
Umami	1.9	1.9	2.1	2	1.9	2	2	1.9
Astringent	2.7	2.6	2.8	2.6	2.5	2.9	2.8	2.6

* Lowercase superscripts represent significant differences ($P \leq 0.05$) between samples according to the Tukey's HSD means test. The absence of letters indicate no significant differences.

2.9.6 Correlation of Analytical Flavor and Sensory Analysis of Stored HRW and IWG Flour

Due to the complexity of the analysis of flavor compounds, flavor analysis is usually done in tandem with sensory evaluation to better relate off-flavor development to lipid oxidation by using correlation data (Bett and Grim, 1994). Most of the VOACs identified in the stored HRW and IWG flour have been generated via lipid rancidity; however, it is important to understand how these VOACs affect sensory perception.

Principal component analysis (PCA) is a commonly used multivariate analytical statistical technique that can be applied to flavor and sensory analysis that reduces the dependent variables (i.e., flavor compounds and DA attributes) to smaller variables (factors) based on patterns of correlation among the original variables (Lawless and Heymann, 1998). The resulting PCA can be used to predict and summarize data between the two methods of evaluating flavor. PCA identified two significant principal components that accounted for 50% of the variance between the flavor and sensory analysis (**Figure 15**). The first component (PC1) represented 32% of the variability. PC1 marginally separated the VOACs, indicating strong association, whereas the aroma and flavor attributes had more separation.

The second component (PC2) represented 18% of the variation and separated the VOACs from the aroma and flavor attributes. The separation of VOACs on PC2 appears to be based on molecular weight (MW). Flavor compounds pentanal, heptanal, hexanal, 3-methylbutanal, and 1-pentanol all eluted in the first half of the GC run, due to their lower MW, and negatively correlated on PC2, whereas heavier compounds, such as decanal, [E]-2-nonenal, and octanal positively correlated on PC2. Lower MW compounds positively correlated with an earthy aroma, Play-doh aroma, and raw dough flavor attributes. Heavier MW compounds associated more with cardboard, grassy, rancid oil, and nutty aromas.

Popcorn and beef aroma attributes were separated from all the VOACs in the PCA. Beef aroma had the lowest intensity scores in descriptive analysis (intensity rating <2 out of 20), whereas popcorn aroma was very consistent among IWG samples (i.e., did not change with treatment) (**Table 8** and **9**). Beef and popcorn aromas could be a result of sample preparation of the tortillas. Since all samples were made the same, this could explain the consistent intensity scores for beef and popcorn aromas.

Over storage, the intensities of the flavor compounds formed increased significantly, and panelists from DA were able to detect an increase in earthy and Play-doh aromas and raw dough flavor, which the PCA was able to correlate with known lipid rancidity flavor compounds. The PCA confirmed the relationship between the flavor compounds and the sensory attributes that have been previously discussed.

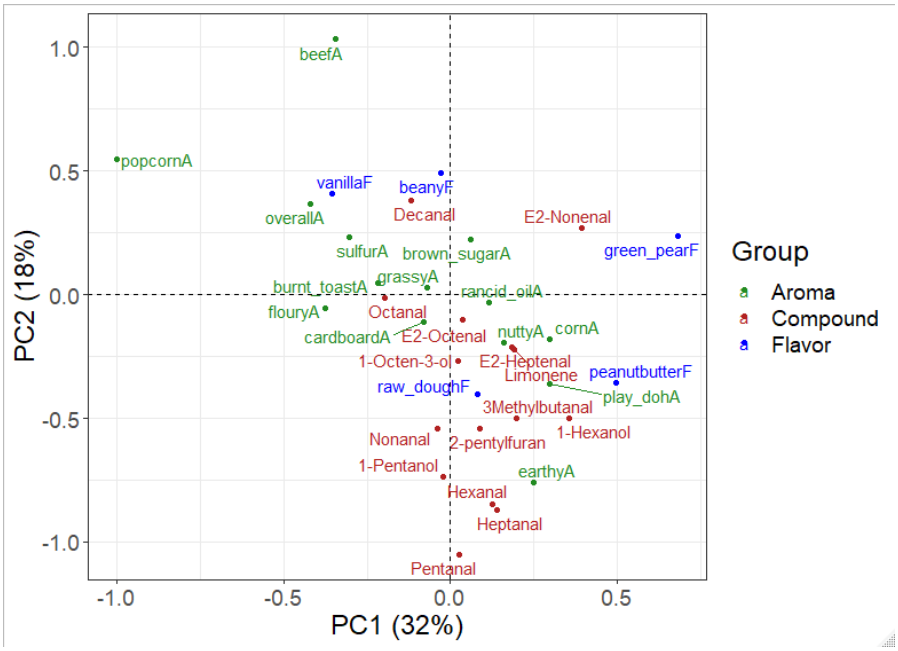


Figure 15: Principal component analysis of correlation between flavor compounds, aroma attributes, and flavor attributes of stored (0, 4.5, and 9 months) HRW and IWG flour at various refinement (whole, partially refined, and refined) and steamed or not-steamed. The first 2 principal components accounted for 50% of the total variance.

2.7 Conclusions

Refinement and thermal treatment of IWG had a significant impact on the development of odor-active VOACs formed through lipid rancidity. Over storage, intensities of VOACs from lipid rancidity increased. Partial refinement of IWG significantly reduced the intensity of key lipid oxidation VOACs, such as hexanal. Compared to whole and partially refined flour, refined flour samples had relatively similar intensities of VOACs at the end of storage, which could be attributed to the loss of antioxidants. These results suggested that partial refinement can be used to achieve a longer shelf-life while maintaining beneficial nutrients (fiber and antioxidants) found in the bran and germ.

Steam treatment significantly reduced lipase and LOX activity; thus, resulted in lower VOAC intensities in whole and partially refined IWG flour samples. In whole IWG flour, steam treatment reduced all but one VOAC. These results confirm that steam treatment is effective in reducing VOACs' development over storage.

Results from sensory evaluation showed that IWG samples had more intense earthy, grassy, and Play-doh aromas, overall flavor, and taste compared to HRW samples, which is likely due to higher fat and phenolic content in IWG. Refinement of IWG resulted in lower flavor intensities; however, it did not impact aroma attributes. Thermal treatment resulted in significantly lower overall aroma, flavor, taste, and aftertaste intensities in IWG samples. Correlations made between flavor analysis and sensory evaluation confirmed that the identified lipid oxidation compounds in the flour samples contributed to several detected flavors and aromas in the tortillas samples.

Both flavor analysis and sensory evaluation results confirmed that the primary concern in the storage of IWG flour is lipid rancidity. Findings further confirmed that partial refinement and steam treatment can be both used in tandem to slow down lipid rancidity in IWG flour and prolong its storage stability for commercial use.

Chapter 3: The Effects of Bran Content and Thermal Treatment on Dough Functionality of Stored Intermediate Wheatgrass Flour

3.1 Overview

Perennial intermediate wheatgrass (IWG), *Thinopryum intermedium*, has long been grown as a crop for forage and animal feed but has recently been recognized for its potential to be integrated into the food system. Previous research on IWG has been directed towards characterizing and determining its functionality in food application. Research on the changes in functionality over storage of IWG flour has not yet been conducted. The aim of this study was to evaluate the effects of refinement, steam treatment, and relative humidity on changes of functionality over the storage of IWG flour compared to hard red wheat (HRW). Over storage, IWG showed increased dough development time, dough stability, resistance to extension, and gluten aggregation denoting an increase in dough strength. IWG also had improvements over storage in starch pasting viscosities, including peak, hold, and final viscosity. Partial refinement of IWG resulted in improved functionality compared to whole IWG, due to less interference of bran with gluten development. Refined IWG had the weakest dough rheology compared to whole IWG, due to deficiency in high molecular weight (HMW) glutenin proteins, but had the highest pasting viscosities due to a higher relative starch content compared to whole and partially refined samples. Steam treatment had a positive effect on dough development time, stability, and resistance to extension during storage, a mild effect on gluten strength, and a slightly negative on effect starch pasting viscosities during storage. Although the overall functionality of IWG remained inferior to HRW throughout storage, data from this work suggested that partial refinement can help make IWG more functional and marketable. Steam treatment was not detrimental to functional properties of IWG flour over storage. Overall, results demonstrated a positive effect of storage on the functionality of IWG flour.

This study provided additional incentives to farmers and food manufacturers alike by highlighting IWG's acceptable storage stability.

3.2 Introduction

The current agricultural landscape of cereal grains consists predominantly of rice, wheat, and maize, all of which are annual crops. Annual crops dominate the global cropland and are known to cause soil erosion, water-run off, nitrogen leaching, and nutrient loss (Cox *et al.*, 2006). Considering the effects of climate change and the need to feed a human population that is expected to reach 10 billion by 2050, development of more sustainable crops is warranted. Perennials are sustainable crops that have been associated with many environmental benefits such as soil conservation, carbon sequestration, nitrogen cycling efficiency, and soil nutrient enrichment (Culman *et al.*, 2013; Lewandowski, 2016). Intermediate wheatgrass (IWG), a perennial crop, was chosen for further domestication due to its potential agronomic benefits attributed to its large root system (Wagoner, 1990; DeHaan *et al.*, 2005) and its nutritional profile (Tyl and Ismail, 2018). Current breeding efforts are underway to improve desirable traits, such as ease of harvest and yield, in order to expedite the commercialization of IWG (Zhang *et al.*, 2016). However, for the successful adoption of IWG into the current agricultural landscape, farmers and consumers need to know it is marketable. Functionality and storage stability are essential factors that will affect the marketability of IWG.

As a cereal grain, IWG is genetically related to wheat and has the potential to be utilized in similar applications. Wheat is often considered the "gold standard;" therefore, cereal grain functionality is often compared to that of wheat. Globally, wheat has become a staple ingredient due to its superior viscoelasticity and ability to trap gas allowing it to be used in a vast array of products (Žilić, 2013). The viscoelastic properties are attributed to the presence of two types of storage

proteins, gliadins and glutenins. Together these two proteins can form a network known as gluten when hydrated and mixed (Dhaka and Khatkar, 2015).

Previous research on the functionality of IWG in comparison to hard red wheat (HRW) was conducted by Rahardjo et al. (2018). The authors found that IWG, while rich in gliadins, is deficient in high molecular weight glutenins (HMWG), leading to a weaker gluten matrix with reduced gas holding capacity. Furthermore, due to its smaller seed size, IWG has a higher bran to endosperm ratio resulting in a higher dietary fiber content, which contributed to a lower loaf volume and height (Rahardjo *et al.*, 2018). Although IWG performance in leavened products does not compare to that of wheat, it can be utilized in non-leavened products such as flatbreads, tortillas, and crackers that require more extensibility.

Starch pasting characteristics also impact rheological properties and end-use applications. Marti et al. (2015) and Rahardjo et al. (2018) both found that IWG has lower starch pasting properties. This observation was attributed to the relatively higher fiber and protein content in IWG. Proteins can interact by forming disulfide bonds and creating a matrix around starch granules, which can prevent swelling, leading to reduced viscosity and increased pasting temperature (Hamaker and Griffin, 1993). Fiber and proteins can both compete with starch for water, leading to lower viscosity (Collar *et al.*, 2006).

As is the case with other grains, including wheat, the functional properties of IWG grains/flour are impacted by multiple factors including storage and processing conditions. Research into the effects of storage on the functionality of IWG is limited. Mathiowetz (2018) investigated the rheological and functional changes of stored IWG groats and the effects these changes may have on storage stability. Flour from stored IWG groats had improved functional properties, including longer dough development times, increased resistance to extension, and improved loaf volume, all of which correspond to a stronger gluten network. Flour from stored IWG groats also had increased starch pasting viscosities, including

peak and hold viscosities (Mathiowetz 2018). These improvements can be attributed to several changes in proteins, starches, and lipids over storage.

Different processing approaches have been utilized to increase the storage stability of grains and flour. Steam treatment is one processing approach that is used in the cereal industry to prolong shelf life (Rose *et al.*, 2008). The primary purpose of steam treatment is to inactivate the enzymes that catalyze hydrolytic and oxidative rancidity, namely lipase and lipoxygenase, respectively. IWG has a higher lipid content than wheat (Tyl and Ismail 2018; Mathiowetz 2018), which makes it more susceptible to lipid rancidity over storage. Steam treatment can potentially be used to extend the shelf-life of IWG flour.

Steam treatment can be beneficial for IWG storage stability, but it is also important to consider the implications. Thermal treatment can cause protein denaturation and pre-gelatinization of starch, which may negatively impact dough functionality and usability (Arntfield *et al.*, 1997; Fasina *et al.*, 1999; Sun *et al.*, 2006; Yadav *et al.*, 2012; Runyon *et al.*, 2015; Deepa and Umesh Hebbar, 2017). Therefore, optimizing a steaming method that can significantly reduce unwanted enzyme activity while maintaining functionality is desired. The indirect steaming method (use of proofing oven) followed by Mathiowetz (2018) may not have been sufficient, as the delivery of the steam was not effective in denaturing the enzymes of concern. A more direct steam treatment will be used in the present study to determine the impacts on the functionality of IWG flour over storage.

Another approach that is used in wheat to extend shelf life is refinement. By removing the bran and germ fractions of the flour, unwanted enzymes found in these fractions are removed, and the lipid and fiber contents are decreased. Consumers prefer refined flour due to its light color and pleasant sensory attributes; however, refined flour lacks the added nutritional benefits found in whole grain flour. Consumption of whole-grain products among consumers has increased due to their association with various health benefits (Slavin *et al.*, 2001). Whole

grain flour has a shorter shelf life due to the retention of bran and germ that contains the lipids and problematic enzymes. Furthermore, bran has been known to interfere with flour functionality. Storage stability of IWG flour at various levels of refinement has not yet been assessed.

Therefore, the objective of this study was to evaluate the effects of prior grain storage, bran content, and steam treatment on the functionality (dough rheology, gluten strength, and starch pasting properties) of IWG flour over storage at 43% and 65% relative humidity. We hypothesized that steam treatment would not have a negative effect on dough functionality. Furthermore, we hypothesized that refinement of IWG flour would enhance its storage stability, while partial refinement would improve functionality.

3.3 Materials

3.3.1 Intermediate Wheatgrass and Hard Red Wheat Grains

IWG and HRW grains were obtained as described in **Chapter 2, section 2.3.1**.

3.3.2 Chemicals

Enzymes and assay kits for total dietary fiber (K-TDFR-100A/K-TDFR-200A 04/17), total starch (K-TSTA-50A/K-TSTA-100A 06/17), and amylose/amylopectin ratio (K-AMYL 12/16) were purchased from Megazyme International Co. (Wicklow, Ireland). Celatom® (diatomaceous earth), tris(hydroxymethyl)aminomethane (TRIS), and 2(*N*-morpholino) ethanesulfonic acid (MES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). TRIS - HCl 4-15% polyacrylamide gel (345-0028), laemmli sample buffer (161-0737), broad range molecular weight standard (345-0024), and concentrated tris-tricine-sodium dodecyl sulfate running buffer (161-0744) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Coomassie brilliant blue R250 (786-498) was purchased from G-Biosciences (St.

Louis, MO, USA). Calcium chloride was purchased from Sigma Aldrich (St. Louis, MO, USA)

3.4 Experimental Design

The experimental design was as described in **Chapter 2 section 2.4**. However, relative humidity was added as a factor with 2 levels, 43% and 65%. Flour samples from freshly harvested grains were stored at both RH and were tested at 0, 3, 6, and 9 months of storage. Flour from one-year-old grains were stored at 43% RH and tested at 0, 3, and 6 months of storage.

3.5 Steam Treatment, Milling, and Sample Preparation

Steam treatment, milling, and sample preparation were performed as described in **Chapter 2 section 2.5**.

3.6 Flour Storage

IWG and HRW samples were stored at 43% RH as described in **Chapter 2, section 2.6**. Additionally, flour samples from freshly harvested grains were stored at 65% RH, which corresponds to a moisture content of ≥14% (Pixton and Warburton, 1971). A CSZ temperature and humidity chamber (Cincinnati Sub-Zero, Cincinnati, OH) was used to store the samples at 65% RH and at ambient temperature. In industry, storage conditions may fluctuate. Grains are stored in conditions below 14% moisture to prevent microbial growth and to slow down grain respiration (Pixton and Warburton, 1971; Butt *et al.*, 2004). A higher RH was chosen to imitate the higher end of storage and its potential effects over storage.

3.7 Methods

3.7.1 Protein Profiling

Protein profiling was done using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), following the procedure developed by Tatham (Marsh *et al.*, 2003) and modified by Rahardjo et al. (2018). Two grams of each flour sample, in 50 mL centrifuge tubes, were defatted using 20 mL of water-saturated butanol. The mixture was placed on a shaker for 1 hour at room temperature, then centrifuged (Beckman J2-MC, Brea, CA) at 9,000 g for 10 min at 20°C. The supernatant was discarded, and the pellet was washed with water-saturated butanol two more times. Next, water and salt soluble proteins (albumins and globulins) were removed by washing with 20 mL of 0.5M sodium chloride. The mixture was placed on a shaker for 1 hour at room temperature and centrifuged at 9,000 g for 10 min at 20°C. The supernatant was discarded, and the pellet was washed two more times with the salt solution. Residual salt was removed with 20 mL of double-distilled water (DDW) added to the pellet to disperse it. The dispersed sample was placed on a shaker for 30 minutes at room temperature, and centrifuged at 9000 g for 10 min at 20°C. The supernatant was discarded, and DDW wash of the pellet was repeated two more times. Glutenins and gliadins were then extracted from the pellet using 20 mL of aqueous 1-propanol (50% v/v), beta-mercaptoethanol (2% v/v), and acetic acid (1% v/v). Each sample was placed on a shaker for 1 hour at room temperature, followed by centrifugation at 9,000 g for 10 min at 20°C. The supernatant, which contains gluten forming proteins (glutenins and gliadins), was then collected into a 250 mL round bottom flask. The pellet was washed two more times with the same solvent and the supernatants were combined. A rotary evaporator was used to evaporate the solvent at 60°C (Buchi Rotavapor R-124 and Waterbath B-481, New Castle, DE). Extracted gluten forming proteins were then lyophilized and stored at -20°C. The lyophilized protein extracts were analyzed for their protein content following the Dumas method, as

outlined in **Section 2.7.1**. Protein profile of the extracts was monitored by SDS-PAGE under reducing conditions. The amount of sample needed to obtain 0.25 mg protein/5 µL, determined by Dumas, was weighed and mixed with 190 µL SDS Laemmli buffer and 10 µL beta-mercaptoethanol, stirred for 30 sec, boiled at 100°C for 5 min, and cooled down to room temperature. The sample was centrifuged at 9000 g (Eppendorf 5415D, Hamburg, Germany) for 5 min. Aliquots (5 µL) of the extracts were loaded into the wells of a 4-15% Criterion Tris-HCl gel. The gel was electrophoresed at 200 V for approximately 45 min, followed by staining using a coomassie blue staining solution (45% v/v methanol, 10% v/v glacial acetic acid, 45% v/v DDW, and 3g/L coomassie brilliant blue R250), and destaining using a destaining solution (10% v/v glacial acetic acid, 5% v/v methanol, and 85% v/v DDW). Gels were scanned using Molecular Image Gel Doc XR system (BioRad, Hercules, CA).

3.7.2 Carbohydrate Composition

3.7.2.1 Dietary Fiber

Total dietary fiber, soluble fiber, and insoluble fiber were analyzed following the AOAC 991.43 method using the Megazyme Total Dietary Fiber Assay Kit (AOAC International, 2016). In duplicate, 1 g of flour sample was dissolved in 40 mL 0.05 M MES-Tris buffer (pH 8.2) and sequentially digested with heat-stable α-amylase for 30 min at 95-100°C, protease for 30 min at 60°C, and amyloglucosidase for 30 min at 60°C. Each digested sample was filtered through an even layer of Celatom® and washed with 78% ethanol, 95% ethanol, and acetone sequentially. The residue, comprising of insoluble dietary fiber (IDF), was dried and weighed. Soluble dietary fiber (SDF) present in the filtrate was precipitated with 95% ethanol and was subsequently filtered through an even layer of Celatom®. The SDF residue was washed with 78% ethanol, 95% ethanol, and

acetone, sequentially, and then was dried and weighed. Total dietary fiber results were corrected for protein and ash by Kjeldahl (AOAC 981.10) and dry ashing (AOAC 942.05), respectively (AOAC International, 2016).

3.7.2.2 Total Starch

Total starch was analyzed in triplicate following the AOAC 996.11 method using the Megazyme Total Starch Assay Kit (AOAC International, 2016). The method used was as outlined in Section “E” of the Megazyme procedure, titled “Determination of starch in samples which also contain D-glucose and/or maltodextrins.” This method included an ethanol wash step (80% v/v) to remove endogenous glucose from flour, before digestion of the starch into glucose. Total starch in the flour was quantified, after hydrolysis by alpha-amylase and amyloglucosidase, spectrophotometrically as a function of glucose concentration following reaction with glucose oxidase-peroxidase reagent (GOPOD), using standard concentrations of D-glucose.

3.7.2.3 Amylose/Amylopectin Ratio

Amylose/amylopectin ratio was analyzed following the concanavalin A (ConA) precipitation procedure using the Amylose/Amylopectin Megazyme kit. First, samples in triplicate were dispersed by heating at 100°C for ca. 1 minute in dimethyl sulfoxide (DMSO), and then lipids were removed by precipitating the starch in ethanol and recovering the precipitated starch. After re-solubilizing the starch in sodium acetate buffer (100 mM sodium acetate buffer, pH 4.5), amylopectin was precipitated out of solution with ConA solution. The supernatant (after amylopectin precipitation, containing amylose), and an aliquot of the solubilized starch in a sodium acetate buffer (representative of total starch), were digested separately by α-amylase and amyloglucosidase to produce D-glucose.

Following reaction with GOPOD, the concentration of D-glucose in the samples was determined spectrophotometrically at 510 nm, compared to a similarly treated starch control with a known amylose/amylopectin ratio. The ratio of D-glucose in the supernatant aliquot (amylose) to the total starch aliquot was used to calculate the percentage of amylose (% w/w).

3.7.2.4 Starch Damage

The content of starch damage was measured in triplicate following the AACCI 76-31.01 method using the Megazyme Starch Damage Assay Kit (AACC International, 2010). In flour, the damaged starch granules present were hydrolyzed to maltosaccharides and dextrins by alpha-amylase at 40°C for exactly 10 minutes. This controlled digestion allows only the damaged starch granules to be digested to dextrins. Amyloglucosidase digestion at 40°C for 10 minutes was then used to convert the dextrins to glucose. Finally, the content of glucose was quantified spectrophotometrically following reaction with GOPOD, using standard concentrations of D-glucose. The content of damaged starch was reported on a dry basis, as a percentage of total starch.

3.7.3 Dough Functionality

3.7.3.1 Dough Rheology/Mixing Properties

The method was adapted from Mathiowetz (2018). Dough behavior during mixing was determined in duplicates for IWG and HRW flour samples using a Farinograph® – AT (C.W. Brabender, Duisburg, Germany) following AACCI method 54-21.02 (International, 2010). A 10 g mixing bowl set at 30 ± 0.2°C was used to determine the water absorption and stability time required to reach an optimum dough consistency of 500 BU (Brabender Units). About 10 g (corrected

to a default moisture content of 14%) was mixed with a variable amount of water corresponding to % water absorption, in the mixing bowl. The amount of water needed to be added for water absorption was calculated experimentally based on adjustments to reach optimal dough consistency. The dough was mixed for 20 minutes, and dough consistency was measured and plotted as a function of time to create a farinogram (**Figure 16**). The farinogram was then used to determine dough stability, arrival and departure, development time, and duration of stability. Stability arrival is the time (in min) for a sample to reach optimal consistency of 500 BU following the addition of water, which is reflective of the flour's ability to absorb water, and departure is the point at which the curve drops below 500 BU, which is when the dough is overmixed, and gluten breaks down. Dough development time is the time (in min) required for a dough to reach peak consistency starting from the time water is added. Dough development time is indicative of ideal mixing time. Dough stability is a measure of the time (in min) a sample maintains optimal consistency at 500 BU (time difference between stability arrival and departure) and shows a sample's tolerance to mechanical action by mixing.

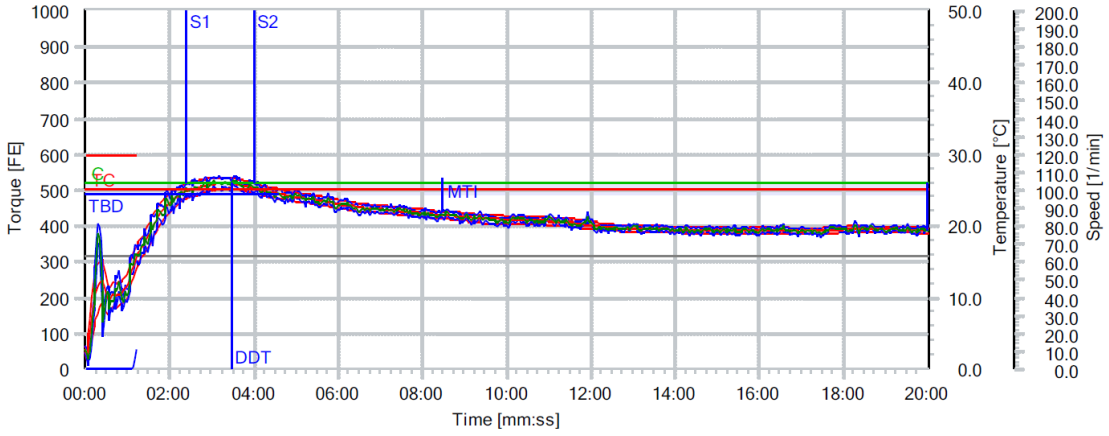


Figure 16: Example of a farinogram of whole IWG flour, used to plot dough consistency over time as an index of mixing properties of dough as measured using a farinograph. S1: stability arrival; S2: stability departure; S2-S1: dough stability time; DDT: dough development time.

