BAKING PROPERTIES AND RESISTANT STARCH CONTENTS OF GLUTEN-FREE BUCKWHEAT-RICE BREAD FORMULATIONS

By

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ABSTRACT

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Celiac disease drastically limits the dietary options