3.7.3.2 Extensibility Using Kieffer Rig

Dough extensibility and resistance to extension of IWG and HRW dough samples prepared by the Farinograph, were tested using a Kieffer Dough and Gluten Extensibility Rig attached to a TA-XT2i texture analyzer (Texture Technologies Corp, Scarsdale, NY, USA) following the method outlined by Mathiowetz (2018). The analysis was conducted in duplicate with 10 sub-replicates. Dough consistency was first optimized using a Farinograph® – AT, as described in **Section 3.7.3.1** with slight modifications. Salt (0.2 g) was added to flour samples (~10 g corrected to a default moisture content of 14%), and the water absorption value necessary to achieve optimal consistency of 500 BU was obtained experimentally for each sample via the Farinograph. Upon reaching optimal consistency at 500 BU, mixing was promptly stopped, and a dough ball was formed and allowed to rest in a closed container for 20 minutes. The fresh dough was then pressed and molded into individual strips in the Kieffer mold to approximately 4 mm in width and 50 mm in length. The dough strips, about 10 strips per dough sample/replicate, were rested for an additional 40 minutes in the mold. Each strip was then placed in the Kieffer micro extension rig and stretched vertically. The dough strips were pulled at a speed of 3.3 mm/s for a max distance of 75 mm. The data was then automatically generated by the Texture Exponent 32 version 6.1.4.0 software (Texture Technologies, Corp. Scarsdale, NY, USA) to provide measurements of dough resistance to extension (mN) and extensibility (mm). The dough resistance to extension (mN) is the measure of the force needed to break the dough, measured by force generated against the hook attached to the texture analyzer, as shown in **Figure 17**. Dough resistance to extension (mN) is an indicator of dough strength. Extensibility (mm) is the distance at which the dough breaks apart, or a measure of dough deformation before it ruptures (Wang *et al.*, 2004).

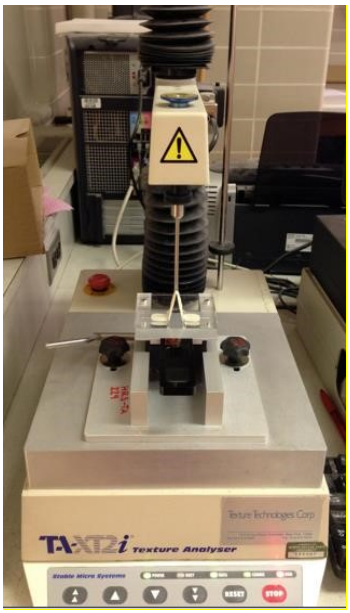


Figure 17: Dough stretched vertically using the Kieffer rig attached to a TA-Texture Analyzer.

3.7.3.3 Gluten Strength and Aggregation

Gluten strength and aggregation was measured in triplicate for IWG and HRW samples using GlutoPeak (C.W. Brabender Instruments, South Hackensack, NJ) following the procedure reported by Marti *et al.* (2015a). Approximately 8.5 g of flour was mixed with 9.5 mL of 0.5 mol/L $CaCl_2$ solution (amount corrected for default moisture content of 14%) in a sample cup that was maintained at 34°C by circulating water. A paddle was set to rotate at 1900 rpm, and the test was carried out for 7 minutes. The main indices that were automatically evaluated by the software were peak maximum time (PMT, expressed in sec), which corresponds to the time before torque falls off due to gluten break down, maximum torque (MT, expressed as Brabender Equivalents – BE), which is the torque BE at PMT, and energy to maximum torque known as the aggregation energy (expressed as arbitrary units – AU) corresponding to the area of the curve from beginning of the test to MT. An example of a GlutoPeak curve is shown in **Figure 18**.

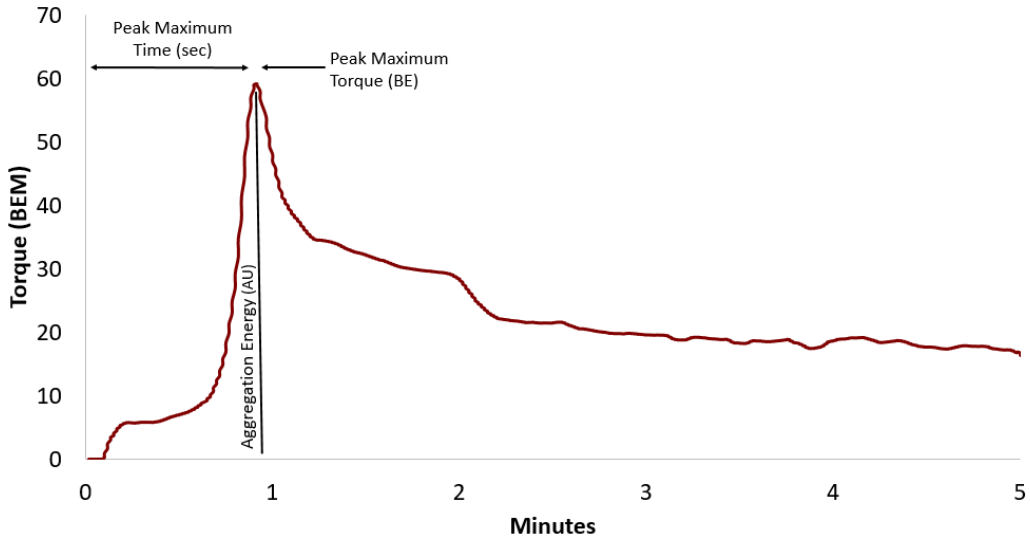


Figure 18: Example of GlutoPeak curve of whole HRW flour used to determine gluten strength. The indices of importance are peak maximum time (PMT), peak maximum torque (BE), and the area under the curve up to peak maximum time known as the aggregation energy (AU).

3.7.3.4 Pasting Properties

Starch pasting properties were measured in triplicate for IWG and HRW samples using the MicroVisco-Amylograph® (MVAG) (C.W. Brabender®, South Hackensack, NJ, USA) following the method outlined by Mathiowetz (2018). Approximately 15g of flour (corrected to 14% moisture) was mixed with 100 g of DDW in a rotating, heated bowl with a paddle operating at a speed of 250 min^{-1}. The following temperature profile was applied: 30°C for 1 min, heating from 30°C to 95°C at a rate of 7.5°C/min, holding at 95°C for 5 min, cooling at a rate of -7.5°C/min, and holding at 30°C for 1 min. The following indices were collected: pasting temperature (the temperature at which the initial starch granules being to swell and thicken), peak viscosity (the maximum viscosity reached during heating), breakdown value (extent of the decrease in viscosity during the holding period), hold viscosity (lowest viscosity after peak viscosity and before final viscosity), final

viscosity, and the setback value (extent of the increase in viscosity during cooling) (Marti et al., 2015). Example starch pasting profile can be seen in **Figure 19**.

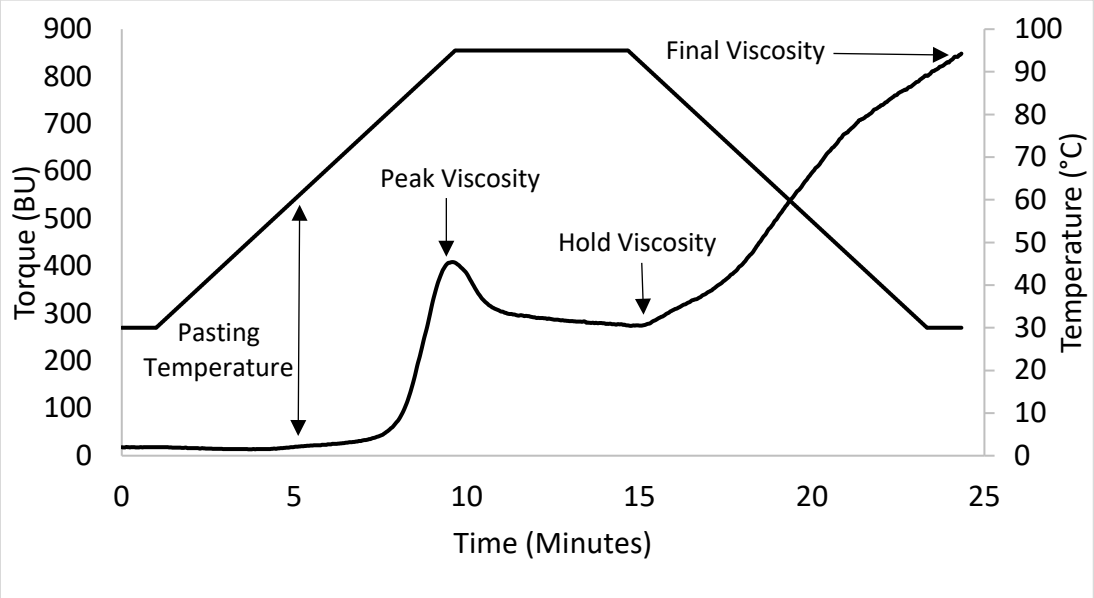


Figure 19: Example starch pasting profile of partially refined IWG flour obtained using a Micro-Visco-Amylograph® (MVAG).

3.8 Statistical Analysis

Analysis of variance (ANOVA) was done using SPSS Software version 25 (IBM Corporation, Armonk, NY) to determine differences among samples at a single time point or within a sample type across all time points of storage. Differences among the means were determined using Tukey-Kramer Honest Significant Difference (HSD) test ($P < 0.05$). Two-way ANOVA was carried out to asses for interaction effects among treatment variables, including storage time, thermal treatment, bran content, and relative humidity based on various dependent variables. ANOVA summary tables can be found in **Appendix F, Tables 76-89**. Independent two-sample t-test was performed to analyze differences between steamed and not-steamed samples. Pearson's product-moment correlation coefficients ($P < 0.05$) were calculated using residuals with R Version 1.1.463

(Rstudio Inc, Boston, MA) to test for linear relationships between dependent variables. Correlation coefficients depicting relationships among dough rheology, gluten extensibility and resistance, pasting properties, and gluten strength can be found in **Appendix G, Figure 25** and **Table 90**.

3.9 Results and Discussion

3.9.1 Profile of Gluten Forming Proteins in IWG vs. HRW

Gluten is a matrix comprised of polymeric glutenin and monomeric gliadin. Glutenin is responsible for the elasticity and strength, whereas gliadin is responsible for the viscosity and extensibility in wheat doughs (Žilić, 2013). When hydrated and mixed, these two proteins interact with each other to form a coherent protein matrix with visco-elastic properties capable of trapping gas (Dhaka and Khatkar, 2015). Glutenin can further be categorized into high molecular weight glutenin (HMWG) and low molecular weight glutenin (LMWG), where HMWG can readily form large polymers that are important for creating a strong gluten network (Žilić, 2013) contributing to larger bread loaf volume (Ohm *et al.*, 2010). Annual wheat is the gold standard for use in leavened baked goods due to its balanced content of glutenin and gliadin proteins. Thus, profiling the gluten-forming proteins of IWG is important to understand its functional properties in comparison to wheat.

With continual breeding of IWG, the protein profile may change with each breeding cycle. IWG from 2016 and 2017 are comparable to previous lines of IWG (2004 and 2015) in protein profile (Rahardjo *et al*., 2018). In comparison to wheat, IWG samples were deficient in HMWG, contained some LMWG, and were rich in α-, β-, and γ- gliadins (**Figure 20**). The difference in the protein profile of IWG compared to wheat suggests that IWG will have weak gas holding capacity and elasticity, leading to less than desirable dough characteristics due to a deficiency of HMWG proteins (>60 kDa). Furthermore, although IWG is higher in protein

content (16.6-18% vs. 11.8-12.6% in HRW), the considerable differences in the protein profile confirm that the content of protein does not verify the presence of functional proteins necessary for dough development.

Zhang *et al.* (2014 & 2015) investigated the HMWG proteins of 60 IWG genotypes and their effects on mixing characteristics. They isolated five HMWG genes from several varieties of IWG that shared similar structures to that of wheat and known to confer gluten strength. Of those isolated, IWG had one unique HMWG with an additional cysteine residue, which is important in the formation of disulfide bonds and affects the size of protein polymers (Shewry *et al.*, 2002). Although these HMWG genes were found in IWG, they were smaller in molecular weight (≤ 60 kDa) than those in wheat.

The functionality of IWG was studied by Rahardjo *et al.* (2018), who found that IWG had relatively poor dough stability, which can be attributed to its deficiency in HMWG. Furthermore, IWG was unable to form a strong gluten network with both elastic and extensible qualities, likely due to the imbalance between glutenin and gliadin proteins. Investigation of the effects of protein found and their molecular interactions in possible applications is necessary for understanding what markets IWG is best suited for. As a relative to common wheat, knowing the protein profile of IWG can give additional insight on its potential impact on functionality and how it can change over storage.

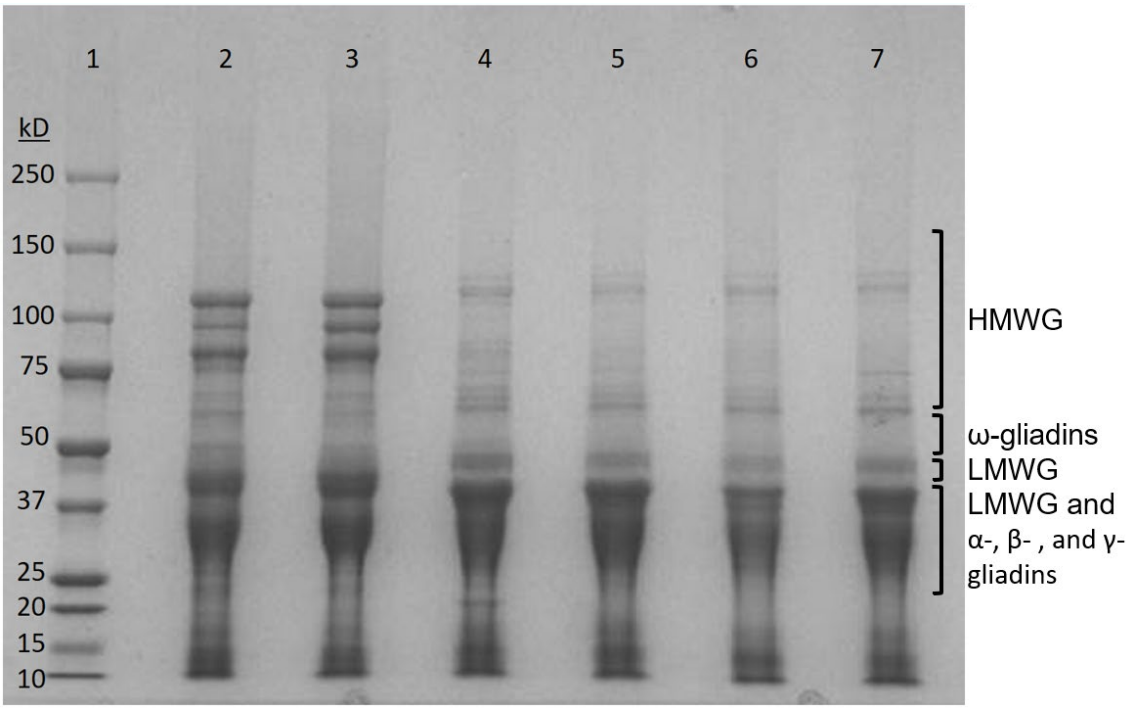


Figure 20: SDS-PAGE gel with coomassie blue staining, visualizing the protein profile of hard red wheat (HRW), and intermediate wheatgrass (IWG) samples under reducing conditions. Lane 1: molecular weight marker; Lane 2: HRW 2015; Lane 3: HRW 2017; Lane 4: IWG 2004; Lane 5: IWG 2015; Lane 6: IWG 2016; Lane 7: IWG 2017. HMWG: High molecular weight glutenins; LMGW: Low molecular weight glutenins.

3.9.2 Carbohydrate Composition of IWG and HRW Flour

Whole IWG flour from both freshly harvested and one-year-old grains had significantly higher insoluble and total dietary fiber yet lower total starch content compared to HRW flour (**Tables 10** and **11**). This observation is in agreement with the previous reports on IWG composition (Becker *et al.*, 1991; Rahardjo *et al.*, 2018; Tyl and Ismail, 2018). The differences in the observed fiber and starch in IWG compared to HRW are due to its smaller seed size that results in higher bran to endosperm ratio (**Table 22**, **Appendix B**) (Dehaan and Ismail, 2017; Rahardjo *et al.*, 2018). Dietary fiber (DF) is mainly found in the bran fraction whereas starch is located in the endosperm fraction (Lineback and Rasper, 1988), which explains

the higher amount of dietary fiber and lower amount of starch in IWG compared to HRW.

Table 10: Dietary fiber and starch composition (on dry basis) of hard red wheat and intermediate wheatgrass from freshly harvested grains at different levels of refinement.

Sample	Total Dietary Fiber†	Insoluble Fiber†	Soluble Fiber†	Total Starch†	Amylose‡	Amylopectin‡	Damaged Starch‡
Whole HRW	12.3*^{b}	10.1^{b}	2.11^{a}	62.8^{c}	21.1^{a}	78.9^{a}	7.87^{c}
Refined HRW	3.00^{d}	2.09^{d}	0.91^{b}	75.5^{a}	20.3^{a}	79.7^{a}	7.39^{c}
Whole IWG	16.9^{a}	14.3^{a}	2.53^{a}	51.2^{e}	20.3^{a}	79.7^{a}	13.5^{a}
Partial IWG	12.4^{b}	10.4^{b}	2.02^{ab}	53.1^{d}	20.7^{a}	79.3^{a}	13.4^{a}
Refined IWG	4.33^{c}	3.37^{c}	0.96^{b}	68.7^{b}	20.7^{a}	79.3^{a}	11.0^{b}

† Total dietary fiber, insoluble fiber, soluble fiber, and total starch are on a g/100 g flour dry basis
‡ Amylose, amylopectin, and damaged starch are on a g/100 g starch dry basis
*Lowercase superscripts represent significant differences (P ≤0.05) between samples according to the Tukey's HSD means comparison test.
^ Whole = 100% bran; Partial = 75% bran; Refine = 0% bran

Table 11: Dietary fiber and starch composition (on dry basis) of hard red wheat and intermediate wheatgrass from one-year-old grains at different levels of refinement.

Sample	Total Dietary Fiber†	Insoluble Fiber†	Soluble Fiber†	Total Starch†	Amylose‡	Amylopectin‡	Damaged Starch‡
Whole HRW	12.7*^{c}	11.1^{c}	1.62^{b}	56.3^{a}	22.0^{a}	77.9^{c}	8.84^{b}
Whole IWG	18.2^{a}	15.7^{a}	2.42^{a}	46.0^{b}	21.4^{b}	78.6^{b}	9.21^{b}
Partial IWG	15.3^{b}	13.1^{b}	2.22^{ab}	55.9^{a}	20.6^{c}	79.4^{a}	9.81^{a}

† Total dietary fiber, insoluble fiber, soluble fiber, and total starch are on a g/100 g flour dry basis
‡ Amylose, amylopectin, and damaged starch are on a g/100 g starch dry basis
*Lowercase superscripts represent significant differences (P ≤0.05) between samples according to the Tukey's HSD means comparison test.
^ Whole = 100% bran; Partial = 75% bran; Refine = 0% bran

High dietary fiber content can be of nutritional significance (Slavin *et al.*, 2001); however, it may negatively affect IWG dough functionality due to competition with gluten for water, which was demonstrated by Banjade *et al.*

(2019). Whole flours had higher insoluble, soluble, and total dietary fiber compared to partially refined and refined samples. Refinement of the flours resulted in a significant reduction in total and insoluble fiber content. Soluble dietary fiber (SDF) contributes to dough viscosity upon water absorption. HRW flour samples had a higher proportion of SDF compared to IWG flour samples (17% vs. 15% in whole samples, respectively), which may contribute to higher dough viscosity (Daou and Zhang, 2014). Insoluble Dietary Fiber (IDF) was the main component of DF in the samples, which may affect water absorption capacity and gluten network formation. Wang, Rosell, and de Barber (2002) found that wheat flour samples with higher IDF have higher water absorption capacity compared to samples with higher SDF. With higher IDF and total DF in IWG samples, a higher water absorption may be observed.

Starch, as a major component in cereals and flours, plays a significant role in the quality of baked products. In bread, starch can absorb up to 50% of its dry weight during mixing (Goesaert *et al.*, 2005; Calvin, 2016). During baking, starch molecules swell and gelatinize then re-associate upon cooling building viscosity. The total starch content in HRW was higher than IWG flour from both fresh and one-year-old grains (**Tables 10** and **11**). With refinement, total starch content significantly increased due to the removal of bran. With higher starch content, higher viscosity results are expected for HRW and refined samples.

The amylose component of starch is associated with bread firmness as it retrogrades during baking, while the amylopectin component impacts bread firmness as it retrogrades over storage (Singh *et al.*, 2003b; Alcázar-Alay and Meireles, 2015). The amylose to amylopectin ratio between HRW and IWG was similar in flour from freshly harvested grains (**Tables 10** and **11**). In flour from one-year-old grains, refinement of IWG caused a significant reduction of amylose and significant increase in amylopectin. Although statistically different in one-year-old grain, the ratios in IWG and HRW samples were similar. With similar amylose to amylopectin ratio among the samples, there should be minimal differences in

bread firmness or staling rates; however, the starch structure of IWG in comparison to HRW was not analyzed, and may have a significant impact on bread firmness and/or staling rate.

Starch damage was higher in IWG samples in both flours from freshly harvest and one-year-old grains compared to HRW samples (**Tables 10** and **11**). These results are different from those reported by Rahardjo *et al.* (2018), where HRW samples had higher damaged starch content. The amount of starch damaged is directly related to kernel hardness (Mok and Dick, 1991; Bass, 1998). Differences in starch damage between the two studies, however, were mostly attributed to the type of milling, ball milling by Rahardjo et al. (2018) vs. milling using Brabender Quadrumat Junior mill in this study. Higher damaged starch content may impact functionality and baking quality. With a higher damaged starch content, water absorption increases, thus less water will be available for the gluten network, which will result in lower bread specific volume (Goesaert *et al.*, 2005; Barrera *et al.*, 2007).

3.9.3 Changes in Dough Rheology of IWG and HRW Flour over Storage

3.9.3.1 Dough Rheology as Affected by Bran Content of Stored Flour from Freshly Harvested Grains

Example farinograms showing the difference between IWG and HRW, changes over storage, and the effects of refinement can be seen in **Figure 21 A**, **B**, and **C**. IWG samples had significantly higher water absorption values than HRW samples at 0, 6, and 9 months of storage, which is contrary to the results found by (Mathiowetz, 2018) and Rahardjo *et al.* (2018) (**Table 12**). The difference is likely due to variations in the characteristics of grains from different harvests and breeding cycles. Grains were all harvested at different years and from different locations. IWG samples from this study are also from a newer and improved breeding line that could have better functional properties. On the other hand, IWG

had a higher IDF and total DF fiber content than wheat, which could explain the higher water absorption values (**Tables 10** and **11**). However, refined IWG contains a significantly lower amount of DF in comparison to whole IWG but had similar water absorption to whole IWG. Thus, the higher water absorption observed in IWG samples could be attributed to the type of fibers that are present. Arabinoxylans (AX) are commonly found in cereals and have a significant role in dough development (Koehler and Wieser, 2013). AXs are capable of absorbing 15-20 times their weight in water. Further analysis of the content and types of AXs found in IWG would be needed to assert this association. Higher water absorption is desirable, as it has been shown to correlate with dough stability, which is a measure of dough strength, and higher bread loaf volume (Aydoğan *et al.*, 2015). An initial drop in water absorption at 3 months of storage, followed by a significant increase was observed in the flours as storage progressed. At the end of storage water absorption values were similar to those at the beginning of storage, except for partially refined IWG flour, which saw a significant increase. Aged flour typically has improved water absorption and dough functionality due to changes in the flour components, such as oxidation of protein, resulting in higher water absorption (Wang and Flores, 1999), but this was not the case for most of the samples. Refinement did not have any clear trends for water absorption.

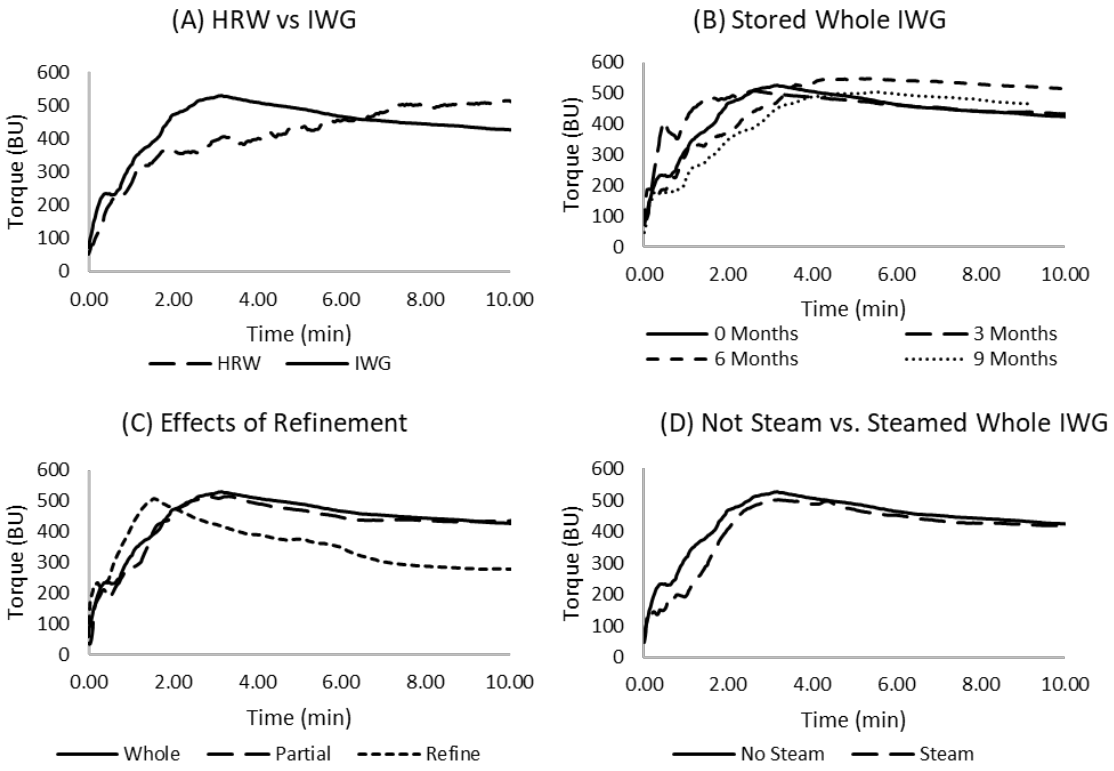


Figure 21: Example farinograms showing differences between (A) HRW and IWG, (B) changes over storage, (C) effects of refinement, and (D) effects of steam treatment.

Table 12. Corrected water absorption, dough development time, and dough stability of IWG and HRW flour from freshly harvested grains at different refinement levels (whole, partially refined, and refined), and thermal treatment as measured by Farinograph over storage at 43% and 65% relative humidity.

43% Relative Humidity

Sample			Corrected water absorption for default moisture content 14% (%)				Dough Development Time (min)				Dough Stability (min)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	60.7^{Aa}	57.9^{Ab}	60.4^{Aab}	58.5^{Aab}	3.99^{b}	4.74^{Ab}	5.55^{Ab}	20.73^{Aa}	3.15^{b}	4.79^{b}	10.81^{Aa}	5.57^{Aab}
	Refined	Not Steamed	52.8^{B}	50.9^{B}	52.8^{B}	51.9^{B}	3.25	2.12^{B}	1.42^{B}	1.29^{B}	4.51^{ab}	3.03^{ab}	5.25^{Ba}	2.14^{Bb}
IWG	Whole	Not Steamed	63.4^{Ba}	57.1^{b}	63.6^{a}	$61.8^{Ba^{\wedge}}$	3.98^{Aab}	2.83^{Ab}	$4.96^{Aa^{\wedge}}$	5.23^{Aa}	$1.99^{ab^{\wedge}}$	1.54^{Ab}	3.58^{Aa}	2.73^{Aab}
	Partial	Not Steamed	63.1^{Bb}	$57.7^{c^{\wedge}}$	$64.0^{ab^{\wedge}}$	64.6^{Aa}	3.23^{Aab}	$2.68^{Ab^{\wedge}}$	$3.36^{Bab^{\wedge}}$	3.62^{Ba}	$1.33^{\wedge}$	1.38^{A}	1.53^{B}	$1.74^{AB^{\wedge}}$
	Refined	Not Steamed	$64.3^{Aa^{\wedge}}$	57.0^{b}	$62.0^{a^{\wedge}}$	$61.6^{Ba^{\wedge}}$	1.46^{B}	1.19^{B}	1.53^{C}	1.12^{C}	0.93	0.37^{B}	$0.73^{B^{\wedge}}$	$0.80^{B^{\wedge}}$
	Whole	Steamed	63.3^{a}	53.3^{c}	62.0^{Aa}	58.4^{Bb}	3.02^{Ac}	3.92^{Abc}	7.11^{Aa}	5.07^{ABb}	1.30	2.44	3.57^{A}	2.42^{B}
	Partial	Steamed	63.4^{a}	56.7^{c}	59.7^{Ab}	63.1^{Aa}	2.69^{Ab}	3.17^{Ab}	4.61^{Bb}	8.25^{Aa}	1.13^{b}	1.48^{b}	2.03^{ABb}	4.72^{Aa}
	Refined	Steamed	59.9	56.6	54.5^{B}	54.9^{C}	1.09^{B}	1.23^{B}	1.03^{C}	1.15^{C}	0.84	0.74	0.42^{B}	0.43^{C}

65% Relative Humidity

Sample			Corrected water absorption for default moisture content 14% (%)				Dough Development Time (min)				Dough Stability (min)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	60.7^{Aa}	54.9^{BCb}	56.2^{Bb}	56.2^{Cb}	3.99^{c}	17.43^{Aa}	17.46^{Aa}	12.05^{Ab}	3.15^{b}	13.74^{Aa}	10.18^{Aa}	7.68^{Aab}
	Refined	Not Steamed	52.8^{Ba}	49.3^{Db}	52.2^{Cab}	52.5^{Dab}	3.25	1.18^{B}	1.14^{B}	1.34^{B}	4.51^{a}	2.13^{Bb}	2.15^{Bb}	1.48^{Cb}
IWG	Whole	Not Steamed	63.4^{Ba}	57.6^{ABb}	$61.8^{a^{\wedge}}$	59.2^{Bb}	3.98^{Ab}	$5.04^{Aab^{\wedge}}$	6.33^{Aa}	$6.10^{Aa^{\wedge}}$	$1.99^{\wedge}$	$3.14^{A^{\wedge}}$	1.76	2.50^{A}
	Partial	Not Steamed	63.1^{Ba}	$59.3^{Ac^{\wedge}}$	$60.9^{bc^{\wedge}}$	$61.8^{Aab^{\wedge}}$	3.23^{Ac}	4.15^{b}	4.84^{Ba}	4.65^{Bab}	$1.33^{\wedge}$	2.50^{A}	1.94	$2.33^{AB^{\wedge}}$
	Refined	Not Steamed	$64.3^{Aa^{\wedge}}$	52.4^{Bd}	$60.5^{b^{\wedge}}$	$58.8^{Bc^{\wedge}}$	1.46^{Ba}	0.75^{Cb}	1.15^{Cab}	1.19^{Ca}	0.93	$0.40^{B^{\wedge}}$	0.73	$0.52^{B^{\wedge}}$
	Whole	Steamed	63.3^{a}	56.5^{Ac}	59.3^{Ab}	57.3^{Ac}	3.02^{Ac}	3.98^{Ab}	7.49^{Aa}	7.67^{Aa}	1.30^{b}	2.08^{Aab}	3.53^{Aa}	4.25^{Aa}
	Partial	Steamed	63.4^{a}	52.8^{ABc}	58.4^{Ab}	57.2^{Ab}	2.69^{Ac}	4.43^{Ab}	5.82^{Bab}	6.24^{Ba}	1.13^{b}	2.20^{Aab}	2.56^{ABab}	3.20^{ABa}
	Refined	Steamed	59.9^{a}	52.5^{Bb}	53.5^{Bb}	52.1^{Bb}	1.09^{Ba}	1.15^{Ba}	1.17^{Ca}	1.18^{Ca}	0.84	0.54^{B}	0.56^{B}	0.83^{C}

*Upper case letters indicates significant differences across samples per thermal treatment and grain type within a single time point and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 2. Absence of letters indicate no significant differences.

^Denotes significant difference ($P \leq 0.05$) between not steamed samples and their respective steamed samples according to independent-samples t-test.

Dough development time (DDT) is a measure of mixing time needed to achieve a stable protein network. A higher DDT is associated with greater dough strength (Cenkowski *et al.*, 2000). Over storage, whole HRW flour samples had significantly longer DDT compared to IWG samples at 3, 6, and 9 months (**Table 12**). These results are similar to those found by Mathiowetz (2018) and were expected as IWG is deficient in HMWG (**Figure 20**). Whole and partially refined IWG samples had significantly higher DDT than refined IWG samples. Whole grain IWG flours required longer mixing time due to the interactions that occur between fibers and gluten, which prevents the hydration of proteins and affects the aggregation and disaggregation of HMW proteins (Rosell *et al.*, 2006; Boita *et al.*, 2016). Partially refined IWG samples had shorter DDT than whole IWG flour, due to less interference from the fiber with gluten formation; however, it was not significantly different until 6 and 9 months of storage. DDT increased over storage, with samples stored at 65% RH having significantly longer DDT than those stored at 43% (**Table 12** and **Table 77**, **Appendix F**). With higher water activity, the aging effect of the flours was more significant most likely due to accelerated chemical changes, such as increased oxidation of thiols that contribute to a stronger protein network resulting in longer DDT (Karel, 1980; Wang and Flores, 1999).

HRW samples, whole and refined, had significantly higher dough stability time than IWG samples (**Table 12**). This is consistent with the results of Marti *et al*. (2015), Rahardjo *et al.* (2018), and Mathiowetz (2018). Dough stability time correlates with the strength of the dough (Aydoğan *et al.*, 2015). The low dough stability of IWG samples is attributed to the deficiency of HMWG protein (**Figure 20**). Whole IWG had significantly longer stability than refined IWG, which had the shortest. The stability of IWG, as impacted by bran content, was similar to the results reported by Wang *et al. (*2002) and Sanz Penella *et al.* (2008). Due to the deficiency of HMW proteins that would otherwise contribute to stronger dough stability, the stability of the whole and partially refined IWG is likely due to fiber. The fiber, mostly AXs, can build viscosity and create a dough that shows good

tolerance for mixing and strength. Partially refined IWG samples had longer stability time at 3, 6, and 9 months of storage at 65% RH. Whole IWG samples saw an increase in stability at 3 months of storage that was significantly higher at 65% RH than 43%; however, with storage, stability was lower than that of 43% RH (**Table 12** and **Table 78, Appendix F**). As mentioned before, 65% RH likely contributed to higher oxidation of proteins leading to better dough functionality for short term storage; however, with prolonged storage, too much oxidation of thiols can lead to aggregation resulting in decreased functionality (Tipples, 1995).

HRW flour samples, whole and refined, had significantly higher resistance to extension and extensibility than IWG (**Table 13**). This is consistent with the results of Rahardjo *et al.* (2018) and Mathiowetz (2018). Wheat has a balance of both gluten forming proteins, glutenins and gliadins, which contribute to elasticity and extensibility, respectively, and produces an overall stable, high-quality dough (Žilić, 2013). HMW glutenins, in particular, contribute to a strong gluten network through the formation of strong protein-protein interactions (Ohm *et al.*, 2010). As a result of the deficiency of HMW glutenins, IWG has significantly less resistance to extension and extensibility than HRW. At the beginning of storage, whole and partially refined IWG samples had significantly better resistance to extension than refined IWG samples, which is likely caused by having a thicker dough due to higher fiber content. However, with storage, refined IWG samples had significant increases in resistance to extension that was statistically comparable to whole and partially refined samples. All samples saw a significant improvement in resistance to extension at 3 months of storage, followed by a significant decrease at 6 months for most samples. Differences between HRW and IWG samples, over storage, could also be due to the higher presence of accessible thiols in IWG (Becker *et al.*, 1991; Marti *et al.*, 2015b). As thiols are oxidized over storage, they form disulfide bridges between glutenin and gliadin proteins that further stabilize the gluten matrix and thus, enhances gluten strength (Tipples, 1995). Samples stored at 65% RH had, for the most part, significantly higher resistance to extension than those stored

at 43% RH. Refined IWG had significantly higher extensibility than whole and partially refined IWG samples. Bran, specifically the dietary fiber content, interferes with the hydration of gluten forming proteins and disrupts the formation of a cohesive gluten network. The fiber was likely able to contribute to resistance to extension but resulted in decreased dough extensibility in IWG. Partially refined IWG samples were comparable to whole IWG samples in extensibility over storage at both 43% and 65% RH. Although partial refinement resulted in a significant decrease in total DF content, the presence of DF can still interfere with gluten extensibility. Extensibility had a significant negative correlation with DDT (r = -0.79, **Figure 25** and **Table 90,** both in **Appendix G**), although both saw increases over storage. The negative correlation is likely related due to the effects of fiber, which caused an increase in DDT and a decrease in extensibility in whole and partially refined IWG.

3.9.3.2 Impact of Steam Treatment on Dough Rheology of Stored Flour from Freshly Harvested Grains

The effects of steam treatment on dough rheology are illustrated in **Figure 21 D** and **Table 12**. Steam treatment resulted in lower water absorption values with varying significance, which is contrary to what Mathiowetz (2018) found, likely due to the difference in the steam treatment method. Prakash and Rao (1999) saw decreased water absorption in steamed wheat flour due to the denaturation of gluten protein. DDT of flour from steamed grains was higher, on average, than not-steamed samples in stored whole and partially refined IWG samples. Mathiowetz (2018) saw that steamed treated IWG samples had higher DDT times, which corroborates with the results found by Caprez *et al. (*1986) and Prakash and Rao (1999). Caprez et al. (1986) found that wheat bran exposed to heat treatment resulted in prolonged DDT. Steam treatment resulted in higher stability times for whole IWG samples stored at 65%. Otherwise, steam treatment did not have a significant impact on dough stability in IWG samples. This result is consistent with

those found by Mathiowetz (2018). The direct steam treatment was short enough to denature problematic enzymes and not completely denature proteins and cause loss of dough stability.

With storage, steaming generally resulted in significantly increased resistance to extension in whole and partially refined IWG samples stored at 43% RH and in whole, partially refined, and refined IWG samples at stored 65% RH (**Table 13**). Mathiowetz (2018) did not see any significant differences between steamed and not steamed IWG samples in resistance to extension or extensibility. Prakash and Rao (1999) measured significant increases to resistance to extension in steam-treated wheat flour. However, Prakash and Rao (1999) saw a considerable decrease in the extensibility due to steaming, which was the opposite trend seen in steamed IWG in this study. Prakash and Rao (1999) related the loss in extensibility to denaturation of gluten forming proteins with heat. Thermal treatments of isolated gluten (Cuq *et al.*, 2000) have confirmed the results seen by Prakash and Rao (1999). Cuq and Boutrot (2000) linked the resistance to extension increases upon heating due to the “rigidification” of the protein network via covalent cross-linkage formation. They argued that the same phenomenon is responsible for a decrease in extensibility. The differences seen in this study and by Mathiowetz (2018), Prakash and Rao (1999), and Cuq and Boutrot (1999) are likely due to different steam treatment conditions having varying effects on gluten-forming proteins. Mathiowetz (2018) used a proofing oven at 100°C at 95% RH for 60 minutes on groats, Prakash and Rao (1999) treated their wheat samples using an autoclave at atmospheric pressure for 5, 15, and 30 minutes, while Cuq and Boutrot (2002) treated isolated gluten using a heating press under 20 MPa for 10 minutes at various temperatures (80-150°). The steam treatment used in the present study was likely short enough to impart positive improvements and not completely denature the gluten forming proteins.

Table 13. Resistance to extension and extensibility of IWG and HRW flour from freshly harvested grains at different refinement levels (whole, partially refinement, refine) and thermal treatment over storage at 43% and 65% relative humidity.

Sample			43% Relative Humidity							
			Resistance to Extension (g)				Extensibility (mm)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	17.9^{Ac}	34.3^{b}	33.6^{Bb}	42.0^{Aa}	49.8^{Bb}	59.0^{Ba}	59.5^{Ba}	19.5^{Bc}
	Refined	Not Steamed	15.9^{Bc}	32.7^{b}	41.6^{Aa}	39.9^{Ba}	69.7^{Ab}	74.8^{Aa}	72.5^{Aab}	73.1^{Aa}
IWG	Whole	Not Steamed	$12.1^{Ad\wedge}$	$28.7^{Aa\wedge}$	16.3^{c}	$20.2^{Ab\wedge}$	$7.8^{Bd\wedge}$	12.1^{Ba}	10.0^{Cc}	10.9^{Bb}
	Partial	Not Steamed	$8.3^{Bd\wedge}$	24.4^{Ba}	$17.2^{b\wedge}$	14.4^{Cc}	5.1^{Bd}	13.2^{Ba}	$10.8^{Bb\wedge}$	10.1^{Cc}
	Refined	Not Steamed	$7.1^{Cd\wedge}$	14.2^{Cc}	15.9^{b}	$18.0^{Ba\wedge}$	18.2^{Aa}	$18.3^{Aa\wedge}$	17.0^{Aab}	15.4^{Ab}
	Whole	Steamed	12.0^{Ad}	31.1^{Aa}	24.1^{Ac}	25.4^{Ab}	8.0^{Bd}	13.0^{Ba}	11.8^{Cc}	12.4^{Bb}
	Partial	Steamed	12.2^{Ac}	26.0^{Ba}	24.4^{Ab}	12.9^{Cc}	8.8^{Bc}	13.2^{Ba}	13.0^{Ba}	9.7^{Cb}
	Refined	Steamed	6.9^{Bc}	16.9^{Cb}	19.0^{Ba}	20.1^{Ba}	22.7^{Aa}	20.8^{Aab}	19.2^{Cbc}	18.2^{Ac}
Sample			65% Relative Humidity							
			Resistance to Extension (g)				Extensibility (mm)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	17.9^{Ad}	50.4^{Ab}	44.2^{Ac}	60.6^{Aa}	49.8^{Ba}	19.2^{Bb}	19.5^{Bb}	19.9^{Bb}
	Refined	Not Steamed	15.9^{Bc}	37.8^{Bb}	36.1^{Bb}	46.4^{Ba}	69.7^{Aa}	70.1^{Aa}	70.6^{Aa}	50.4^{Ab}
IWG	Whole	Not Steamed	12.1^{Ac}	$31.0^{Aa\wedge}$	$18.1^{Bb\wedge}$	$30.0^{Aa\wedge}$	7.8^{Bc}	$11.7^{Ba\wedge}$	$9.5^{Cb\wedge}$	$12.2^{Ba\wedge}$
	Partial	Not Steamed	$8.3^{Bd\wedge}$	$21.6^{Ba\wedge}$	$19.1^{Ac\wedge}$	$20.4^{Bb\wedge}$	$5.1^{Bc\wedge}$	$10.8^{Cab\wedge}$	$10.4^{Bb\wedge}$	$11.1^{Ca\wedge}$
	Refined	Not Steamed	7.1^{Cc}	$17.4^{Cab\wedge}$	$16.5^{Cb\wedge}$	$18.3^{Ca\wedge}$	$18.2^{Aa\wedge}$	$15.3^{Ab\wedge}$	$15.7^{Ab\wedge}$	15.3^{Ab}
	Whole	Steamed	12.0^{Ad}	44.3^{Aa}	22.5^{Ac}	27.5^{Ab}	8.0^{Bc}	12.5^{Ca}	10.7^{Cb}	11.1^{Bb}
	Partial	Steamed	12.2^{Ad}	29.0^{Ba}	21.7^{Ac}	23.8^{Bb}	8.8^{Bc}	13.0^{Ba}	11.4^{Bb}	11.6^{Bb}
	Refined	Steamed	6.9^{Bc}	26.9^{Ca}	12.2^{Bb}	27.1^{Aa}	22.7^{Aa}	16.4^{Ab}	12.3^{Ac}	14.8^{Ab}

*Upper case letters indicates significant differences across samples per thermal treatment and grain type within a single time point and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 24. Absence of letters indicate no significant differences.
^Denotes significant difference ($P \leq 0.05$) between not steamed samples and their respective steamed samples according to independent-samples t-test.

3.9.3.3 Changes in Dough Rheology of Stored Flour from One-Year-Old Grains

Flour from one-year-old grains had similar water absorption to that from freshly harvested grains (**Table 14**). An increase in water absorption was observed over storage and was significant at 6 months of storage for not steamed whole and partially refined IWG, with no initial drop that was observed in flour from freshly harvested grains. Flour from one-year-old grains had significantly lower DDT and dough stability at 0 months of storage compared to flour from freshly harvested grains. However, with storage, DDT and dough stability were higher than those of freshly harvested grain samples at 3 months and were similar at 6 months of storage, likely due to the effects of aging.

There were no significant differences in DDT between whole and partially refined IWG samples, but the values significantly increased from 0 to 3 months of storage for both IWG samples. Although not statistically significant, whole IWG samples had longer stability time than the partially refined sample. Because IWG is deficient in HMW gluten forming proteins needed to form a stable dough, its stability is primarily due to its high fiber content. The trends in DDT and dough stability were similar to the results of samples from freshly harvested grain samples.

Steam treatment resulted in significant increases in water absorption for whole IWG at all stages of storage, and at 0 and 3 months of storage for partially refined IWG, an observation that was contrary to the results from freshly harvested samples, but similar to the results found by Mathiowetz (2018). Freshly harvested grains did not go through any aging that could have improved the kernels prior to milling; thus the kernels from freshly harvested grains could have been more prone to the effects of the steam treatment. Steam treatment in one-year-old grains had similar trends seen in freshly harvested grains for DDT and stability. Steam treatment significantly increased DDT in both whole and partially refined IWG

samples. Steam treated IWG samples had higher stability times than not-steamed IWG samples; however, it was mostly not significantly different. Partial denaturation of gluten forming proteins can lead to the exposure of hydrophobic regions, which allows for the rearrangement of disulfide bonds. As a result, gluten aggregates can form and produce a stronger dough (Hu *et al.*, 2017). The steam treatment used in this study was gentle enough to not completely denature the proteins, resulting in improved dough stability instead of negatively affecting it.

One-year-old grain samples had similar trends to those of the freshly harvested grains in resistance to extension and extensibility over storage (**Table 14**). Resistance to extension and extensibility significantly increased from 0 to 3 months of storage for all samples, and significantly increased from 3 to 6 months for some of the samples. HRW had the highest resistance to extension and extensibility compared to IWG samples. Whole and partially refined IWG samples had similar resistance to extension over storage. Partially refined IWG had lower extensibility than whole samples, which is different from the results found in samples from freshly harvested grains. With less bran, there is less interference with gluten and should result in increased extensibility, as seen in the results from freshly harvested samples. However, this trend was not observed for samples from one-year-old grains, which could be due to changes in fiber content due to increased seed size from breeding. Freshly harvested grains had a larger seed size and lower fiber content than one-year-old grains (**Figure 23, Appendix A** and **Tables 10** and **11**). Both batches of IWG samples were from different breeding cycles, where one-year-old grains originated from 2006 to 2008 cycle and freshly harvested grains from 2010 to 2015 cycle. Steam treatment increased resistance to extension, similar to previous findings, but did not have an effect on extensibility in both whole and partially refined IWG samples. This observation indicates that steam treatment was strong enough to affect the protein network leading to increased resistance in extention from protein rigidification, discussed in the previous section, but gentle enough not to affect the extensibility.

Table 14. Corrected water absorption, dough development time, and dough stability, as measured by Farinograph, and resistance to extension and extensibility, as measured by Kieffer, of IWG and HRW flour from one-year-old grains at different refinement levels (whole and partially refined) and thermal treatment over storage at 43% relative humidity.

Sample		Farinograph								
		Water Absorption Corrected for Default Moisture Content)14%)			Dough Development Time (min)			Dough Stability (min)		
		0 Months	3 Months	6 Months	0 Months	3 Months	6 Months	0 Months	3 Months	6 Months
IWG Whole	Not Steamed	61.2^{Ab*}	62.2^{Aab}	62.7^{Ca}	1.43^{Cb}	4.48^{Ca}	4.95^{Ca}	0.63^{Bb}	2.68^{BCab}	3.64^{Ba}
	Steamed	62.6^{Aa}	62.1^{ABa}	64.4^{Ba}	4.67^{ABb}	9.36^{ABa}	7.69^{Ba}	1.97^{ABb}	3.36^{Bab}	5.42^{Ba}
IWG Partial	Not Steamed	57.3^{Bb}	56.7^{Cb}	62.7^{Ca}	1.62^{Cb}	3.25^{Cab}	4.84^{Ca}	0.77^{Bb}	1.65^{Cb}	3.05^{Ba}
	Steamed	63.3^{Aa}	60.7^{ABc}	62.1^{Db}	4.22^{Bb}	8.30^{Ba}	5.82^{Cab}	2.24^{ABb}	3.98^{Ba}	4.22^{Ba}
HRW Whole	Not Steamed	60.4^{ABb}	60.3^{Bb}	67.3^{Aa}	6.62^{Ac}	12.99^{Ab}	18.68^{Aa}	4.29^{Ab}	9.30^{Aa}	9.38^{Aa}

Sample		Kieffer					
		Resistance to Extension (g)			Extensibility (mm)		
		0 Months	3 Months	6 Months	0 Months	3 Months	6 Months
IWG Whole	Not Steamed	10.2^{Cc}	11.6^{Db}	12.4^{Ca}	9.03^{Bb}	12.24^{Ba}	12.78^{Ca}
	Steamed	13.4^{Bc}	18.1^{Ba}	14.9^{Bb}	8.4^{BCb}	12.6^{Ba}	11.6^{Ca}
IWG Partial	Not Steamed	10.7^{Cb}	11.9^{Da}	10.0^{Db}	7.1^{CDc}	8.0^{Cb}	12.1^{BCa}
	Steamed	10.3^{Cc}	13.4^{Cb}	15.2^{Ba}	6.4^{Dc}	8.3^{Cb}	12.5^{BCa}
HRW Whole	Not Steamed	32.5^{Ab}	49.0^{Aa}	26.6^{Ac}	34.9^{Aa}	22.5^{Ac}	25.2^{Ab}

*Upper case letters indicates significant differences across samples within a single time point and lowercase letters indicates significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), Farinograph n = 3, Kieffer n = 20.

3.9.4 Changes in Gluten Strength of IWG and HRW Flour over Storage

3.9.4.1 Gluten Strength as Affected by Bran Content of Stored Flour from Freshly Harvested Grains

HRW samples had significantly higher gluten strength than IWG samples with higher peak maximum time (PMT), peak maximum torque (MT), and gluten aggregation energy (**Figure 22 A** and **Table 15**). These results are similar to those found by Marti et al. (2015). The lower gluten strength and aggregation is likely due to the deficiency HMW glutenin and a smaller glutenin-to-gliadin ratio in IWG. PMT is indicative of the time required for gluten to aggregate and exhibit maximum torque on the mixing blade before gluten breaks down. PMT is directly related to

the glutenin-to-gliadin ratio (Melnyk *et al.*, 2012). Refined HRW samples had higher PMT compared to whole HRW flour, which is likely due to less interference from the bran. There were no significant differences in PMT among whole, partially refined, and refined IWG flour over storage at both RH (**Figure 22 C** and **Table 15**). PMT did not change from 0 to 6 months of storage; however, it was significantly higher at 9 months of storage (**Figure 22 B**). This increase is likely due to the oxidation of thiols over the storage of the gluten forming proteins (Tipples, 1995; Wieser, 2007). The disulfide bridges formed between cysteine residues of gliadin and glutenin proteins lead to polymerization, thus increasing gluten strength (Ariyama and Khan, 1990; Wrigley and Bekes, 1999). PMT had a significant positive correlation with DDT (r = 0.35), where higher values of both indicate stronger gluten development (**Figure 25** and **Table 87, Appendix G**).

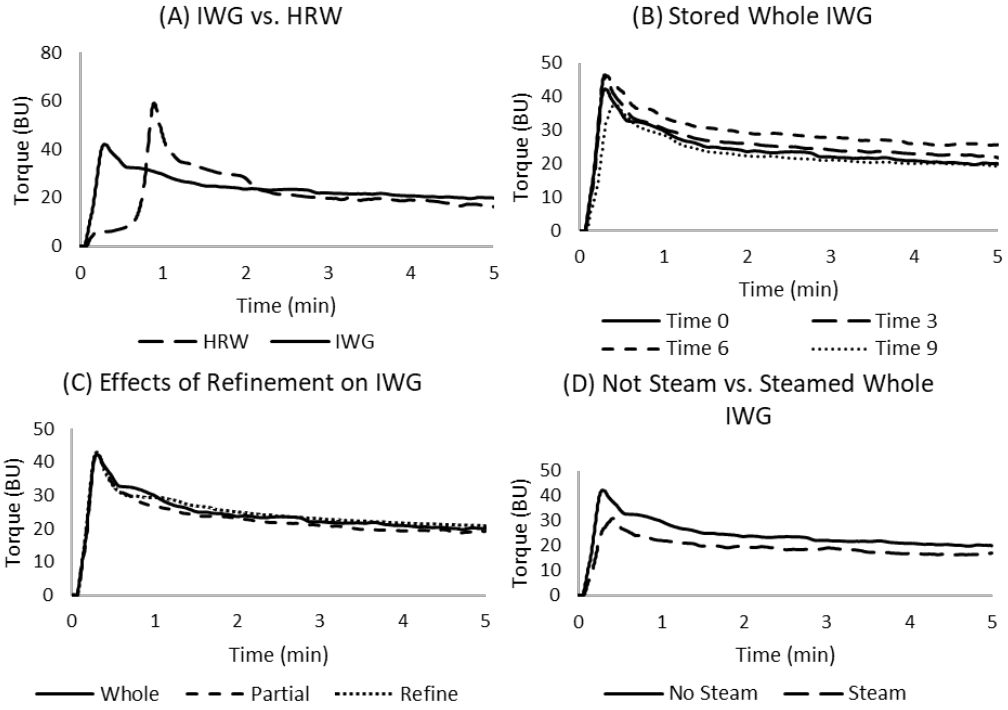


Figure 22: Example Glutopeak results showing differences between (A) HRW and IWG, (B) changes over storage, (C) effects of refinement, and (D) effects of steam treatment.

MT is the torque at the moment of the gluten network breakdown due to continuous mechanical stress, which is mainly determined by glutenin content (Melnyk *et al.*, 2012). There were no statistical differences in MT for refined or whole HRW. MT of IWG samples was significantly lower than that of HRW samples, mostly attributed to the deficiency of HMW glutenins (**Figure 20**). For the most part, partially refined and refined IWG samples had higher MT than whole IWG flour, likely due to less interference from the bran; however, this trend was not consistently significant over storage at 43% RH. Similar MT trends were seen for samples stored at 65% RH. Previous research by Goldstein *et al.* (2010) and Marti *et al.* (2014) found the addition of bran fraction resulted in the weakening of the gluten network.

The area under the curve to MT represents the total time and energy required, known as the gluten aggregation energy, and is expressed in arbitrary units. HRW samples had higher aggregation energy than IWG samples, with no significant differences observed between whole or refined HRW (**Table 15**). There were no clear differences with refinement on aggregation energy. Whole IWG samples stored at 43% had a significant increase in aggregation energy over storage up to 6 months, followed by a significant decrease after 9 months of storage. This trend, though not statistically significant, can be seen among the other IWG samples. The increase in aggregation energy over storage is likely due to the previously mentioned changes in protein-protein interaction in the gluten proteins (Kozlova and Nekrasov, 1956). Understanding the changes in the gluten strength for IWG is very important in determining the baking quality and other end-use.

3.9.4.2 Impact of Steam Treatment on Gluten Strength of Stored Flour from Freshly Harvested Grains

Steam treatment had varied effects on gluten strength, with similar storage trends discussed in the previous section (**Table 15**). Refined IWG at all stages of

storage at 43% RH and 0 and 9 months of storage at 65% RH had higher PMT than not-steamed refined samples. The effects of storage for gluten strength was mostly not significant for PMT, MT, and aggregation energy, however values over storage increased. In general, steam treatment increased PMT, likely due to the rearrangement of disulfide bonds through partial denaturation of the protein causing a longer time for the protein to aggregate. On the other hand, steam treatment resulted in decreased MT and aggregation energy for most of the IWG samples stored at both RH, which was significant only for whole IWG, indicating that thermal treatment weakened the gluten network (**Figure 22 D**). Steam treatment likely resulted in exposure of thiol groups leading to further protein aggregation. With less thiols available for the gluten forming proteins to aggregate, the time it took for the gluten to develop increased while MT decreased. The decrease is likely attributed to changes in the protein. Previous studies have shown that thermally treated grains resulted in changes in the protein profile. Sun *et al.* (2006) reported significant decreases in monomeric proteins and soluble glutenins, and significant increases in insoluble glutenin and residue insoluble proteins in wheat subjected to Micronization at 100°C. It was concluded that the changes in the protein profile are related to the denaturation of heat-sensitive monomeric proteins with subsequent exposure of hydrophobic groups, leading to protein aggregation, the formation of disulfide bonds, and protein insolubility. Disruption of the proteins that comprise the gluten network in IWG, which is already lacking in gluten strength compared to HRW, can lead to undesirable repercussions on quality. Since effects of steam treatment on partially refined IWG flour was not significant, the steam treatment used in this study can potentially be used to enhance the storage stability of IWG without significant negative effects on quality and end-use applications.

3.9.4.3 Changes in Gluten Strength of Stored Flour from One-Year-Old Grains

Flour from one-year-old grains had similar trends to those found in flour from freshly harvested grains (**Table 16**). HRW had significantly higher PMT, MT, and aggregation energy than IWG samples. There were no significant changes in PMT over storage for IWG samples, with the exception of partially refined IWG flour from steamed grains. Although not significant, average PMT did increase over storage, similar to the results from freshly harvested grains. MT values at the beginning of and at 3 months of storage were not significantly different between whole and partially refined IWG. However, at 6 months of storage partially refined IWG flour had significantly higher PMT than the whole flour. Aggregation energy did not have significant changes in whole IWG flour samples, but significant changes were noted in partially refined IWG from 0 to 3 months of storage. Aggregation energy did increase, on average, over storage for all samples.

Although the trends are similar to those of the flour from freshly harvested grains, MT and aggregation energy were lower. Stored grains typically have improved functionality strength, including gluten strength, however, this was not the case in this study. The difference between freshly harvested grains and one-year-old grains is likely due to breeding efforts for improved traits, such as seed size (**Table 23, Appendix B**) and protein quality, leading to the differences observed in the gluten strength values. Both batches of IWG were from two different breeding cycles as previously mentioned in **Section 3.9.3.3**.

Table 15. Peak maximum time, peak maximum torque, and aggregation energy of IWG and HRW flour from freshly harvested grain at different refinement levels (whole, partial refinement, refine) and thermal treatment over storage at 43% and 65% relative humidity.

Sample			43% Relative Humidity											
			Peak Maximum Time (s)				Peak Maximum Torque (BE)				Aggregation Energy (AU)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	55^{Bb}	50^{Bb}	49^{Bb}	85^{Ba}	59	62	65^{a}	54	1169	1198	1257	1190
	Refined	Not Steamed	73^{Ab}	63^{Ab}	64^{Ab}	104^{Aa}	52^{a}	56^{a}	58^{a}	40^{b}	1109	1171	1171	1069
IWG	Whole	Not Steamed	19^{b}	19^{b}	20^{b}	27^{a}	40^{Bab}	$47^{a^}$	$46^{ABab^}$	34^{b}	903^{Aab}	$979^{ABab^}$	$1035^{a^}$	815^{b}
	Partial	Not Steamed	18^{ab}	$17^{b^}$	21^{ab}	$22^{a^}$	43^{AB}	50	42^{B}	49	887^{B}	$1014^{A^}$	919	$1046^{^}$
	Refined	Not Steamed	$18^{b^}$	$20^{b^}$	$18^{b^}$	$24^{a^}$	44^{Aab}	36^{b}	$51^{Aa^}$	39^{ab}	957^{B}	735^{B}	1009	836
	Whole	Steamed	23	21^{B}	23	25	32	39^{A}	37^{B}	40	887	845^{A}	812	896^{A}
	Partial	Steamed	21^{b}	20^{Bb}	22^{b}	28^{a}	38^{ab}	43^{Aab}	44^{Aa}	36^{b}	807	886^{A}	667	791^{AB}
	Refined	Steamed	24^{c}	41^{Aa}	23^{c}	29^{b}	36^{a}	22^{Bb}	40^{Ba}	34^{a}	751^{ab}	544^{Bb}	831^{a}	710^{Bab}
Sample			65% Relative Humidity											
			Peak Maximum Time (s)				Peak Maximum Torque (BE)				Aggregation Energy (AU)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	55^{Bb}	57^{Bb}	59^{b}	94^{a}	59	63	65	60	1169^{b}	1255^{ab}	1321^{a}	1295^{ab}
	Refined	Not Steamed	73^{Ab}	68^{Ab}	67^{b}	92^{a}	52^{b}	59^{ab}	62^{a}	54^{ab}	1109^{b}	1250^{a}	1293^{a}	1276^{a}
IWG	Whole	Not Steamed	19^{b}	19^{b}	21^{ab}	28^{a}	$40^{B^}$	$45^{^}$	44^{B}	43^{B}	$903^{A^}$	$995^{^}$	$1048^{^}$	1068
	Partial	Not Steamed	18^{b}	$19^{b^}$	19^{b}	24^{a}	43^{AB}	48	$52^{A^}$	52^{A}	887^{Bb}	1075^{ab}	$1105^{ab^}$	$1243^{a^}$
	Refined	Not Steamed	$18^{b^}$	19^{b}	19^{b}	$25^{a^}$	44^{A}	45	52^{A}	$48^{AB^}$	874^{B}	1003	1075	$1063^{^}$
	Whole	Steamed	23	22	22	24^{B}	32^{b}	39^{Aa}	42^{Aa}	42^{a}	727^{c}	852^{Ab}	910^{ab}	944^{a}
	Partial	Steamed	21^{b}	22^{b}	23^{b}	30^{ABa}	38	39^{A}	26^{B}	40	807	852^{A}	867	968
	Refined	Steamed	24	29	24	33^{A}	36	26^{B}	42^{A}	41	751	591^{B}	865	916

*Upper case letters indicates significant differences across samples per thermal treatment and grain type within a single time point and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 2. Absence of letters indicates no significant differences.
^Denotes significant difference ($P \leq 0.05$) between not steamed samples and their respective steamed samples according to independent-samples t-test.

Table 16. Peak maximum time, peak maximum torque, and aggregation energy of IWG and HRW flour from one-year-old grains at different refinement levels (whole and partial refinement) and thermal treatment over storage at 43% relative humidity.

Sample		Peak Maximum Time			Peak Maximum Torque			Aggregation Energy		
		0 Months	3 Months	6 Months	0 Months	3 Months	6 Months	0 Months	3 Months	6 Months
IWG Whole	No Steam	21.3^{B}	23.0^{B}	23.7^{BC}	30.0^{B}	28.0^{B}	28.7^{C}	696.8^{B}	677.9^{B}	717.3^{BC}
	Steam	23.3^{B}	23.7^{B}	26.5^{B}	26.7^{B}	27.3^{B}	26.7^{C}	657.4^{B}	661.0^{B}	689.8^{C}
IWG Partial	No Steam	20.3^{B}	21.0^{B}	22.0^{C}	30.7^{B}	31.3^{B}	32.3^{B}	691.5^{Bb}	747.6^{Ba}	776.3^{Ba}
	Steam	22.0^{Bb}	22.7^{Bab}	$24.7B^{Ca}$	28.7^{B}	28.7^{B}	29.7^{BC}	665.8^{B}	686.1^{B}	737.2^{BC}
HRW Whole	No Steam	50.3^{A}	46.7^{A}	50.7^{A}	58.3^{A}	60.0^{A}	60.3^{A}	1295^{A}	1340^{A}	1347^{A}

* Upper case letters indicate significant differences across samples within a single time point and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 3. The absence of letters indicates no significant differences.

3.9.5 Changes in Starch Pasting Properties of IWG and HRW Flour over Storage

3.9.5.1 Starch Pasting Properties as Affected by Bran Content of Stored Flour from Freshly Harvested Grains

Prior to storage, HRW flour samples had significantly higher values of several measured indices compared to IWG (**Figure 23 A** and **Tables 17** and **18**). IWG has similar amylose to amylopectin ratio to HRW; however, the starch content of IWG is overall lower, contributing to lower pasting viscosities. Furthermore, the higher protein and fiber content in IWG can interfere with starch gelatinization and swelling, thus lowering the viscosity of a starch slurry (Collar *et al.*, 2006; Marti *et al.*, 2015b). In fact, Lim *et al.* (1999) found that a higher protein content has a negative correlation with peak viscosity. Protein polypeptides can form disulfide bonds in and around starch granules and prevent swelling and disruption of the starch granules, lowering viscosity values (Juliano, 1965).

The viscosity values for IWG flour in this study are lower than those reported by Rahardjo *et al.* (2018) and Mathiowetz (2018), which could be attributed to the freshness of the grain upon milling. Grains are typically stored prior to usage for improved functionality. Over the storage of grains, lipid hydrolysis by lipase result in the production of mono- and di-acylglycerols and free fatty acids (FFA), which interact with amylose to form insoluble amylose-lipid (AM-L) complexes upon heating (Kugimiya *et al.*, 1980). These AM-L complexes aggregate in an aqueous system and build an inter-granular network that results in increased pasting values (Conde-Petit and Escher, 1992; Putseys *et al.*, 2010). Freshly harvested grains lack the benefits of the AM-L complexes that are formed over storage. Rahardjo *et al.* (2018) and Mathiowetz (2018) worked with stored IWG groats that had higher pasting values.

Samples stored at 65% RH exhibited higher viscosity values than those stored at 43% RH, which is likely due to an accelerated storage effect caused by an increase in water activity. Increased water activity has been shown to increase lipase enzyme activity, which catalyzes the hydrolytic rancidity of acylglycerols to form FFAs (Wehtje and Adlercreutz, 1997). With increased lipase activity and development of FFAs and smaller acylglycerols, the formation of AM-L complexes resulted in higher viscosities seen in flour stored at 65% RH.

Pasting temperature indicates the minimum temperature necessary to produce an increase in viscosity of the starch slurry; thus, it can be used to identify processing conditions for various applications. Starch pasting temperatures from this study are aligned with the results reported by Marti *et al*. (2015), where IWG samples had higher pasting temperatures than HRW (**Table 17**). Marti *et al*. (2015) determined that the differences in pasting temperature between IWG and HRW were likely related to starch characteristics and not due to the addition of bran. However, refined IWG flour samples from this study had significantly lower in pasting temperatures than whole IWG and the stored partially refined flour suggesting that bran content may influence pasting temperature (**Table 17**). Refined HRW flour also had lower pasting temperatures than whole HRW flour by ~2°C but was not significant until the end of storage. The lower pasting temperatures found in the refined flour sample was likely due to less competition for water between the fiber and starch granules during swelling. Pasting temperatures of IWG samples were far lower than those reported (79.6°C) by Rahardjo *et al.* (2018). This discrepancy is likely due to the use of different instrumentation, as Rahardjo *et al.* (2018) employed a rapid visco analyzer (RVA) in place of an MVAG, which was used in the present study and by Marti et al. (2015).

A high peak viscosity is an indicator of good water holding capacity and granule swelling ability, which both directly impact the ease of processing and

baking (Sahlstrùm *et al.*, 2003). HRW samples had significantly higher peak viscosity than IWG samples (**Table 17**), similar to previous observations by Marti et al. (2015), Rahardjo *et al.* (2018), and Mathiowetz (2018). Refined IWG flour had higher peak viscosity than whole and partially refined samples. The peak viscosity of refined IWG flour was significantly higher than that of whole IWG flour at 3 months of storage and that of whole and partially refined flour at 6 months of storage at both RH. At the end of storage, refined IWG stored at 43% RH had a lower peak viscosity and was lower than both whole and partially refined IWG. However, refined IWG flour samples stored at 65% RH were significantly higher in peak viscosity. This decrease in peak viscosity is likely caused by starch degradation. Starch degradation over storage has been associated with changes in the amylose to amylopectin ratio, shortened amylopectin average chain lengths, and shift in chain-length distribution to short branch chains caused by amylolytic enzymes (Patindol *et al.*, 2005). Alternatively, the drop in viscosity could be caused by strong protein-protein interactions around starch molecules leading to lower swelling and gelatinization, thus lower viscosity values (Zhou *et al.*, 2003). Further evaluation of the starch structure would be needed to fully understand changes over storage.

IWG samples had significantly lower hold viscosity and breakdown values compared to HRW, which is similar to the observations by Marti et al. (2015), Rahardjo *et al.* (2018), and Mathiowetz (2018) (**Tables 17** and **18**). The breakdown value is an indicator of the rigidity of the swollen starch granules and the degree of susceptibility of the starch granules to disintegrate (Ma and Baik, 2018). Marti et al. (2015) demonstrated that the difference between IWG and HRW is independent of variations in starch concentration and attributed the lower breakdown value of IWG to its high protein and fiber content, which compete with starch for water. Refinement of IWG resulted in higher hold viscosity and breakdown values, which were significant in stored IWG samples, similar to the trend mentioned previously. The trends seen in hold viscosity and breakdown

value suggests that bran refinement may be beneficial for HRW and IWG in terms of baking ability due to increased water holding capacity and granule swelling ability that would contribute to a more cohesive dough and larger bread volume (Sahlstrùm *et al.*, 2003; Alvarez-Jubete *et al.*, 2010)

HRW samples had significantly higher final viscosity and setback values than IWG samples (**Tables 17** and **18**). Final viscosity, also known as “cold paste viscosity,” is the stabilized viscosity of the starch paste upon cooling. The setback is the difference between the final viscosity and hold viscosity (minimum viscosity) and represents the extent of an increase in viscosity upon cooling. Together, final viscosity and setback value indicate the susceptibility to retrogradation and syneresis. Retrogradation is when the leached amylose molecules re-associate to form double helices and amylopectin recrystallizes, which together manifest as staling and hardening of the final product (Singh *et al.*, 2003b; Alcázar-Alay and Meireles, 2015). Retrogradation can be undesirable as it causes staling, loss of crispness in baked goods, changes in flavor and aroma, and reduces starch digestibility (Morris, 1990). However, retrogradation can be desirable in products such as pasta as it increases hardness and reduces stickiness (Farhat, 2004). Amylose re-associates faster than amylopectin and therefore has a more substantial influence on retrogradation (Singh and Anderson, 2004). Syneresis is the process in which a starch gel contracts and exudes unbound water (Wang *et al.*, 2015). This process causes inconsistent textures in end products as well as contributes to the staling of baked goods. Lower final viscosity and setback values indicate strong interactions between the dispersed amylose and amylopectin and thus a lowered tendency for retrogradation and syneresis (Ji *et al.*, 2010; Wang *et al.*, 2015). Therefore, the lower final viscosity and setback values of IWG compared to HRW can be beneficial for the textural stability over storage of products made with IWG. The refinement of HRW and IWG resulted in higher final viscosity and setback values. Refinement of IWG samples had higher final viscosity and setback values that increased over storage at both 43% and 65% RH. Significant

differences due to refinement were mainly observed after 3 to 6 months of storage, however by end of storage refined IWG samples at 43% had a significant decrease compared to whole and partially refined samples.

Overall, refinement had a positive impact on starch characteristics. A higher bran proportion decreases the total starch content, which was seen in the total starch composition of the samples (**Tables 10** and **11**). The lower starch content, therefore, resulted in lower starch pasting values that were measured. Partially refined IWG had a positive effect on starch pasting properties compared to whole IWG, indicating that partial refinement can be used to improve starch functionality of IWG, while maintaining the beneficial properties of the bran.

Table 17. Pasting temperature, peak viscosity, hold viscosity, and final viscosity of IWG and HRW flour from freshly harvested grains at different refinement levels (whole, partial refinement, refine) and thermal treatment over storage at 43% and 65% relative humidity.

Sample			43% Relative Humidity															
			Pasting Temperature (°C)				Peak Viscosity (cP)				Hold Viscosity (cP)				Final Viscosity (cP)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	62.1	62.2	62.0	62.1^{A}	979.0^{Bb}	1042.7^{Ba}	1016.7^{Bab}	1050^{Ba}	619.7^{Bb}	669.7^{B}	671.0^{B}	694.0^{B}	1407.7^{Bb}	1442.0^{Bab}	1452.3^{Bab}	1507.3^{Ba}
	Refined	Not Steamed	60.5	60.2	60.8	60.0^{B}	1140.0^{A}	1128.3^{A}	1166.3^{A}	1163.3^{A}	693.3^{Ac}	716.7^{Ab}	740.7^{Aa}	755.0^{Aa}	1640.0^{Ac}	1678.7^{Abc}	1737.0^{Ab}	1834.7^{Aa}
IWG	Whole	Not Steamed	$63.9^{A\wedge}$	67.2^{A}	63.1^{A}	63.7^{A}	$109.3^{a\wedge}$	116.3^{B}	118.7^{B}	125.3	$39.3^{a\wedge}$	39.0	47.0^{B}	48.3^{A}	$100.67^{\wedge}$	121.0	126.0^{B}	134.0^{A}
	Partial	Not Steamed	$63.9^{AB\wedge}$	62.4^{AB}	63.2^{A}	63.6^{A}	$120.7^{\wedge}$	$137.0^{AB\wedge}$	123.7^{B}	139.3	$41.7^{a\wedge}$	$50.3^{\wedge}$	47.0^{B}	48.0^{A}	$107.0^{b\wedge}$	$140.0^{a\wedge}$	131.7^{Bab}	147.67^{Aa}
	Refined	Not Steamed	58.3^{B}	57.7^{B}	57.9^{B}	58.0^{B}	141.0^{ab}	160.5^{Aab}	164.3^{Aa}	$121.7^{b\wedge}$	45.0^{b}	$52.0^{ab\wedge}$	56.3^{Aa}	$29.0^{Bc\wedge}$	116.5^{b}	$145.5^{a\wedge}$	161.7^{Aa}	$84.7^{Bc\wedge}$
	Whole	Steamed	60.9	65.5^{A}	63.7^{A}	63.9^{A}	85.7^{b}	100.7^{Bab}	107.3^{Bab}	121.0^{Ba}	28.0^{b}	33.3^{Bab}	44.3^{ab}	49.7^{a}	68.3^{Bc}	100.7^{Bb}	119.3^{ab}	140.3^{ab}
	Partial	Steamed	59.4^{c}	61.1^{ABbc}	62.2^{Bab}	63.6^{Aa}	91.0^{c}	106.3^{Bbc}	114.0^{Bab}	129.0^{ABa}	28.0^{c}	39.0^{Bb}	43.0^{ab}	49.5^{a}	74.3^{ABc}	103.0^{Bb}	116.3^{ab}	140.0^{a}
	Refined	Steamed	60.0	57.1^{B}	58.0^{C}	58.4^{B}	107.0^{b}	165.3^{Aa}	145.7^{Aab}	156.0^{Aab}	30.3^{b}	65.7^{Aa}	50.7^{ab}	42.7^{ab}	106.3^{Ab}	199^{Aa}	144.3^{ab}	128.0^{b}

Sample			65% Relative Humidity															
			Pasting Temperature (°C)				Peak Viscosity (cP)				Hold Viscosity (cP)				Final Viscosity (cP)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	Not Steamed	62.1	63.1	61.2	61.8	979.0^{Bb}	1041.3^{Bav}	1058.0^{Ba}	1062.0^{Ba}	619.7^{Bb}	673.7^{Bb}	696.7^{Bb}	750.3^{Ba}	1407.7^{Bb}	1430.3^{Bab}	1438.3^{Bab}	1477.7^{Ba}
	Refine	Not Steamed	60.5	61.6	60.0	61.0	1140.0^{A}	1135.3^{Ab}	1165.3^{Ab}	1216.0^{Aa}	693.3^{Ac}	723.0^{Ac}	769.0^{Ab}	791.3^{Aa}	1640.0^{Ac}	1737.0^{Abc}	1790.3^{Aa}	1745.3^{Aab}
IWG	Whole	Not Steamed	$63.9^{A\wedge}$	68.9^{A}	64.9^{A}	$63.8^{A\wedge}$	$109.3^{\wedge}$	120.0^{Bb}	118.0^{Bb}	153.3^{Ba}	$39.3^{a\wedge}$	44.0^{b}	47.3^{Bb}	72.3^{Ba}	$100.67^{\wedge}$	147.7^{Bb}	150.3^{Bb}	227.0^{Ba}
	Partial	Not Steamed	$63.9^{AB\wedge}$	64.5^{A}	$63.8^{A\wedge}$	63.6^{A}	$120.7^{\wedge}$	133.0^{Bb}	133.0^{ABb}	171.0^{Ba}	$41.7^{a\wedge}$	53.3^{b}	57.0^{ABb}	$78.7^{Ba\wedge}$	$107.0^{b\wedge}$	155.7^{ABb}	163.0^{ABb}	$242.0^{Ba\wedge}$
	Refine	Not Steamed	58.3^{B}	57.2^{B}	58.3^{C}	57.9^{B}	141.0^{ab}	182.7^{Aab}	172.3^{Ab}	237.0^{Aa}	45.0^{b}	66.0^{b}	62.7^{Ab}	100.0^{Aa}	116.5^{b}	187.0^{Ab}	181.7^{Ab}	316.5^{Aa}
	Whole	Steamed	60.9^{b}	68.4^{a}	64.8^{Aab}	65.1^{Aab}	85.7^{b}	109.3^{b}	114.7^{Bb}	145.0^{Ba}	28.0^{b}	41.3^{cb}	49.7^{b}	68.0^{Ba}	68.3^{Bc}	125.7^{b}	144.3^{b}	219.0^{Ba}
	Partial	Steamed	59.4^{b}	64.4^{a}	64.7^{Aa}	64.2^{Aa}	91.0^{c}	127.0^{b}	113.0^{Bb}	159.0^{Ba}	28.0^{c}	52.3^{b}	50.3^{b}	74.7^{Ba}	74.3^{ABc}	145.0^{b}	143.3^{b}	230.0^{Ba}
	Refine	Steamed	60.0	58.3	58.9^{B}	57.8^{B}	107.0^{b}	152.3^{ab}	170.7^{Aab}	249.5^{Aa}	30.3^{b}	54.0^{b}	59^{b}	126.5^{Aa}	106.3^{Ab}	146.0^{b}	168.7^{b}	400.5^{Aa}

*Upper case letters indicate significant differences across samples per thermal treatment and grain type within a single time point and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 3. Absence of letters indicate no significant differences.

^Denotes significant difference ($P \leq 0.05$) between not steamed samples and their respective steamed samples according to independent-samples t-test.

Table 18. Breakdown and setback values of IWG and HRW flour from freshly harvested grains at different refinement levels (whole, partial refinement, refine) and thermal treatment over storage at 43% and 65% relative humidity.

43% Relative Humidity									
Sample			Breakdown (Peak Viscosity - Hold Viscosity) (cP)				Total Setback (Final Viscosity - Hold Viscosity) (cP)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	No Steam	359.3^{Bab}	373.0^{Ba}	345.7^{Bb}	356.0^{Bab}	788.0^{B}	772.3^{B}	781.3^{B}	813.3^{B}
	Refined	No Steam	446.7^{A}	411.7^{A}	425.7^{A}	408.3^{A}	946.7^{Ab}	962.0^{Ab}	996.3^{Ab}	1079.7^{Aa}
IWG	Whole	No Steam	$70.0^{\wedge}$	$77.3^{B\wedge}$	$71.7^{B\wedge}$	77.0^{B}	$61.3^{b\wedge}$	$82.0^{a\wedge}$	79.0^{Ba}	85.7^{Ba}
	Partial	No Steam	$79.0^{\wedge}$	$86.7^{AB\wedge}$	76.7^{B}	91.3^{A}	$65.3^{b\wedge}$	$89.7^{a\wedge}$	84.7^{Ba}	99.7^{Aa}
	Refined	No Steam	89.0	95.0^{A}	108.0^{A}	$92.7^{A\wedge}$	71.0^{b}	$93.5^{a\wedge}$	105.3^{Aa}	$55.7^{Cc\wedge}$
	Whole	Steam	57.7^{b}	67.3^{Ba}	63.0^{Bab}	71.3^{Ba}	40.3^{Bc}	67.3^{Bb}	75.0^{ab}	90.7^{a}
	Partial	Steam	63.0^{b}	67.3^{Bab}	71.0^{Bab}	79.5^{Ba}	46.3^{Bc}	64.0^{Bb}	73.3^{b}	90.5^{a}
	Refined	Steam	76.7^{b}	99.7^{Aab}	95.0^{Aab}	113.3^{Aa}	76.0^{Ab}	133.3^{Aa}	93.7^{ab}	85.3^{b}
65% Relative Humidity										
Sample			Breakdown (Peak Viscosity - Hold Viscosity) (cP)				Total Setback (Final Viscosity - Hold Viscosity) (cP)			
			0 Months	3 Months	6 Months	9 Months	0 Months	3 Months	6 Months	9 Months
HRW	Whole	No Steam	359.3^{B}	367.7^{B}	361.3^{B}	311.7^{B}	788.0^{Ba}	756.7^{Bab}	741.7^{Bab}	727.3^{Bb}
	Refined	No Steam	446.7^{Aa}	412.3^{Aab}	396.3^{Ab}	424.7^{Aab}	946.7^{A}	983.0^{A}	1021.3^{A}	954.0^{A}
IWG	Whole	No Steam	$70.0^{\wedge}$	76.0^{B}	70.7^{B}	81.0^{B}	$61.3^{c\wedge}$	$103.7^{Bb\wedge}$	103.0^{b}	154.7^{Ba}
	Partial	No Steam	$79.0^{ab\wedge}$	79.7^{Bab}	$76.0^{Bb\wedge}$	92.3^{Ba}	$65.3^{c\wedge}$	$102.3^{Bb\wedge}$	$106.0^{b\wedge}$	163.3^{Ba}
	Refined	No Steam	89.0	116.7^{A}	109.7^{A}	137.0^{A}	58.0^{c}	121.0^{Ab}	119.0^{b}	$216.5^{Aa\wedge}$
	Whole	Steam	57.7^{c}	68.0^{b}	65.0^{Bab}	77.0^{Ba}	40.3^{Bc}	84.3^{b}	94.7^{b}	151.0^{Ba}
	Partial	Steam	63.0^{b}	74.7^{a}	62.7^{Bb}	84.3^{Ba}	$46.3^{B}c$	92.7^{b}	93.0^{b}	155.3^{Ba}
	Refined	Steam	76.7	98.3	111.7^{A}	123.0^{A}	76.0^{Ab}	92.0^{b}	109.7^{b}	274.0^{Aa}

*Upper case letters indicate significant differences among samples per thermal treatment and grain type within a single time point and lowercase letters indicates significant different within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 3. Absence of letters indicate no significant differences.

^Denotes significant difference ($P \leq 0.05$) between not steamed samples and their respective steamed samples according to independent-samples t-test.

3.9.5.2 Impact of Steam Treatment on Pasting Properties of Stored IWG from Freshly Harvested Grain

Starch pasting properties of steamed and not-steamed flour samples can be found in **Figure 23 D** and **Tables 17** and **18.** Steam treatment of IWG grain samples over storage did not have a major impact on pasting temperature. At the beginning of storage, steamed whole and partially refined IWG samples exhibited lower starch pasting temperatures than not-steamed counterparts; however, after storage, there were little to no significant differences between steamed and not-steamed samples. Steam treatment had no impact on pasting temperatures for refined IWG samples. Results indicated that steam treatment was gentle enough to not significantly change starch morphology over storage.

All starch pasting indices for whole and partially refined IWG samples at both RH were lower, on average, for steamed samples than not-steamed samples. The effects of steam treatment over storage were mostly not significant for whole and partially refined IWG flour sample. However, at 3 months of storage, steam treatment did have a significant negative impact on all pasting properties of partially refined IWG samples stored at 43% RH, but with continued storage, the difference became insignificant. Steamed refined IWG flour samples did not have a clear trend, where pasting values decreased at 0 and 6 months, but significantly increased at 3 and 9 months. By the end of storage at both RH, steamed refined IWG samples had higher pasting viscosities than not-steamed counterparts. An increase in peak viscosities was observed in steamed HRW by Hu *et al.* (2010) and was attributed to changes in starch morphology caused by the steam. Further investigation would be needed to understand the cause of this, such as using scanning electron microcopy to see changes in starch morphology. On the other hand, Mathiowetz (2018) saw significant effects of steam treatment on peak and hold viscosity and breakdown value of IWG samples resulting in lower viscosities. According to Rasper (1980), the decrease in starch pasting viscosities following

hydrothermal treatment of flour is likely due to the disintegration of starch granules. However, Mathiowetz (2018) did not see a significant effect on either the final viscosity or the setback value of IWG samples. The varied results between the two studies are likely due to the difference in steam treatment. Mathiowetz (2018) steamed grain samples in a proofing oven at 100°C and 95% RH for 60 minutes, whereas the steaming performed in this study was a direct treatment.

Although pasting properties were, on average, lower for most of the steam treated IWG samples, a significant difference was mainly observed around 3 months of storage and became insignificant over storage. The trends observed between steamed and not-steamed samples suggest that with direct steam treatment starch pasting properties can be mildly impacted but would plateau over extended storage. Therefore, direct steam treatment can be used to improve shelf-life while having little significant changes in starch functionality in IWG samples.

3.9.5.3 Changes in Starch Pasting Properties of Stored Flour from One-Year-Old Grain

Starch pasting properties of stored flour from one-year-old grains were similar to those reported by Marti et al. (2015) and Mathiowetz (2018) (**Tables 19** and **20**). The measured viscosities were higher than those of the flour from freshly harvested grains, which is likely due to aging of the starch granules and formation of AM-L complexes.

Pasting temperatures of whole and partially refined IWG samples from one-year-old at beginning of storage were closer to those observed by Mathiowetz (2018) and Marti *et al.* (2015). From 0 to 3 months of storage, a significant increase in pasting temperature was observed in IWG samples, unlike the observations for the flour from freshly harvested grains. This spike in pasting temperature is higher than those observed by Mathiowetz (2018) and Marti *et al. (2015),* but lower than those reported by Rahardjo *et al.* (2018). Aging of grains could result in increased

protein-protein interactions around starch granules resulting in increased pasting temperatures (Juliano, 1965). Partial refinement did not have a significant impact on pasting temperature, similar to the trend observed for the flour from freshly harvested grains. Steaming resulted in a significant increase in pasting temperature, opposite to what was observed for flour from freshly harvested grains, and contrary to the results reported by Mathiowetz (2018). Higher pasting temperatures have been observed after hydrothermal treatment in starch from multiple plant sources and are due to associations between the chains in the amorphous region of the starch granule as well as changes in the crystallinity during hydrothermal treatment, such as pre-gelatinization (Watcharatewinkul *et al.*, 2009; Bahrani *et al.*, 2012). These changes in the starch granule reinforce the intra-granular bonds and require more heat for structural disintegration and paste formation (Watcharatewinkul *et al.*, 2009; Bahrani *et al.*, 2012). Freshly harvested grains have yet to go through significant changes in starch structure caused through storage and thus may have been more resistant to the effects of steam.

Flour from one-year-old grains had higher starch pasting viscosities than those seen in flour from freshly harvested grains, likely due to the aging of the grains forming AM-L complexes. Changes, however, due to bran refinement, steam treatment, and storage followed similar trends to what was observed for flour from freshly harvested grains. HRW samples had significantly higher pasting viscosity values than IWG samples. Bran refinement resulted in significantly higher pasting values due to an increase in endosperm-to-bran ratio. Steam treatment resulted in lower pasting viscosities caused by disintegration of starch molecules, as previously discussed. Short term storage of flour from one-year-old grains resulted in improved starch properties due to changes in the starch, such as formation of AM-L complexes. Results indicated that prior storage of groats before milling can be beneficial for starch pasting viscosities and can be used in tandem with refinement and steam treatment resulting in better functionality and storage stability.

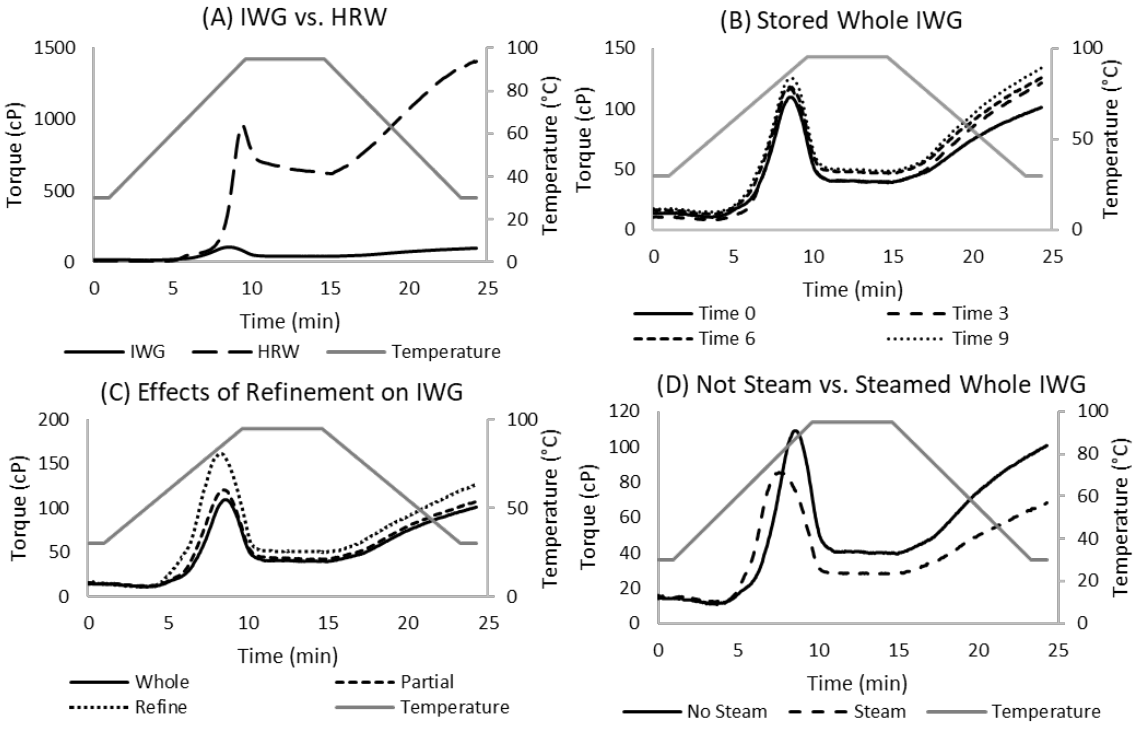


Figure 23: Example starch pasting profiles showing differences between (A) HRW and IWG, (B) changes over storage, (C) effects of refinement, and (D) effects of steam treatment.

Table 19. Pasting temperature, peak viscosity, hold viscosity, an final viscosity of IWG and HRW flour from one-year-old grains at different refinement levels (whole and partially refined) and thermal treatment over storage at 43% relative humidity.

Sample		Pasting Temperature (°C)			Peak Viscosity (cP)			Hold Viscosity (cP)			Final Viscosity (cP)		
		0 Months	3 Months	6 Months	0 Months	3 Months	6 Months	0 Months	3 Months	6 Months	0 Months	3 Months	6 Months
IWG Whole	Not Steamed	60.0^{CDb*}	69.7^{BCa}	68.8^{Ba}	267.3^{BCb}	357.5^{Ca}	365.7^{Ca}	154.7^{Cb}	214.5^{Ca}	217.0^{CDa}	465.7^{Cb}	688.0^{Ca}	654.0^{Ca}
	Steamed	61.7^{Bb}	72.5^{Aa}	72.1^{Aa}	238.7^{BCb}	337.7^{Ca}	337.3^{Da}	145.3^{Cb}	197.3^{Ca}	207.3^{Da}	431.3^{Cb}	610.0^{Ca}	606.0^{Ca}
IWG Partial	Not Steamed	59.2^{Db}	67.8^{Ba}	67.6^{Ba}	297.0^{Bb}	430.3^{Ba}	423.0^{Ba}	191.0^{Bb}	268.3^{Ba}	267.7^{Ba}	579.7^{Bb}	824.3^{Ba}	782.0^{Ba}
	Steamed	60.3^{Cc}	72.2^{ABa}	69.5^{Bb}	225.7^{Cb}	350.9^{Ca}	373.7^{Ca}	141.0^{Cb}	223.3^{Ca}	229.0^{Ca}	438.3^{Cb}	683.3^{Ca}	660.0^{Ca}
HRW Whole	Not Steamed	63.3^{Aa}	63.2^{Dab}	63.0^{Cb}	760.7^{Ac}	881.7^{Ab}	940.0^{Aa}	482.7^{Ab}	569.7^{Aa}	592.3^{Aa}	1340.0^{Aa}	1412.0^{Aa}	1363.0^{Aa}

*Upper case letters indicate significant differences across samples within a single time point and lowercase letters indicate significant differences within a single sample across time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), $n = 3$.

Table 20: Breakdown and setback values of IWG and HRW flour from one-year-old grains at different refinement levels (whole and partial refinement) and thermal treatment over storage at 43% relative humidity.

Sample		Breakdown (Peak Viscosity – Hold Viscosity) (cP)			Setback (Final Viscosity – Hold Viscosity) (cP)		
		0 Months	3 Months	6 Months	0 Months	3 Months	6 Months
IWG Whole	Not Steamed	112.7^{Bb}	143.0^{Ba}	148.7^{Ba}	198.3^{Cc}	330.5^{CBa}	288.3^{Cb}
	Steamed	93.3^{Bb}	140.3^{Ba}	130.0^{Cab}	192.7^{Cb}	272.3^{Ca}	268.7^{Ca}
IWG Partial	Not Steamed	106.0^{Bb}	162.0^{Ba}	155.3^{Ba}	282.7^{Bb}	394.0^{Ba}	359.0^{Bab}
	Steamed	84.7^{Bb}	135.7^{Ba}	144.7^{CBa}	212.7^{Cb}	324.3^{CBa}	286.3^{Ca}
HRW Whole	Not Steam	278.0^{Ab}	312.0^{Aab}	347.7^{Aa}	579.7^{Aa}	531.0^{Aa}	423.3^{Ab}

*Upper case letters indicate significant differences among samples within a single time point and lowercase letters indicates significant different among a single sample among time points according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n = 3.

3.10 Conclusions

Over storage, some improvement in IWG functionality was observed. Increases in dough development time, stability, resistance to extension, and gluten aggregation were noted over storage, which denotes improvement in dough strength. Partial refinement of IWG resulted in improvements in dough functionality due to less fiber interference with the formation of gluten. Due to the deficiency of HMW glutenin proteins in IWG, complete refinement of IWG resulted in poor dough performance. Steam treatment resulted in improved dough rheology properties, but on the other hand, saw reduced gluten strength and starch pasting properties, but was mild enough not to have significant negative effects over storage.

Improvements in starch pasting properties were also observed over the storage of IWG flour samples. The noted improvement could result in a better specific volume of baked goods as a function of improved starch gelling ability (Ma and Baik, 2018). Partial refinement of IWG had a significantly positive effect on the starch pasting properties of IWG, resulting in higher viscosities. Steam treatment had a negative impact on starch pasting properties due to changes in starch granule structure and possible disintegration; however, the impact was not significant in whole grain samples. Together, refinement can be used to improve starch pasting profiles in various applications and steam treatment can be used in tandem to improve storage stability. It is important to consider treatments that improve or do not markedly affect starch pasting properties of IWG to maximize the applications and use in products.

Overall, these results demonstrated a positive effect of storage on the functionality of IWG flour. Partial refinement of IWG can be used to help improve dough strength for end-use applications, while maintaining beneficial nutrients that are of interest to consumers. Steam treatment can be used to extend the storage stability of IWG without significant detriment to dough rheology. Although the

overall functionality of IWG remains inferior to HRW through storage, the use of different processes can be utilized to improve its end-use performance.

Chapter 4: Conclusions, Implications, and Recommendations

The use of refinement and steam treatment in the present study was effective at reducing the development of off-flavors and sensory attributes caused by lipid rancidity. Over the storage of intermediate wheatgrass (IWG) and hard red wheat (HRW) flour, the primary changes in flavor were caused by secondary lipid oxidation products from lipid rancidity. Due to higher fat content, intensities of volatile odor active compounds (VOAC) in IWG flour at the end of storage were higher than those observed in stored HRW flour, and contributed to higher intensities of floury, earthy, and Play-doh aromas. However, IWG contains a significant amount of antioxidants, such as carotenoids and hydroxycinnamic acids, which likely prolonged the induction period of the VOACs, an observation not seen in HRW flour. This observation implied competitive short term storage stability of IWG flour compared to wheat flour.

Refinement significantly reduced the fat content, while steam treatment significantly reduced lipase and lipoxygenase activity in IWG flour. These treatments resulted in significantly fewer VOACs formed from lipid catabolism, namely alkyl aldehydes, enal aldehydes, furans, and alcohols. Hexanal, a secondary metabolite of lipid oxidation and a noteworthy off-flavor in grains, significantly correlated with the development of most of the lipid oxidation VOACs. Hexanal significantly decreased in intensity with refinement and steam treatment of IWG. Sensory results showed that refinement significantly decreased the intensity of overall flavor, raw dough flavor, beany flavor, bitter aftertaste, and overall aftertaste, due in part to the removal of phenolic compounds found in the brans in IWG and HRW flour. Steam treatment resulted in decreased overall aroma, overall flavor, and bitter taste and aftertaste intensities in IWG.

While refinement and steam treatment significantly reduced the intensities of unwanted flavor compounds and sensory attributes, it is important to consider their effects on functionality over storage. Storage of IWG flour had a positive impact on functionality with improved dough development time, dough stability, resistance to extension, gluten aggregation energy, and pasting viscosities. Partial refinement of IWG resulted in improved dough functionality and gluten aggregation compared to whole IWG flours due to less fiber competing with gluten forming proteins for water, and less interference with the development of the gluten matrix. Completely refined IWG had poor dough functionality due to the deficiency in high molecular weight glutenins that would contribute to a strong gluten network, thus whole and partially refined IWG's dough stability was partially attributed to its high fiber content. Starch pasting viscosity values increased with refinement due to a higher starch to non-starch ratio. Steam treatment of IWG resulted in improved dough development time and dough stability, but slightly decreased gluten strength and starch pasting viscosities. With these results, we accept our hypothesis that steam treatment and a moderate reduction in bran content will enhance the storage stability of IWG in terms of functionality.

The present study contributed to the understanding of IWG storage stability and illustrated best processing practices for enhanced shelf-life. By establishing a more effective method of steam treatment and exploring the changes to the overall performance and quality of IWG with refinement, a complete picture of IWG's storage stability will help guide future end-use applications. With continual breeding efforts to increase seed size, the composition of IWG may continue to change and thus warrant continual storage research on IWG groats and flour. Genetic selection of IWG lines with lower lipase and lipoxygenase content is recommended, as well as lines with a more favorable protein profile for dough functionality. Additional studies should investigate the consumer acceptability of products made with IWG. The current study described the flavor profile of IWG compared to HRW, but did not investigate the overall acceptability of IWG.

Furthermore, additional studies should investigate fiber and starch components unique to IWG as well as textural changes in end products made from stored IWG flour to understand the relationship between functionality (dough rheology and pasting properties) and end-use applications. The results from this study can help elucidate changes seen in end products.

IWG has garnered recognition for its environmental benefits, and was identified as a promising perennial crop for commercial food use, owing to its relatively superior agronomic properties in comparison to other perennials as well as its excellent nutrient profile (Wagoner, 1990; Becker *et al.*, 1991). Integrating a perennial cereal grain into the food market is a novel idea that will require collaboration among agronomists, breeders, farmers, and food scientists. Breeders have so far been successful in increasing seed size and yield, reducing seed shattering, and shortening plant height to prevent lodging (Glover *et al.*, 2010; Zhang *et al.*, 2016). Food scientists have made progress towards characterizing IWG and evaluating ways to improve IWG for end-use applications as a stand-alone grain or in combination with other cereals (Marti *et al.*, 2015b; Rahardjo *et al.*, 2018; Tyl and Ismail, 2018; Banjade *et al.*, 2019). This study provided foundational information on how to improve the storage stability of IWG through refinement and steam treatment. The present study illustrated the potential challenges with flavor development and how to maximize IWG's storage stability for end-use applications. Data gathered will further boost the marketability of IWG, thus providing an incentive for farmers to grow the crop.

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Appendix A: Steam Treatment Optimization

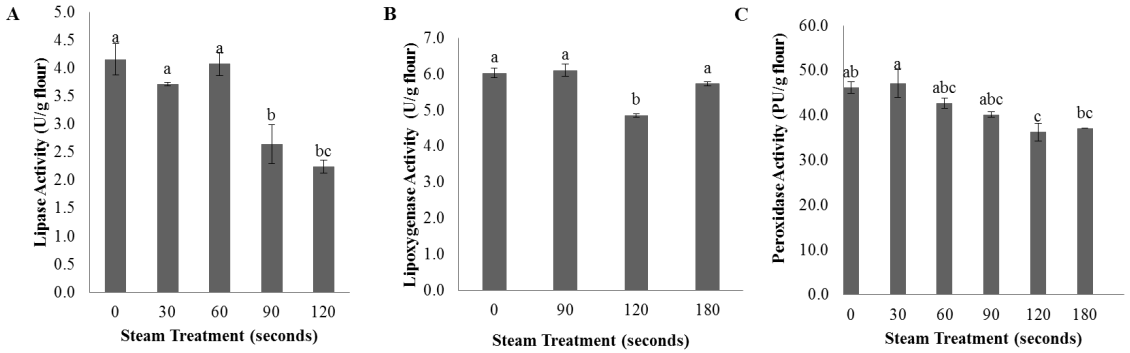

Figure 24. Enzymatic activity (A) lipase activity, (B) lipoxygenase activity, (C) peroxidase activity) of IWG flours from one-year-old grains with varying steam times. Lowercase superscripts represent significant differences ($P \leq 0.05$) according to Tukey's HSD means comparison test and error bars represent standard error (n=3).

Table 21: Antioxidant content in IWG grains subjected to various steaming times.

Steam Treatment (seconds)	Hydroxycinnamic Acids (ug/g Flour)			Carotenoids (mg/100g Flour)	
	p-Coumaric Acid	Ferulic Acid	Sinapic Acid	Lutein	Zeaxanthin
0	39.0^{a*}	658.6^{ab}	37.6^{ab}	3.48^{b}	0.761^{ab}
30	34.9^{a}	762.7^{a}	43.5^{a}	3.65^{a}	0.812^{a}
60	34.8^{a}	523.7^{bc}	30.5^{b}	3.54^{ab}	0.775^{ab}
90	41.3^{a}	679.2^{ab}	$38.7a^{b}$	3.41^{ab}	0.740^{b}
120	35.8^{a}	622.2^{abc}	37.8^{ab}	3.45^{ab}	0.764^{ab}

*Lowercase superscripts represent significant differences ($P \leq 0.05$) according to Tukey's HSD means comparison test.

Appendix B: Endosperm to Bran Ratio and Seed Size

Table 22: Endosperm to Bran ratio of freshly harvested IWG.

Rep	Endosperm (g)	Bran (g)	Total (g)	Loss (g)	Endosperm + Loss (g)	Endosperm Ratio (#/100g)	Bran Ratio (#/100g)
1	49.9	45.0	94.9	5.1	55.0	55.0	45.0
2	49.9	45.6	95.5	4.4	54.4	54.4	45.6
3	45.0	45.0	90.0	10	55.0	55.0	45.0
4	54.9	43.1	98.0	2	57.0	56.9	43.1
					Average	55.3	44.7
					SD	1.11	1.11
					CV	2.01	2.49

Table 23: Seed size of one-year-old and freshly harvested HRW and IWG.

Sample	Weight (g/100 seeds)	Weight (g/1000 seeds)	Average	SD	CV
	3.266	32.66			
HRW 2016	3.455	34.55	33.98	1.14	3.36
	3.471	34.71			
	2.747	27.47			
HRW 2017	2.759	27.59	27.38	0.28	1.03
	2.706	27.06			
	0.5349	5.349			
IWG 2016	0.5309	5.309	5.38	0.10	1.80
	0.5493	5.493			
	0.7455	7.455			
IWG 2017	0.7095	7.095	7.34	0.21	2.89
	0.7469	7.469			

Appendix C: Kovats Retention Index

Table 24. The retention time of alkane standards for the calculation of Kovats Index.

Alkane	# of Carbons	Retention Time (Min)
Hexane	6	2.000
Heptane	7	2.724
Octane	8	3.640
Nonane	9	5.124
Decane	10	7.032
Undecane	11	9.063
Dodecane	12	11.103
Tridecane	13	13.068
Tetradecane	14	14.941
Pentadecane	15	16.718
Hexadecane	16	18.396
Heptadecane	18	19.864

Table 25. Calculated Kovats Index (KI) of odor active compounds found in stored IWG and HRW flour determined through gas chromatography-olfactometry-mass spectrometry.

Compounds	Average Retention Time "i"	n-alkane	N-alkane	trn	trN	Kovats Index	Literature Kovats Index*
2-Methyl Butanol	2.196	6	7	2	2.73	627	664-700
Pentanal	2.528	6	7	2	2.73	673	671-677
1-Pentanol	3.549	7	8	2.72	3.65	789	744-801
Hexanal	3.93	8	9	3.64	5.12	819	769-823
1-Hexanol	4.96	8	9	3.64	5.12	889	839-889
Heptanal	5.49	9	10	5.12	7.03	919	855-921
E-2-Heptanal	6.54	9	10	5.12	7.03	974	927-978
1-Octen-3-ol	7.00	9	10	5.12	7.03	998	958-999
2-pentyl Furan	7.15	10	11	7.03	9.06	1006	977-1012
Octanal	7.43	10	11	7.03	9.06	1020	973-1021
Limonene	7.99	10	11	7.03	9.06	1047	1010-1060
[E]-2-Octenal	8.55	10	11	7.03	9.06	1075	1034-1076
Nonanal	9.50	11	12	9.06	11.10	1121	1073-1130
[E]-2-Nonenal	10.63	11	12	9.06	11.10	1177	1129-1180
Decanal	11.57	12	13	11.10	13.07	1224	1183-1231

*Range from the National Institute of Standards and Technology Mass Spectrometry Data Center
n = carbon number of alkane which elutes before "i"
N = carbon number of alkane which elutes after "i"
tr_i = Retention time of "i"
tr_n = Retention time of the alkane which elutes before "i"
tr_N = Retention time of the alkane which elutes after "i"

Appendix D: Analysis of Variation of VOACs from GC-O-MS Analysis and Descriptive Analysis

Table 26: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on hexanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	########	68.016	5.41E-10
Refinement	2	########	2.850	7.03E-02
Steam	1	########	48.093	3.01E-08
Time	2	########	166.356	1.60E-19
Grain * Refinement	1	########	36.625	4.83E-07
Grain * Steam	0			
Grain * Time	2	########	31.471	8.68E-09
Refinement * Steam	2	########	7.800	1.45E-03
Refinement * Time	4	########	10.233	1.01E-05
Steam * Time	2	########	13.102	4.70E-05
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	########	19.375	1.58E-06
Grain * Steam * Time	0			
Refinement * Steam * Time	4	########	5.112	2.14E-03
Grain * Refinement * Steam * Time	0			
Error	38	########		

Table 27: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on 3-methylbutanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	5.62E+15	37.509	4.26E-07
Refinement	2	4.08E+14	2.724	7.88E-02
Steam	1	3.58E+15	23.868	2.01E-05
Time	2	3.85E+15	25.679	1.01E-07
Grain * Refinement	1	5.72E+15	38.196	3.58E-07
Grain * Steam	0			
Grain * Time	2	2.62E+15	17.520	4.43E-06
Refinement * Steam	2	2.06E+15	13.726	3.47E-05
Refinement * Time	4	1.70E+14	1.135	3.55E-01
Steam * Time	2	1.21E+15	8.069	1.23E-03
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.69E+15	17.981	3.50E-06
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.32E+15	8.810	4.22E-05
Grain * Refinement * Steam * Time	0			
Error	37	1.50E+14		

Table 28: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on pentanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	3.14E+15	69.233	8.24E-10
Refinement	2	1.83E+14	4.045	2.63E-02
Steam	1	4.53E+14	9.988	3.24E-03
Time	2	2.75E+15	60.662	4.22E-12
Grain * Refinement	1	1.59E+15	35.118	9.66E-07
Grain * Steam	0			
Grain * Time	2	1.38E+15	30.427	2.20E-08
Refinement * Steam	2	1.01E+15	22.222	5.89E-07
Refinement * Time	4	7.47E+13	1.649	1.84E-01
Steam * Time	2	4.31E+14	9.517	5.00E-04
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.00E+15	22.123	6.15E-07
Grain * Steam * Time	0			
Refinement * Steam * Time	4	7.94E+14	17.524	5.57E-08
Grain * Refinement * Steam * Time	0			
Error	35	4.53E+13		

Table 29: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on 1-pentanol intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	8.73E+15	44.127	6.65E-08
Refinement	2	1.75E+15	8.845	6.80E-04
Steam	1	1.19E+16	60.202	1.99E-09
Time	2	1.64E+16	82.724	9.32E-15
Grain * Refinement	1	3.43E+15	17.316	1.69E-04
Grain * Steam	0			
Grain * Time	2	5.30E+15	26.767	4.82E-08
Refinement * Steam	2	6.55E+14	3.309	4.71E-02
Refinement * Time	4	1.69E+15	8.556	4.68E-05
Steam * Time	2	6.30E+15	31.828	6.36E-09
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	5.85E+15	29.562	1.54E-08
Grain * Steam * Time	0			
Refinement * Steam * Time	4	8.11E+14	4.096	7.25E-03
Grain * Refinement * Steam * Time	0			
Error	39	1.98E+14		

Table 30: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on 1-hexanol intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.45E+15	42.308	1.16E-07
Refinement	2	4.28E+13	1.249	2.98E-01
Steam	1	2.65E+15	77.500	1.04E-10
Time	2	3.49E+15	101.772	5.48E-16
Grain * Refinement	1	8.33E+14	24.320	1.64E-05
Grain * Steam	0			
Grain * Time	2	6.86E+14	20.019	1.15E-06
Refinement * Steam	2	1.64E+14	4.799	1.39E-02
Refinement * Time	4	1.80E+14	5.253	1.81E-03
Steam * Time	2	6.27E+14	18.312	2.70E-06
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	8.84E+14	25.811	8.32E-08
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.51E+14	4.410	5.01E-03
Grain * Refinement * Steam * Time	0			
Error	38	3.43E+13		

Table 31: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on heptanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.72E+15	109.789	6.71E-13
Refinement	2	9.58E+13	6.117	4.89E-03
Steam	1	1.28E+15	81.484	4.28E-11
Time	2	1.90E+15	121.310	1.81E-17
Grain * Refinement	1	3.16E+14	20.201	6.08E-05
Grain * Steam	0			
Grain * Time	2	8.16E+14	52.149	9.53E-12
Refinement * Steam	2	1.35E+14	8.648	7.79E-04
Refinement * Time	4	1.79E+14	11.432	3.10E-06
Steam * Time	2	4.14E+14	26.468	5.47E-08
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.05E+14	13.089	4.47E-05
Grain * Steam * Time	0			
Refinement * Steam * Time	4	8.84E+13	5.646	1.10E-03
Grain * Refinement * Steam * Time	0			
Error	39	1.57E+13		

Table 32: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on [E]-2-heptanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	5.08E+13	7.910	7.58E-03
Refinement	2	1.30E+13	2.028	1.45E-01
Steam	1	1.82E+14	28.383	4.15E-06
Time	2	3.37E+14	52.441	6.62E-12
Grain * Refinement	1	7.00E+13	10.903	2.03E-03
Grain * Steam	0			
Grain * Time	2	1.41E+13	2.202	1.24E-01
Refinement * Steam	2	2.59E+13	4.038	2.53E-02
Refinement * Time	4	3.14E+13	4.881	2.67E-03
Steam * Time	2	3.38E+13	5.262	9.36E-03
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	3.24E+13	5.038	1.12E-02
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.78E+13	2.765	4.04E-02
Grain * Refinement * Steam * Time	0			
Error	40	6.42E+12		

Table 33: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on 1-octen-3-ol intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	4.60E+15	156.249	2.14E-15
Refinement	2	8.53E+14	28.978	1.66E-08
Steam	1	2.67E+15	90.731	7.70E-12
Time	2	2.48E+15	84.088	4.71E-15
Grain * Refinement	1	1.99E+15	67.663	3.92E-10
Grain * Steam	0			
Grain * Time	2	2.29E+15	77.717	1.66E-14
Refinement * Steam	2	8.77E+14	29.791	1.20E-08
Refinement * Time	4	8.03E+14	27.276	5.83E-11
Steam * Time	2	7.61E+14	25.848	6.23E-08
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.20E+15	40.709	2.27E-10
Grain * Steam * Time	0			
Refinement * Steam * Time	4	5.28E+14	17.916	1.67E-08
Grain * Refinement * Steam * Time	0			
Error	40	2.94E+13		

Table 34: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on 2-pentylfuran intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	4.60E+15	156.249	2.14E-15
Refinement	2	8.53E+14	28.978	1.66E-08
Steam	1	2.67E+15	90.731	7.70E-12
Time	2	2.48E+15	84.088	4.71E-15
Grain * Refinement	1	1.99E+15	67.663	3.92E-10
Grain * Steam	0			
Grain * Time	2	2.29E+15	77.717	1.66E-14
Refinement * Steam	2	8.77E+14	29.791	1.20E-08
Refinement * Time	4	8.03E+14	27.276	5.83E-11
Steam * Time	2	7.61E+14	25.848	6.23E-08
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.20E+15	40.709	2.27E-10
Grain * Steam * Time	0			
Refinement * Steam * Time	4	5.28E+14	17.916	1.67E-08
Grain * Refinement * Steam * Time	0			
Error	40	2.94E+13		

Table 35: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on octanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.81E+14	17.571	1.54E-04
Refinement	2	4.34E+12	0.421	6.60E-01
Steam	1	1.91E+14	18.578	1.07E-04
Time	2	2.28E+14	22.141	3.76E-07
Grain * Refinement	1	3.81E+13	3.699	6.18E-02
Grain * Steam	0			
Grain * Time	2	3.23E+13	3.131	5.48E-02
Refinement * Steam	2	4.06E+13	3.943	2.76E-02
Refinement * Time	4	4.02E+13	3.904	9.25E-03
Steam * Time	2	3.55E+13	3.440	4.21E-02
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.17E+13	1.138	3.31E-01
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.47E+13	1.422	2.45E-01
Grain * Refinement * Steam * Time	0			
Error	39	1.03E+13		

Table 36: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on limonene intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	2.75E+14	3.469	6.99E-02
Refinement	2	3.30E+14	4.162	2.28E-02
Steam	1	1.68E+15	21.237	4.09E-05
Time	2	1.80E+15	22.735	2.54E-07
Grain * Refinement	1	2.41E+14	3.048	8.85E-02
Grain * Steam	0			
Grain * Time	2	5.85E+13	0.738	4.84E-01
Refinement * Steam	2	1.39E+15	17.495	3.48E-06
Refinement * Time	4	2.46E+14	3.107	2.56E-02
Steam * Time	2	1.01E+15	12.695	5.38E-05
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.23E+14	1.556	2.24E-01
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.22E+14	1.534	2.11E-01
Grain * Refinement * Steam * Time	0			
Error	40	7.92E+13		

Table 37: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on [E]-2-octenal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	9.94E+13	18.849	1.01E-04
Refinement	2	1.01E+13	1.909	1.62E-01
Steam	1	2.09E+14	39.621	2.25E-07
Time	2	1.92E+14	36.307	1.53E-09
Grain * Refinement	1	1.91E+13	3.628	6.44E-02
Grain * Steam	0			
Grain * Time	2	3.77E+13	7.144	2.32E-03
Refinement * Steam	2	1.36E+13	2.569	8.98E-02
Refinement * Time	4	4.70E+13	8.917	3.52E-05
Steam * Time	2	1.73E+13	3.280	4.85E-02
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	4.58E+12	0.868	4.28E-01
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.53E+13	4.805	3.09E-03
Grain * Refinement * Steam * Time	0			
Error	38	5.27E+12		

Table 38: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on nonanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.88E+14	61.225	1.62E-09
Refinement	2	9.90E+12	3.220	5.08E-02
Steam	1	7.59E+13	24.699	1.38E-05
Time	2	1.14E+14	37.013	9.74E-10
Grain * Refinement	1	2.95E+12	0.959	3.33E-01
Grain * Steam	0			
Grain * Time	2	4.48E+13	14.577	1.87E-05
Refinement * Steam	2	2.43E+13	7.920	1.30E-03
Refinement * Time	4	2.06E+13	6.708	3.29E-04
Steam * Time	2	1.42E+13	4.625	1.58E-02
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	9.91E+12	3.223	5.07E-02
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.07E+13	3.473	1.61E-02
Grain * Refinement * Steam * Time	0			
Error	39	3.07E+12		

Table 39: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on [E]-2-nonenal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	9.39E+10	0.241	0.626
Refinement	2	2.12E+12	5.431	0.009
Steam	1	1.91E+11	0.491	0.488
Time	2	3.04E+12	7.794	0.001
Grain * Refinement	1	1.58E+10	0.040	0.842
Grain * Steam	0			
Grain * Time	2	3.94E+11	1.011	0.374
Refinement * Steam	2	1.59E+11	0.408	0.668
Refinement * Time	4	2.63E+11	0.674	0.614
Steam * Time	2	6.72E+11	1.725	0.192
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.97E+11	0.763	0.474
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.06E+12	5.280	0.002
Grain * Refinement * Steam * Time	0			
Error	37	3.90E+11		

Table 40: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on decanal intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	6.35E+12	9.641	0.004
Refinement	2	4.08E+11	0.619	0.544
Steam	1	2.39E+12	3.633	0.064
Time	2	4.16E+12	6.311	0.004
Grain * Refinement	1	3.37E+11	0.511	0.479
Grain * Steam	0			
Grain * Time	2	5.02E+10	0.076	0.927
Refinement * Steam	2	3.88E+12	5.887	0.006
Refinement * Time	4	1.78E+12	2.705	0.044
Steam * Time	2	5.76E+11	0.875	0.425
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.23E+12	3.387	0.044
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.87E+12	2.841	0.037
Grain * Refinement * Steam * Time	0			
Error	39	6.58E+11		

Table 41: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on overall aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	15.428	1.966	0.162
Refinement	2	0.013	0.002	0.998
Steam	1	23.488	2.993	0.085
Time	2	1.440	0.183	0.832
Grain * Refinement	2	4.544	0.579	0.561
Grain * Steam	0			
Grain * Time	1	16.537	2.107	0.148
Refinement * Steam	2	2.683	0.342	0.711
Refinement * Time	4	7.371	0.939	0.441
Steam * Time	2	2.375	0.303	0.739
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.185	0.151	0.860
Grain * Steam * Time	0			
Refinement * Steam * Time	4	5.370	0.684	0.603
Grain * Refinement * Steam * Time	0			
Error	353	7.848		

Table 42: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on brown sugar intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	0.024	0.004	0.952
Refinement	2	1.637	0.239	0.787
Steam	1	0.488	0.071	0.790
Time	2	0.599	0.087	0.916
Grain * Refinement	2	0.563	0.082	0.921
Grain * Steam	0			
Grain * Time	1	0.811	0.118	0.731
Refinement * Steam	2	0.068	0.010	0.990
Refinement * Time	4	1.204	0.176	0.951
Steam * Time	2	2.078	0.303	0.738
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.363	0.053	0.948
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.135	0.020	0.999
Grain * Refinement * Steam * Time	0			
Error	353	6.848		

Table 43: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on nutty aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.256	0.233	0.629
Refinement	2	3.125	0.580	0.560
Steam	1	0.269	0.050	0.823
Time	2	0.151	0.028	0.972
Grain * Refinement	2	0.159	0.030	0.971
Grain * Steam	0			
Grain * Time	1	8.710	1.617	0.204
Refinement * Steam	2	0.604	0.112	0.894
Refinement * Time	4	0.655	0.122	0.975
Steam * Time	2	0.325	0.060	0.942
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.570	0.106	0.900
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.291	0.425	0.790
Grain * Refinement * Steam * Time	0			
Error	353	5.386		

Table 44: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on floury aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	3.216	0.691	0.406
Refinement	2	2.920	0.627	0.535
Steam	1	2.089	0.449	0.503
Time	2	0.794	0.171	0.843
Grain * Refinement	2	2.778	0.597	0.551
Grain * Steam	0			
Grain * Time	1	0.127	0.027	0.869
Refinement * Steam	2	4.175	0.897	0.409
Refinement * Time	4	0.416	0.089	0.986
Steam * Time	2	0.316	0.068	0.934
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.597	0.343	0.710
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.133	0.458	0.766
Grain * Refinement * Steam * Time	0			
Error	353	4.654		

Table 45: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on corn aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	23	2.629	0.359	0.998
	1	3849.333	525.504	0.000
	1	0.614	0.084	0.772
Refinement	2	5.558	0.759	0.469
Steam	1	0.699	0.095	0.758
Time	2	4.020	0.549	0.578
Grain * Refinement	2	0.363	0.049	0.952
Grain * Steam	0			
Grain * Time	1	10.270	1.402	0.237
Refinement * Steam	2	0.430	0.059	0.943
Refinement * Time	4	2.235	0.305	0.874
Steam * Time	2	6.932	0.946	0.389
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.893	0.122	0.885
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.120	0.289	0.885
Grain * Refinement * Steam * Time	0			
Error	353	7.325		

Table 46: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on popcorn aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	15.428	1.966	0.162
Refinement	2	0.013	0.002	0.998
Steam	1	23.488	2.993	0.085
Time	2	1.440	0.183	0.832
Grain * Refinement	2	4.544	0.579	0.561
Grain * Steam	0			
Grain * Time	1	16.537	2.107	0.148
Refinement * Steam	2	2.683	0.342	0.711
Refinement * Time	4	7.371	0.939	0.441
Steam * Time	2	2.375	0.303	0.739
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.185	0.151	0.860
Grain * Steam * Time	0			
Refinement * Steam * Time	4	5.370	0.684	0.603
Grain * Refinement * Steam * Time	0			
Error	353	7.848		

Table 47: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on earthy aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	15.428	1.966	0.162
Refinement	2	0.013	0.002	0.998
Steam	1	23.488	2.993	0.085
Time	2	1.440	0.183	0.832
Grain * Refinement	2	4.544	0.579	0.561
Grain * Steam	0			
Grain * Time	1	16.537	2.107	0.148
Refinement * Steam	2	2.683	0.342	0.711
Refinement * Time	4	7.371	0.939	0.441
Steam * Time	2	2.375	0.303	0.739
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.185	0.151	0.860
Grain * Steam * Time	0			
Refinement * Steam * Time	4	5.370	0.684	0.603
Grain * Refinement * Steam * Time	0			
Error	353	7.848		

Table 48: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on grassy aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	8.085	0.400	0.528
Refinement	2	0.339	0.017	0.983
Steam	1	0.382	0.019	0.891
Time	2	1.631	0.081	0.923
Grain * Refinement	2	9.400	0.465	0.629
Grain * Steam	0			
Grain * Time	1	0.876	0.043	0.835
Refinement * Steam	2	2.157	0.107	0.899
Refinement * Time	4	3.042	0.150	0.963
Steam * Time	2	6.274	0.310	0.733
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.103	0.005	0.995
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.520	0.125	0.974
Grain * Refinement * Steam * Time	0			
Error	353	20.224		

Table 49: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on beef aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.139	0.264	0.608
Refinement	2	0.435	0.101	0.904
Steam	1	0.083	0.019	0.890
Time	2	3.504	0.811	0.445
Grain * Refinement	2	0.463	0.107	0.898
Grain * Steam	0			
Grain * Time	1	17.754	4.106	0.043
Refinement * Steam	2	0.135	0.031	0.969
Refinement * Time	4	1.088	0.252	0.909
Steam * Time	2	10.547	2.440	0.089
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.217	0.050	0.951
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.377	0.087	0.986
Grain * Refinement * Steam * Time	0			
Error	353	4.323		

Table 50: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on PlayDoh aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	7.005	0.753	0.386
Refinement	2	5.092	0.548	0.579
Steam	1	6.794	0.731	0.393
Time	2	0.768	0.083	0.921
Grain * Refinement	2	8.861	0.953	0.387
Grain * Steam	0			
Grain * Time	1	0.568	0.061	0.805
Refinement * Steam	2	8.091	0.870	0.420
Refinement * Time	4	2.233	0.240	0.915
Steam * Time	2	5.493	0.591	0.554
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.103	0.119	0.888
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.576	0.170	0.954
Grain * Refinement * Steam * Time	0			
Error	353	9.299		

Table 51: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on burnt toast aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.979	0.181	0.671
Refinement	2	0.011	0.001	0.999
Steam	1	0.488	0.045	0.833
Time	2	0.576	0.053	0.949
Grain * Refinement	2	3.552	0.324	0.723
Grain * Steam	0			
Grain * Time	1	2.199	0.201	0.654
Refinement * Steam	2	2.973	0.272	0.762
Refinement * Time	4	0.798	0.073	0.990
Steam * Time	2	0.602	0.055	0.947
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.438	0.040	0.961
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.642	0.059	0.994
Grain * Refinement * Steam * Time	0			
Error	353	10.950		

Table 52: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on cardboard aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	1.932	0.331	0.566
Refinement	2	6.049	1.035	0.356
Steam	1	0.674	0.115	0.734
Time	2	1.059	0.181	0.834
Grain * Refinement	2	2.000	0.342	0.710
Grain * Steam	0			
Grain * Time	1	1.163	0.199	0.656
Refinement * Steam	2	0.800	0.137	0.872
Refinement * Time	4	1.041	0.178	0.950
Steam * Time	2	0.384	0.066	0.936
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.799	0.308	0.735
Grain * Steam * Time	0			
Refinement * Steam * Time	4	3.077	0.527	0.716
Grain * Refinement * Steam * Time	0			
Error	353	5.844		

Table 53: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on rancid oil aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	2.414	0.244	0.621
Refinement	2	4.565	0.462	0.630
Steam	1	3.557	0.360	0.549
Time	2	4.175	0.423	0.655
Grain * Refinement	2	2.430	0.246	0.782
Grain * Steam	0			
Grain * Time	1	3.373	0.342	0.559
Refinement * Steam	2	0.608	0.062	0.940
Refinement * Time	4	1.628	0.165	0.956
Steam * Time	2	0.618	0.063	0.939
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.706	0.072	0.931
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.494	0.151	0.962
Grain * Refinement * Steam * Time	0			
Error	353	9.873		

Table 54: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on sulfur aroma intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	6.520	0.692	0.406
Refinement	2	0.148	0.016	0.984
Steam	1	0.679	0.072	0.789
Time	2	6.709	0.712	0.491
Grain * Refinement	2	2.569	0.273	0.762
Grain * Steam	0			
Grain * Time	1	11.880	1.260	0.262
Refinement * Steam	2	1.487	0.158	0.854
Refinement * Time	4	0.487	0.052	0.995
Steam * Time	2	2.389	0.253	0.776
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.689	0.073	0.929
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.793	0.190	0.943
Grain * Refinement * Steam * Time	0			
Error	353	9.426		

Table 55: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on sweet taste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	22.767	3.252	0.072
Refinement	2	2.416	0.345	0.708
Steam	1	0.546	0.078	0.780
Time	2	0.058	0.008	0.992
Grain * Refinement	2	0.109	0.016	0.985
Grain * Steam	0			
Grain * Time	1	7.188	1.027	0.312
Refinement * Steam	2	0.664	0.095	0.910
Refinement * Time	4	2.472	0.353	0.842
Steam * Time	2	1.836	0.262	0.769
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	4.001	0.571	0.565
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.940	0.420	0.794
Grain * Refinement * Steam * Time	0			
Error	353	7.000		

Table 56: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on saltiness taste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	0.241	0.067	0.795
Refinement	2	2.091	0.584	0.558
Steam	1	0.809	0.226	0.635
Time	2	6.604	1.844	0.160
Grain * Refinement	2	0.729	0.204	0.816
Grain * Steam	0			
Grain * Time	1	1.789	0.500	0.480
Refinement * Steam	2	0.285	0.080	0.924
Refinement * Time	4	1.649	0.461	0.765
Steam * Time	2	1.628	0.455	0.635
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.220	0.061	0.940
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.677	0.748	0.560
Grain * Refinement * Steam * Time	0			
Error	353	3.581		

Table 57: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on sourness taste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	25.062	5.568	0.019
Refinement	2	0.883	0.196	0.822
Steam	1	2.214	0.492	0.484
Time	2	1.595	0.354	0.702
Grain * Refinement	2	0.664	0.148	0.863
Grain * Steam	0			
Grain * Time	1	3.070	0.682	0.409
Refinement * Steam	2	0.394	0.087	0.916
Refinement * Time	4	1.522	0.338	0.852
Steam * Time	2	9.430	2.095	0.125
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.221	0.493	0.611
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.179	0.262	0.902
Grain * Refinement * Steam * Time	0			
Error	353	4.501		

Table 58: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on bitterness taste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	40.752	4.992	0.026
Refinement	2	1.754	0.215	0.807
Steam	1	29.986	3.673	0.056
Time	2	6.613	0.810	0.446
Grain * Refinement	2	3.084	0.378	0.686
Grain * Steam	0			
Grain * Time	1	22.951	2.811	0.094
Refinement * Steam	2	2.618	0.321	0.726
Refinement * Time	4	0.933	0.114	0.977
Steam * Time	2	3.464	0.424	0.655
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.429	0.053	0.949
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.689	0.207	0.934
Grain * Refinement * Steam * Time	0			
Error	353	8.163		

Table 59: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on umami taste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	11.722	2.030	0.155
Refinement	2	1.157	0.200	0.819
Steam	1	0.455	0.079	0.779
Time	2	1.793	0.311	0.733
Grain * Refinement	2	1.047	0.181	0.834
Grain * Steam	0			
Grain * Time	1	0.074	0.013	0.910
Refinement * Steam	2	0.920	0.159	0.853
Refinement * Time	4	0.499	0.086	0.987
Steam * Time	2	2.495	0.432	0.649
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.450	0.078	0.925
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.934	0.162	0.958
Grain * Refinement * Steam * Time	0			
Error	353	5.773		

Table 60: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on overall flavor intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	207.020	27.116	0.000
Refinement	2	5.923	0.776	0.461
Steam	1	52.237	6.842	0.009
Time	2	41.545	5.442	0.005
Grain * Refinement	2	1.349	0.177	0.838
Grain * Steam	0			
Grain * Time	1	2.766	0.362	0.548
Refinement * Steam	2	0.803	0.105	0.900
Refinement * Time	4	1.901	0.249	0.910
Steam * Time	2	2.838	0.372	0.690
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.869	0.376	0.687
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.762	0.100	0.982
Grain * Refinement * Steam * Time	0			
Error	353	7.634		

Table 61: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on vanilla flavor intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	8.509	1.356	0.245
Refinement	2	0.096	0.015	0.985
Steam	1	0.004	0.001	0.979
Time	2	5.339	0.850	0.428
Grain * Refinement	2	1.706	0.272	0.762
Grain * Steam	0			
Grain * Time	1	9.757	1.554	0.213
Refinement * Steam	2	0.017	0.003	0.997
Refinement * Time	4	0.768	0.122	0.974
Steam * Time	2	5.201	0.829	0.438
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.568	0.250	0.779
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.909	0.304	0.875
Grain * Refinement * Steam * Time	0			
Error	353	6.277		

Table 62: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on green pear flavor intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	10.045	1.350	0.246
Refinement	2	0.139	0.019	0.981
Steam	1	0.552	0.074	0.785
Time	2	10.270	1.380	0.253
Grain * Refinement	2	0.492	0.066	0.936
Grain * Steam	0			
Grain * Time	1	6.490	0.872	0.351
Refinement * Steam	2	0.204	0.027	0.973
Refinement * Time	4	1.410	0.190	0.944
Steam * Time	2	0.334	0.045	0.956
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.023	0.272	0.762
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.716	0.096	0.984
Grain * Refinement * Steam * Time	0			
Error	353	7.441		

Table 63: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on raw dough flavor intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	0.881	0.163	0.686
Refinement	2	0.265	0.049	0.952
Steam	1	5.348	0.992	0.320
Time	2	10.648	1.974	0.140
Grain * Refinement	2	0.305	0.056	0.945
Grain * Steam	0			
Grain * Time	1	1.853	0.344	0.558
Refinement * Steam	2	0.065	0.012	0.988
Refinement * Time	4	0.180	0.033	0.998
Steam * Time	2	1.091	0.202	0.817
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.316	0.244	0.784
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.701	0.315	0.868
Grain * Refinement * Steam * Time	0			
Error	353	5.393		

Table 64: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on peanut butter flavor intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	3.423	0.503	0.479
Refinement	2	1.252	0.184	0.832
Steam	1	0.074	0.011	0.917
Time	2	5.981	0.878	0.417
Grain * Refinement	2	0.171	0.025	0.975
Grain * Steam	0			
Grain * Time	1	1.562	0.229	0.632
Refinement * Steam	2	3.651	0.536	0.586
Refinement * Time	4	1.274	0.187	0.945
Steam * Time	2	0.532	0.078	0.925
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	1.533	0.225	0.799
Grain * Steam * Time	0			
Refinement * Steam * Time	4	1.860	0.273	0.895
Grain * Refinement * Steam * Time	0			
Error	353	6.812		

Table 65: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on beany flavor intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	41.751	8.147	0.005
Refinement	2	1.284	0.250	0.779
Steam	1	0.045	0.009	0.925
Time	2	21.333	4.163	0.016
Grain * Refinement	2	1.702	0.332	0.718
Grain * Steam	0			
Grain * Time	1	6.803	1.328	0.250
Refinement * Steam	2	0.795	0.155	0.856
Refinement * Time	4	6.029	1.176	0.321
Steam * Time	2	2.812	0.549	0.578
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	2.795	0.545	0.580
Grain * Steam * Time	0			
Refinement * Steam * Time	4	3.848	0.751	0.558
Grain * Refinement * Steam * Time	0			
Error	353	5.125		

Table 66: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on overall aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	191.779	17.907	0.000
Refinement	2	4.137	0.386	0.680
Steam	1	65.472	6.113	0.014
Time	2	22.189	2.072	0.127
Grain * Refinement	2	5.726	0.535	0.586
Grain * Steam	0			
Grain * Time	1	17.011	1.588	0.208
Refinement * Steam	2	2.385	0.223	0.800
Refinement * Time	4	6.213	0.580	0.677
Steam * Time	2	3.451	0.322	0.725
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	3.828	0.357	0.700
Grain * Steam * Time	0			
Refinement * Steam * Time	4	3.703	0.346	0.847
Grain * Refinement * Steam * Time	0			
Error	353	10.710		

Table 67: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on sweet aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	0.308	0.049	0.825
Refinement	2	1.165	0.184	0.832
Steam	1	0.058	0.009	0.924
Time	2	0.819	0.130	0.879
Grain * Refinement	2	1.189	0.188	0.829
Grain * Steam	0			
Grain * Time	1	1.662	0.263	0.609
Refinement * Steam	2	6.199	0.980	0.376
Refinement * Time	4	1.700	0.269	0.898
Steam * Time	2	3.172	0.502	0.606
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.123	0.020	0.981
Grain * Steam * Time	0			
Refinement * Steam * Time	4	2.930	0.463	0.763
Grain * Refinement * Steam * Time	0			
Error	353	6.323		

Table 68: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on salty aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	8.454	2.359	0.125
Refinement	2	0.166	0.046	0.955
Steam	1	0.016	0.004	0.947
Time	2	5.302	1.479	0.229
Grain * Refinement	2	0.068	0.019	0.981
Grain * Steam	0			
Grain * Time	1	0.045	0.013	0.911
Refinement * Steam	2	0.510	0.142	0.867
Refinement * Time	4	0.636	0.178	0.950
Steam * Time	2	1.377	0.384	0.681
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.360	0.100	0.905
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.481	0.134	0.970
Grain * Refinement * Steam * Time	0			
Error	353	3.584		

Table 69: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on sour aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	45.282	7.810	0.005
Refinement	2	0.233	0.040	0.961
Steam	1	5.779	0.997	0.319
Time	2	7.261	1.252	0.287
Grain * Refinement	2	0.264	0.046	0.955
Grain * Steam	0			
Grain * Time	1	1.747E-05	0.000	0.999
Refinement * Steam	2	5.555	0.958	0.385
Refinement * Time	4	0.433	0.075	0.990
Steam * Time	2	9.916	1.710	0.182
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	3.219	0.555	0.574
Grain * Steam * Time	0			
Refinement * Steam * Time	4	3.004	0.518	0.722
Grain * Refinement * Steam * Time	0			
Error	353	5.798		

Table 70: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on bitter aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	143.100	14.703	0.000
Refinement	2	5.045	0.518	0.596
Steam	1	27.934	2.870	0.091
Time	2	8.667	0.890	0.411
Grain * Refinement	2	13.550	1.392	0.250
Grain * Steam	0			
Grain * Time	1	4.198	0.431	0.512
Refinement * Steam	2	1.369	0.141	0.869
Refinement * Time	4	3.676	0.378	0.825
Steam * Time	2	5.249	0.539	0.584
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.319	0.033	0.968
Grain * Steam * Time	0			
Refinement * Steam * Time	4	7.700	0.791	0.531
Grain * Refinement * Steam * Time	0			
Error	353	9.733		

Table 71: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on umami aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	9.286	1.481	0.224
Refinement	2	0.178	0.028	0.972
Steam	1	0.453	0.072	0.788
Time	2	3.272	0.522	0.594
Grain * Refinement	2	0.250	0.040	0.961
Grain * Steam	0			
Grain * Time	1	1.287	0.205	0.651
Refinement * Steam	2	0.056	0.009	0.991
Refinement * Time	4	0.670	0.107	0.980
Steam * Time	2	2.799	0.446	0.640
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.869	0.139	0.871
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.841	0.134	0.970
Grain * Refinement * Steam * Time	0			
Error	353	6.270		

Table 72: Analysis of variance on the individual effects and interactions effects of grain type, refinement, steam treatment, and storage time on astringent aftertaste intensity.

Source of Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	9.286	1.481	0.224
Refinement	2	0.178	0.028	0.972
Steam	1	0.453	0.072	0.788
Time	2	3.272	0.522	0.594
Grain * Refinement	2	0.250	0.040	0.961
Grain * Steam	0			
Grain * Time	1	1.287	0.205	0.651
Refinement * Steam	2	0.056	0.009	0.991
Refinement * Time	4	0.670	0.107	0.980
Steam * Time	2	2.799	0.446	0.640
Grain * Refinement * Steam	0			
Grain * Refinement * Time	2	0.869	0.139	0.871
Grain * Steam * Time	0			
Refinement * Steam * Time	4	0.841	0.134	0.970
Grain * Refinement * Steam * Time	0			
Error	353	6.270		

Appendix E: Flavor Analysis and Sensory Evaluation Correlation

Table 73: Correlation data depicting relationships among volatile odor active compounds over storage.

	3-Methyl Butanal	Pentanal	1-Pentanol	Hexanal	1-Hexanol	Heptanal	E2-Heptenal	1-Octen-3-ol	2-pentyl Furan	Octanal	Limonene	E2-Octenal	Nonanal	E2-Nonenal	Decanal
3-Methyl Butanal	1														
Pentanal	0.47	1													
1-Pentanol	0.86****	0.40	1												
Hexanal	0.65**	0.47	0.81***	1											
1-Hexanol	0.83****	0.46	0.93****	0.89****	1										
Heptanal	0.79***	0.32	0.89****	0.94****	0.90****	1									
E2-Heptenal	0.53*	0.44	0.75***	0.86****	0.86****	0.77***	1								
1-Octen-3-ol	0.10	0.32	0.18	0.50*	0.36	0.30	0.52*	1							
2-pentyl Furan	0.84****	0.32	0.87****	0.76***	0.85****	0.84****	0.61*	0.33	1						
Octanal	0.37	0.12	0.53*	0.69**	0.65**	0.63**	0.74**	0.67**	0.60*	1					
Limonene	0.28	0.25	0.38	0.60*	0.50*	0.51*	0.43	0.45	0.59*	0.32	1				
E2-Octenal	0.42	0.17	0.64**	0.84****	0.76***	0.79***	0.91****	0.60*	0.63**	0.87****	0.48	1			
Nonanal	0.66**	0.24	0.80***	0.89****	0.83****	0.92****	0.79***	0.43	0.88****	0.78***	0.58*	0.87****	1		
E2-Nonenal	0.04	0.18	-0.04	0.21	0.06	0.19	0.19	0.36	0.19	0.21	0.33	0.36	0.31	1	
Decanal	0.56*	0.30	0.66**	0.56*	0.74**	0.56*	0.72**	0.29	0.68**	0.74***	0.23	0.66**	0.72**	0.00	1

*P < 0.05; **P < 0.01; ***P < 0.01.

Table 74: Correlation data depicting relationships between volatile odor active compounds and aroma and flavor attributes over storage.

	3Methyl Butanal	Pentanal	1-Pentanol	Hexanal	1-Hexanol	Heptanal	E2-Heptenal	1-Octen-3-ol	2-pentyl Furan	Octanal	Limonene	E2-Octenal	Nonanal	E2-Nonenal	Decanal
overallA	-0.23	-0.08	-0.14	-0.41	-0.34	-0.35	-0.42	-0.19	-0.11	-0.48	-0.05	-0.43	-0.33	0.14	-0.37
brown_sugarA	0.13	-0.51*	0.01	0.00	0.03	0.17	-0.13	-0.08	0.28	0.31	0.05	0.15	0.31	0.27	0.21
nuttyA	-0.20	-0.19	0.03	0.42	0.16	0.27	0.29	0.44	0.24	0.42	0.68**	0.49	0.44	0.25	0.11
flouryA	-0.18	0.23	-0.13	-0.18	-0.14	-0.30	-0.19	0.04	-0.08	0.04	-0.12	-0.27	-0.20	-0.34	0.16
cornA	0.33	0.14	0.22	-0.21	0.03	-0.05	-0.27	-0.55*	0.10	-0.43	-0.38	-0.46	-0.20	-0.27	-0.06
popcornA	-0.01	-0.36	-0.03	-0.29	-0.23	-0.14	-0.19	-0.18	-0.13	0.05	-0.74**	-0.10	-0.11	-0.12	0.01
earthyA	0.70**	0.47	0.52*	0.55*	0.54*	0.62*	0.30	0.10	0.48	0.05	0.37	0.26	0.37	0.28	0.02
grassyA	0.14	-0.07	0.32	-0.05	0.12	0.07	-0.02	-0.51*	0.05	-0.28	-0.37	-0.17	-0.04	-0.43	0.10
beefA	-0.04	-0.42	0.02	-0.41	-0.14	-0.23	-0.31	-0.46	0.03	-0.27	0.01	-0.30	-0.22	-0.24	-0.05
play_dohA	0.15	0.05	0.37	0.26	0.26	0.30	0.24	-0.26	0.03	-0.24	-0.11	0.09	0.08	-0.19	-0.10
burnt_toastA	0.58*	0.34	0.75***	0.75***	0.79***	0.74**	0.82****	0.39	0.73**	0.67**	0.48	0.81***	0.80***	0.19	0.78***
cardboardA	-0.22	0.13	0.03	0.22	0.06	0.07	0.29	0.34	-0.02	0.12	0.38	0.30	0.08	0.12	-0.08
rancid_oilA	0.17	0.43	0.01	-0.10	-0.04	-0.07	-0.01	-0.12	-0.21	-0.44	-0.36	-0.21	-0.29	0.15	-0.29
sulfurA	0.19	0.14	0.19	-0.08	0.02	0.04	0.03	-0.27	0.05	-0.39	-0.10	-0.09	-0.02	0.14	-0.07
sweetness	0.03	0.47	0.08	0.18	0.09	0.04	0.32	0.30	-0.05	-0.21	0.25	0.12	-0.05	0.18	-0.21
saltiness	0.17	0.34	-0.10	-0.07	0.01	-0.05	0.02	-0.11	-0.28	-0.06	-0.48	-0.10	-0.23	0.12	-0.06
sourness	-0.47	-0.40	-0.63**	-0.48	-0.56*	-0.47	-0.51*	-0.18	-0.63**	-0.25	-0.62*	-0.39	-0.47	-0.11	-0.39
bitterness	0.23	-0.07	0.08	-0.12	0.05	0.03	-0.22	-0.42	0.12	-0.14	-0.24	-0.20	0.00	0.19	0.11
umami	0.44	0.17	0.31	-0.05	0.16	0.12	-0.24	-0.40	0.21	-0.38	-0.10	-0.37	-0.12	-0.17	-0.19
overallF	0.18	0.39	0.10	-0.05	-0.06	-0.06	-0.36	-0.03	0.14	-0.27	0.02	-0.44	-0.16	-0.26	-0.20
vanillaF	0.21	0.48	0.10	-0.27	0.02	-0.26	-0.08	-0.15	-0.04	-0.15	-0.45	-0.33	-0.32	-0.18	0.19
green_pearF	0.40	0.09	0.30	-0.16	0.21	-0.01	-0.08	-0.44	0.18	-0.31	-0.10	-0.32	-0.16	-0.48	0.11
peanutbutterF	0.41	0.30	0.29	0.26	0.39	0.25	0.28	0.29	0.15	0.40	-0.19	0.21	0.07	-0.13	0.21
raw_doughF	-0.05	0.37	0.17	0.51*	0.26	0.33	0.53*	0.49	0.21	0.25	0.56*	0.53*	0.44	0.64**	0.11
beanyF	-0.04	-0.02	-0.03	-0.33	-0.18	-0.24	-0.39	-0.36	0.03	-0.35	-0.06	-0.40	-0.25	0.03	-0.14

*P < 0.05; **P < 0.01; ***P < 0.01.

Table 75: Correlation data depicting relationships between aroma and flavor attributes over storage.

	OverallA	Brown sugarA	nuttyA	flouryA	cornA	popcornA	earthyA	grassyA	beefA	Play dohA	Burnt toastA	cardboard A	Rancid oilA	sulfurA	sweetness	saltiness	sourness	bitterness	umami	overallF	vanillaF	Green pearF	Peanut butterF	Raw doughF	beanyF
OverallA	1																								
brown_sugarA	-0.18	1																							
nuttyA	-0.25	0.21	1																						
flouryA	0.15	-0.33	-0.04	1																					
cornA	0.41	-0.09	-0.57*	0.19	1																				
popcornA	0.14	0.31	-0.49	-0.17	0.22	1																			
earthyA	-0.14	-0.06	-0.14	-0.35	0.04	-0.28	1																		
grassyA	0.40	-0.22	-0.35		0.71**	0.30	-0.16	1																	
beefA	0.53*	0.19	-0.20	0.00	0.23	0.11	-0.16	0.21	1																
play_dohA	0.20	-0.42	-0.12	-0.18	0.42	0.01	0.18	0.77***	-0.13	1															
burnt_toastA	-0.21	-0.05	0.25	0.01	-0.27	-0.17	0.39	0.05	-0.05	0.14	1														
cardboardA	0.13	-0.61*	0.42	0.29	-0.36	-0.49	0.04	-0.07	-0.08	0.23	0.38	1													
rancid_oilA	0.24	-0.57*	-0.65**	-0.16	0.38	0.12	0.41	0.27	-0.26	0.49	-0.07	0.10	1												
sulfurA	0.53*	-0.28	-0.49	-0.34	0.24	0.26	0.25	0.42	0.26	0.43	0.19	0.02	0.62*	1											
sweetness	0.10	-0.70**	0.01	-0.29	-0.15	-0.30	0.28	-0.12	-0.22	0.29	0.13	0.48	0.57*	0.45	1										
saltiness	-0.38	-0.06	-0.58*	-0.15	0.17	0.14	0.30	-0.18	-0.37	-0.06	-0.22	-0.37	0.51*	-0.08	0.00	1									
sourness	-0.17	0.17	-0.21	0.03	0.04	0.33	-0.34	0.04	-0.40	0.03	-0.61*	-0.34	0.14	-0.23	-0.37	0.38	1								
bitterness	0.12	0.61*	-0.27	-0.24	0.47	0.30	0.12	0.24	0.19	-0.02	-0.18	0.76***	-0.03	0.05	-0.49	0.28	0.13	1							
umami	0.44	-0.11	-0.52*	0.11	0.85***	-0.01	0.38	0.50*	0.30	0.39	-0.18	-0.17	0.40	0.22	-0.10	0.18	-0.09	0.31	1						
overallF	0.42	-0.30	-0.26	0.57*	0.40	-0.06	0.24	0.20	0.05	0.00	-0.10	0.03	0.15	0.08	-0.04	-0.17	-0.14	-0.02	0.51*	1					
vanillaF	0.31	-0.35	-0.72**	0.48	0.55*	0.22	0.01	0.28	0.22	-0.07	0.00	-0.14	0.42	0.19	0.02	0.40	-0.16	0.22	0.43	0.42	1				
green_pearF	0.22	-0.24	-0.49	0.24	0.73**	-0.12	0.10	0.52*	0.46	0.30	-0.01	-0.07	0.25	0.19	-0.03	0.07	-0.19	0.07	0.75***	0.24	0.49	1			
peanutbutterF	-0.40	-0.04	-0.30	0.10	0.15	0.07	0.31	-0.17	-0.21	-0.11	0.06	-0.14	0.14	-0.44	-0.11	0.65**	0.04	0.04	0.30	0.04	0.41	0.24	1		
raw_doughF	0.04	-0.13	0.54*	-0.34	-0.42	-0.34	0.13	-0.23	-0.36	0.14	0.34	0.34	0.02	0.18	0.54*	-0.21	-0.38	-0.12	-0.42	-0.26	-0.36	-0.57*	-0.33	1	
beanyF	0.71**	-0.13	-0.21	0.53*	0.55*	-0.10	-0.03	0.42	0.49	0.13	-0.04	0.25	0.13	0.16	-0.22	-0.24	-0.17	0.17	0.60*	0.49	0.46	0.50	-0.14	-0.29	1

*P < 0.05; **P < 0.01; ***P < 0.01.

Appendix F: Analysis of Variance of Functionality Results

Table 76: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on water absorption.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	296.510	500.914	2.22E-27
Refinement	2	172.948	292.173	5.88E-28
Steam	1	219.801	371.323	1.63E-24
RelativeHumidity	1	79.366	134.078	1.24E-15
Time	3	52.182	88.155	9.36E-20
Grain * Refinement	1	54.827	92.623	7.07E-13
Grain * Steam	0			
Grain * RelativeHumidity	1	0.255	0.431	5.15E-01
Grain * Time	2	15.351	25.934	2.08E-08
Refinement * Steam	2	8.407	14.202	1.36E-05
Refinement * RelativeHumidity	2	1.426	2.409	1.00E-01
Refinement * Time	4	8.733	14.753	5.59E-08
Steam * RelativeHumidity	1	0.320	0.541	4.66E-01
Steam * Time	2	8.352	14.109	1.45E-05
RelativeHumidity * Time	2	1.304	2.202	1.21E-01
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	13.975	23.609	1.25E-05
Grain * Refinement * Time	2	0.379	0.641	5.31E-01
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	1.890	3.193	4.97E-02
Refinement * Steam * RelativeHumidity	2	4.618	7.801	1.14E-03
Refinement * Steam * Time	4	11.533	19.484	1.20E-09
Refinement * RelativeHumidity * Time	4	6.342	10.714	2.56E-06
Steam * RelativeHumidity * Time	2	0.656	1.109	3.38E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	0.854	1.443	2.46E-01
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	4.024	6.798	1.96E-04
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	49	0.592		

Table 77: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on dough development time.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	721771	782	8.94E-32
Refinement	2	1652643	1790	1.57E-46
Steam	1	38874	42	4.12E-08
RelativeHumidity	1	125441	136	9.81E-16
Time	3	64140	69	1.15E-17
Grain * Refinement	1	632502	685	1.86E-30
Grain * Steam	0			
Grain * RelativeHumidity	1	36631	40	8.10E-08
Grain * Time	2	23617	26	2.48E-08
Refinement * Steam	2	12816	14	1.68E-05
Refinement * RelativeHumidity	2	68435	74	1.53E-15
Refinement * Time	4	35836	39	1.27E-14
Steam * RelativeHumidity	1	3348	4	6.28E-02
Steam * Time	2	6284	7	2.47E-03
RelativeHumidity * Time	2	125857	136	9.57E-21
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	42364	46	1.49E-08
Grain * Refinement * Time	2	23197	25	3.10E-08
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	118898	129	3.10E-20
Refinement * Steam * RelativeHumidity	2	2872	3	5.35E-02
Refinement * Steam * Time	4	6694	7	1.14E-04
Refinement * RelativeHumidity * Time	4	57790	63	1.19E-18
Steam * RelativeHumidity * Time	2	234	0	7.77E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	122982	133	1.54E-20
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	5848	6	3.44E-04
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	49	923		

Table 78: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on dough stability.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	754757.5	350.5	5.68E-24
Refinement	2	446839.8	207.5	1.20E-24
Steam	1	12116.1	5.6	2.17E-02
RelativeHumidity	1	19009.5	8.8	4.59E-03
Time	3	13730.1	6.4	9.81E-04
Grain * Refinement	1	185629.7	86.2	2.23E-12
Grain * Steam	0			
Grain * RelativeHumidity	1	12513.0	5.8	1.97E-02
Grain * Time	2	30184.5	14.0	1.53E-05
Refinement * Steam	2	3518.7	1.6	2.06E-01
Refinement * RelativeHumidity	2	34858.8	16.2	4.01E-06
Refinement * Time	4	4898.3	2.3	7.45E-02
Steam * RelativeHumidity	1	0.9	0.0	9.84E-01
Steam * Time	2	3867.7	1.8	1.77E-01
RelativeHumidity * Time	2	34007.0	15.8	5.09E-06
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	70303.5	32.6	6.44E-07
Grain * Refinement * Time	2	4490.7	2.1	1.35E-01
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	15679.6	7.3	1.70E-03
Refinement * Steam * RelativeHumidity	2	2893.7	1.3	2.70E-01
Refinement * Steam * Time	4	3060.0	1.4	2.41E-01
Refinement * RelativeHumidity * Time	4	10928.3	5.1	1.68E-03
Steam * RelativeHumidity * Time	2	3358.2	1.6	2.21E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	7374.6	3.4	4.05E-02
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	4259.6	2.0	1.13E-01
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	49	2153.4		

Table 79: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on resistance to extension.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	62805.180	13722.146	0.00E+00
Refinement	2	4947.023	1080.863	7.74E-258
Steam	1	3446.768	753.076	4.90E-126
RelativeHumidity	1	6629.892	1448.548	2.68E-201
Time	2	2133.655	466.177	1.96E-146
Grain * Refinement	1	174.892	38.212	9.00E-10
Grain * Steam	0			
Grain * RelativeHumidity	1	1076.946	235.299	3.64E-48
Grain * Time	2	1101.377	240.637	4.59E-87
Refinement * Steam	2	56.828	12.416	4.67E-06
Refinement * RelativeHumidity	2	1001.982	218.921	1.84E-80
Refinement * Time	4	1153.472	252.019	1.18E-152
Steam * RelativeHumidity	1	89.855	19.632	1.03E-05
Steam * Time	2	228.896	50.011	1.71E-21
RelativeHumidity * Time	2	1846.914	403.528	2.11E-131
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	840.548	183.649	9.78E-39
Grain * Refinement * Time	2	204.240	44.624	2.42E-19
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	152.211	33.256	9.70E-15
Refinement * Steam * RelativeHumidity	2	23.294	5.089	6.31E-03
Refinement * Steam * Time	4	226.297	49.443	3.24E-38
Refinement * RelativeHumidity * Time	4	270.695	59.143	3.69E-45
Steam * RelativeHumidity * Time	2	712.983	155.778	4.02E-60
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	120.219	26.266	7.30E-12
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	204.538	44.689	9.71E-35
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	1073	4.577		

Table 80: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on extensibility.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	190925	28646	0.00E+00
Refinement	2	29773	4467	0.00E+00
Steam	1	145	22	3.59E-06
RelativeHumidity	1	13755	2064	3.36E-252
Time	2	3943	592	6.56E-174
Grain * Refinement	1	32774	4917	0.00E+00
Grain * Steam	0			
Grain * RelativeHumidity	1	10612	1592	3.23E-214
Grain * Time	2	3416	512	5.94E-157
Refinement * Steam	2	0	0	9.70E-01
Refinement * RelativeHumidity	2	605	91	3.95E-37
Refinement * Time	4	128	19	2.57E-15
Steam * RelativeHumidity	1	83	12	4.47E-04
Steam * Time	2	12	2	1.78E-01
RelativeHumidity * Time	2	657	99	4.92E-40
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	2908	436	1.42E-81
Grain * Refinement * Time	2	533	80	4.00E-33
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	287	43	1.02E-18
Refinement * Steam * RelativeHumidity	2	75	11	1.41E-05
Refinement * Steam * Time	4	25	4	4.97E-03
Refinement * RelativeHumidity * Time	4	1420	213	1.61E-134
Steam * RelativeHumidity * Time	2	32	5	7.93E-03
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	3532	530	8.30E-161
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	10	1	2.19E-01
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	1073	7		

Table 81: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on peak maximum time.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	38618.598	2996.910	2.04E-70
Refinement	2	636.297	49.378	2.93E-14
Steam	1	520.001	40.354	5.00E-08
RelativeHumidity	1	56.221	4.363	4.61E-34
Time	2	2915.967	226.287	4.97E-02
Grain * Refinement	1	846.598	65.698	4.03E-10
Grain * Steam	0			
Grain * RelativeHumidity	1	41.070	3.187	1.55E-23
Grain * Time	2	1425.926	110.656	9.26E-02
Refinement * Steam	2	116.616	9.050	4.74E-05
Refinement * RelativeHumidity	2	57.207	4.439	6.36E-03
Refinement * Time	4	54.504	4.230	2.12E-02
Steam * RelativeHumidity	1	7.344	0.570	7.00E-02
Steam * Time	2	38.944	3.022	4.74E-01
RelativeHumidity * Time	2	12.244	0.950	4.26E-01
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	66.270	5.143	9.07E-01
Grain * Refinement * Time	2	1.388	0.108	3.35E-02
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	31.455	2.441	1.15E-01
Refinement * Steam * RelativeHumidity	2	5.143	0.399	2.30E-02
Refinement * Steam * Time	4	42.472	3.296	6.97E-01
Refinement * RelativeHumidity * Time	4	10.839	0.841	5.52E-01
Steam * RelativeHumidity * Time	2	11.411	0.885	4.51E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	21.404	1.661	2.27E-01
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	15.735	1.221	3.58E-01
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	101	12.886		

Table 82: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on peak maximum torque.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	3197.959	167.702	1.05E-22
Refinement	2	246.583	12.931	4.56E-07
Steam	1	1632.716	85.620	1.54E-14
RelativeHumidity	1	208.740	10.946	9.58E-04
Time	2	149.636	7.847	1.68E-03
Grain * Refinement	1	320.257	16.794	2.96E-04
Grain * Steam	0			
Grain * RelativeHumidity	1	0.563	0.030	9.21E-02
Grain * Time	2	48.737	2.556	8.67E-01
Refinement * Steam	2	60.374	3.166	1.89E-03
Refinement * RelativeHumidity	2	116.737	6.122	8.05E-07
Refinement * Time	4	202.096	10.598	4.04E-03
Steam * RelativeHumidity	1	81.046	4.250	8.18E-02
Steam * Time	2	51.220	2.686	4.65E-02
RelativeHumidity * Time	2	99.693	5.228	8.66E-03
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	1.203	0.063	3.73E-03
Grain * Refinement * Time	2	118.532	6.216	8.06E-01
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	14.657	0.769	4.82E-01
Refinement * Steam * RelativeHumidity	2	61.239	3.211	7.51E-02
Refinement * Steam * Time	4	43.807	2.297	5.10E-02
Refinement * RelativeHumidity * Time	4	6.509	0.341	8.59E-01
Steam * RelativeHumidity * Time	2	14.846	0.779	4.77E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	35.580	1.866	1.73E-01
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	108.264	5.677	5.75E-04
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	101	19.069		

Table 83: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on aggregation energy.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	57308	0.095	0.759
Refinement	2	15191	0.025	0.975
Steam	1	2209758	3.651	0.059
RelativeHumidity	1	103608	0.171	0.680
Time	2	143734	0.237	0.789
Grain * Refinement	1	684571	1.131	0.290
Grain * Steam	0			
Grain * RelativeHumidity	1	1031550	1.704	0.195
Grain * Time	2	1398361	2.310	0.104
Refinement * Steam	2	542413	0.896	0.411
Refinement * RelativeHumidity	2	287699	0.475	0.623
Refinement * Time	4	633613	1.047	0.387
Steam * RelativeHumidity	1	527751	0.872	0.353
Steam * Time	2	888287	1.468	0.235
RelativeHumidity * Time	2	96448	0.159	0.853
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	1113488	1.840	0.178
Grain * Refinement * Time	2	1337756	2.210	0.115
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	1421809	2.349	0.101
Refinement * Steam * RelativeHumidity	2	666744	1.102	0.336
Refinement * Steam * Time	4	703371	1.162	0.332
Refinement * RelativeHumidity * Time	4	463592	0.766	0.550
Steam * RelativeHumidity * Time	2	976301	1.613	0.204
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	1272540	2.102	0.127
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	771148	1.274	0.285
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	101	605282		

Table 84: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on pasting temperature.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	0.160	0.000	0.993
Refinement	2	2383.210	1.203	0.304
Steam	1	1867.508	0.943	0.334
RelativeHumidity	1	1809.910	0.913	0.341
Time	2	2116.037	1.068	0.347
Grain * Refinement	1	134.354	0.068	0.795
Grain * Steam	0			
Grain * RelativeHumidity	1	0.411	0.000	0.989
Grain * Time	2	2.790	0.001	0.999
Refinement * Steam	2	1873.352	0.945	0.392
Refinement * RelativeHumidity	2	2710.970	1.368	0.259
Refinement * Time	4	2968.233	1.498	0.208
Steam * RelativeHumidity	1	2999.921	1.514	0.221
Steam * Time	2	2829.265	1.428	0.244
RelativeHumidity * Time	2	2350.445	1.186	0.309
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	3.696	0.002	0.966
Grain * Refinement * Time	2	9.087	0.005	0.995
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	2.643	0.001	0.999
Refinement * Steam * RelativeHumidity	2	2874.721	1.451	0.239
Refinement * Steam * Time	4	2889.892	1.458	0.220
Refinement * RelativeHumidity * Time	4	2903.012	1.465	0.218
Steam * RelativeHumidity * Time	2	2832.063	1.429	0.244
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	0.150	0.000	1.000
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	2930.597	1.479	0.214
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	107	1981.414		

Table 85: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on peak viscosity.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	16103167	88607.440	3.65E-146
Refinement	2	241219.649	583.279	8.45E-43
Steam	1	1871.876	4.526	1.49E-04
RelativeHumidity	1	33762.326	81.639	1.19E-12
Time	2	50106.777	121.160	6.20E-09
Grain * Refinement	1	280746.698	678.857	1.29E-17
Grain * Steam	0			
Grain * RelativeHumidity	1	30160.213	72.929	1.01E-01
Grain * Time	2	2489.656	6.020	1.93E-01
Refinement * Steam	2	2509.356	6.068	4.73E-01
Refinement * RelativeHumidity	2	3823.576	9.246	1.77E-01
Refinement * Time	4	617.709	1.494	1.52E-02
Steam * RelativeHumidity	1	175.634	0.425	1.30E-01
Steam * Time	2	2581.187	6.241	7.68E-01
RelativeHumidity * Time	2	8504.763	20.565	5.98E-06
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	1264.853	3.058	1.89E-02
Grain * Refinement * Time	2	1563.861	3.781	3.40E-02
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	20752.921	50.181	2.67E-02
Refinement * Steam * RelativeHumidity	2	260.312	0.629	4.69E-01
Refinement * Steam * Time	4	1207.145	2.919	5.22E-01
Refinement * RelativeHumidity * Time	4	4549.800	11.002	2.59E-04
Steam * RelativeHumidity * Time	2	542.863	1.313	7.08E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	7463.891	18.048	5.53E-01
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	1521.362	3.679	3.37E-01
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	107	413.558		

Table 86: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on hold viscosity.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	7610518.52	95157.420	1.22E-159
Refinement	2	13478.272	168.524	8.60E-34
Steam	1	330.750	4.136	4.45E-02
RelativeHumidity	1	12784.280	159.847	9.78E-28
Time	2	9404.032	117.582	5.78E-23
Grain * Refinement	1	11632.752	145.449	1.15E-21
Grain * Steam	0			
Grain * RelativeHumidity	1	158.413	1.981	1.77E-10
Grain * Time	2	2231.442	27.901	1.62E-01
Refinement * Steam	2	109.702	1.372	2.58E-01
Refinement * RelativeHumidity	2	279.921	3.500	9.04E-01
Refinement * Time	4	20.671	0.258	3.37E-02
Steam * RelativeHumidity	1	3.167	0.040	6.38E-02
Steam * Time	2	225.890	2.824	8.43E-01
RelativeHumidity * Time	2	3916.905	48.975	7.92E-16
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	1	711.480	8.896	3.12E-02
Grain * Refinement * Time	2	286.534	3.583	3.54E-03
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	345.339	4.318	1.57E-02
Refinement * Steam * RelativeHumidity	2	13.090	0.164	1.62E-01
Refinement * Steam * Time	4	133.590	1.670	8.49E-01
Refinement * RelativeHumidity * Time	4	467.307	5.843	2.72E-04
Steam * RelativeHumidity * Time	2	19.316	0.242	7.86E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	458.605	5.734	4.31E-03
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	158.800	1.986	1.02E-01
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	107	79.978		

Table 87: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on final viscosity.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	36644283.070	88607.440	1.58E-149
Refinement	2	241219.649	583.279	9.62E-169
Steam	1	1871.876	4.526	5.51E-158
RelativeHumidity	1	33762.326	81.639	2.84E-58
Time	2	50106.777	121.160	3.57E-02
Grain * Refinement	1	280746.698	678.857	3.23E-28
Grain * Steam	0			7.74E-15
Grain * RelativeHumidity	1	30160.213	72.929	3.88E-48
Grain * Time	2	2489.656	6.020	
Refinement * Steam	2	2509.356	6.068	3.33E-03
Refinement * RelativeHumidity	2	3823.576	9.246	1.00E-13
Refinement * Time	4	617.709	1.494	3.19E-03
Steam * RelativeHumidity	1	175.634	0.425	2.09E-01
Steam * Time	2	2581.187	6.241	1.98E-04
RelativeHumidity * Time	2	8504.763	20.565	2.73E-03
Grain * Refinement * Steam	0			5.16E-01
Grain * Refinement * RelativeHumidity	1	1264.853	3.058	2.77E-08
Grain * Refinement * Time	2	1563.861	3.781	
Grain * Steam * RelativeHumidity	0			2.59E-02
Grain * Steam * Time	0			8.32E-02
Grain * RelativeHumidity * Time	2	20752.921	50.181	
Refinement * Steam * RelativeHumidity	2	260.312	0.629	
Refinement * Steam * Time	4	1207.145	2.919	4.24E-16
Refinement * RelativeHumidity * Time	4	4549.800	11.002	2.46E-02
Steam * RelativeHumidity * Time	2	542.863	1.313	5.35E-01
Grain * Refinement * Steam * RelativeHumidity	0			1.64E-07
Grain * Refinement * Steam * Time	0			2.73E-01
Grain * Refinement * RelativeHumidity * Time	2	7463.891	18.048	
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	1521.362	3.679	1.76E-07
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	107	413.558		7.57E-03

Table 88: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on breakdown value.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	########	8136.675	8.88E-103
Refinement	2	29704.931	153.664	3.50E-32
Steam	1	2523.000	13.051	4.63E-04
RelativeHumidity	2	393.488	2.036	1.36E-01
Time	1	10.074	0.052	8.20E-01
Grain * Refinement	1	4992.297	25.825	1.60E-06
Grain * Steam	0			
Grain * RelativeHumidity	2	518.520	2.682	7.30E-02
Grain * Time	1	1252.563	6.480	1.23E-02
Refinement * Steam	2	35.071	0.181	8.34E-01
Refinement * RelativeHumidity	4	496.016	2.566	4.23E-02
Refinement * Time	2	395.555	2.046	1.34E-01
Steam * RelativeHumidity	2	126.246	0.653	5.23E-01
Steam * Time	1	11.368	0.059	8.09E-01
RelativeHumidity * Time	2	3.195	0.017	9.84E-01
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	2	616.138	3.187	4.52E-02
Grain * Refinement * Time	1	114.083	0.590	4.44E-01
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	478.049	2.473	8.91E-02
Refinement * Steam * RelativeHumidity	4	42.698	0.221	9.26E-01
Refinement * Steam * Time	2	109.610	0.567	5.69E-01
Refinement * RelativeHumidity * Time	4	565.597	2.926	2.43E-02
Steam * RelativeHumidity * Time	2	109.892	0.568	5.68E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	435.264	2.252	1.10E-01
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	203.739	1.054	3.83E-01
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	107	193.312		

Table 89: Analysis of variance on the individual effects and interactions of grain type, refinement, steam treatment, relative humidity, and storage time on setback value.

Source or Variation	Degrees of Freedom	Mean Square	F Value	Significance (P-Value)
Grain	1	########	39789.723	2.04E-139
Refinement	2	########	515.631	1.16E-55
Steam	1	628.939	2.305	1.32E-01
RelativeHumidity	2	16278.793	59.669	3.91E-18
Time	1	4995.335	18.310	4.10E-05
Grain * Refinement	1	########	652.762	2.37E-47
Grain * Steam	0			
Grain * RelativeHumidity	2	554.206	2.031	1.36E-01
Grain * Time	1	34690.253	127.156	6.59E-20
Refinement * Steam	2	1584.465	5.808	4.03E-03
Refinement * RelativeHumidity	4	621.005	2.276	6.58E-02
Refinement * Time	2	2035.105	7.460	9.27E-04
Steam * RelativeHumidity	2	1280.194	4.693	1.11E-02
Steam * Time	1	131.634	0.482	4.89E-01
RelativeHumidity * Time	2	879.217	3.223	4.37E-02
Grain * Refinement * Steam	0			
Grain * Refinement * RelativeHumidity	2	580.323	2.127	1.24E-01
Grain * Refinement * Time	1	79.053	0.290	5.91E-01
Grain * Steam * RelativeHumidity	0			
Grain * Steam * Time	0			
Grain * RelativeHumidity * Time	2	18389.354	67.406	1.14E-19
Refinement * Steam * RelativeHumidity	4	558.286	2.046	9.30E-02
Refinement * Steam * Time	2	173.389	0.636	5.32E-01
Refinement * RelativeHumidity * Time	4	2152.319	7.889	1.30E-05
Steam * RelativeHumidity * Time	2	371.356	1.361	2.61E-01
Grain * Refinement * Steam * RelativeHumidity	0			
Grain * Refinement * Steam * Time	0			
Grain * Refinement * RelativeHumidity * Time	2	4248.669	15.573	1.16E-06
Grain * Steam * RelativeHumidity * Time	0			
Refinement * Steam * RelativeHumidity * Time	4	702.812	2.576	4.16E-02
Grain * Refinement * Steam * RelativeHumidity * Time	0			
Error	107	272.816		

Appendix G: Functionality Correlation

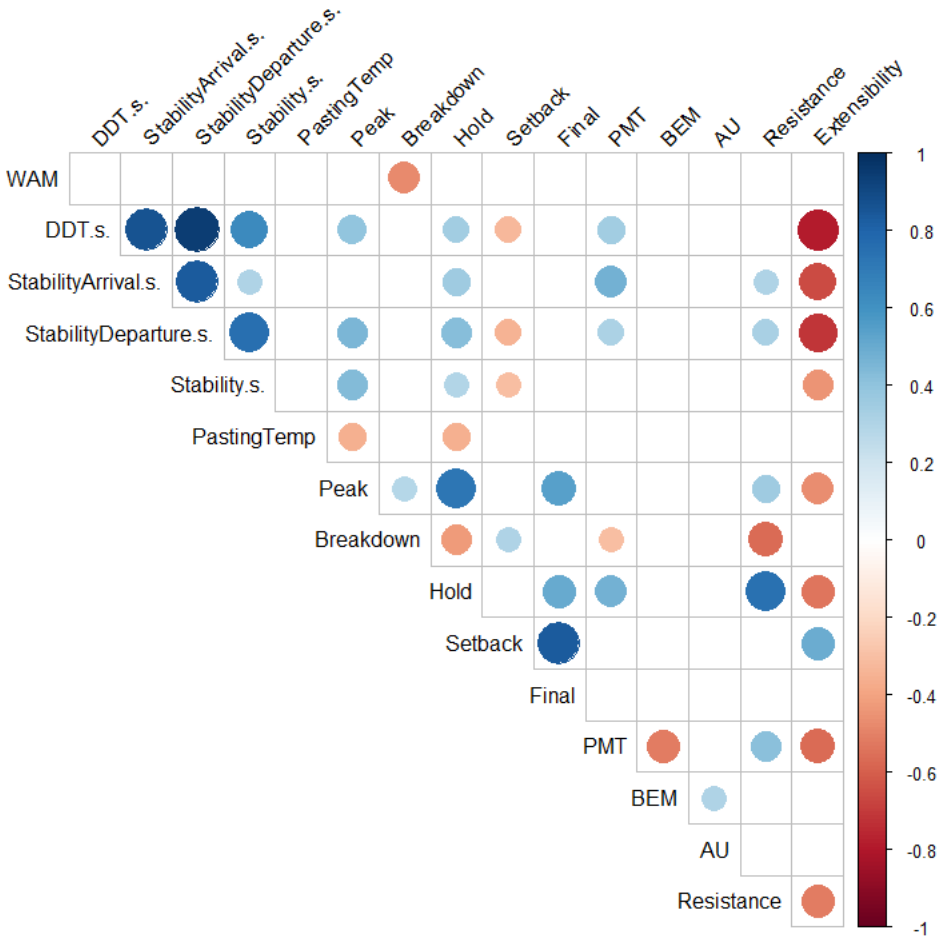

Figure 25: Visual representation of correlation data depicting relationships among functionality properties. The presence of a color dot between two attributes indicates a significant correlation ($P \leq 0.05$) according to the color scale, where blue is a positive correlation, and red is a negative correlation.

Table 90: Correlation data depicting relationships among functionality parameters over storage.

	WAM	DDT	Stability Arrival	Stability Departure	Stability	Pasting Temp	Peak	Breakdown	Hold	Setback	Final	PMT	MT	Aggregation Energy	Resistance to Extension
WAM															
DDT.s.	-0.09														
Stability Arrival.s.	-0.05	0.86****													
Stability Departure.s.	-0.08	0.94****	0.84****												
Stability.s.	-0.07	0.64****	0.31*	0.76****											
PastingTemp	0.10	-0.25	-0.18	-0.26	-0.21										
Peak	-0.26	0.40**	0.28	0.45**	0.43**	-0.36*									
Breakdown	-0.48***	0.02	-0.15	-0.04	0.11	0.06	0.29*								
Hold	0.07	0.34*	0.36*	0.42**	0.30*	-0.36*	0.72****	-0.42**							
Setback	-0.14	-0.33*	-0.26	-0.34*	-0.31*	0.06	0.17	0.30*	-0.05						
Final	-0.08	-0.10	-0.03	-0.06	-0.10	-0.14	0.54****	0.03	0.50***	0.84****					
PMT	0.02	0.35*	0.48***	0.31*	-0.02	-0.20	0.25	-0.31*	0.48***	-0.21	0.08				
BEM	-0.09	-0.15	-0.16	-0.10	0.04	0.13	0.07	0.02	0.04	0.11	0.12	-0.51***			
AU	-0.08	-0.01	-0.04	-0.02	-0.02	0.06	0.11	0.16	-0.04	0.06	0.03	-0.04	0.31*		
Resistance to Extension	0.07	0.28	0.30*	0.32*	0.23	-0.06	0.35*	-0.56****	0.74****	-0.26	0.18	0.42**	0.13	-0.03	
Extensibility	0.14	-0.79****	-0.65****	-0.71****	-0.45**	0.22	-0.46***	0.10	-0.53****	0.50***	0.14	-0.56****	0.17	-0.04	-0.51***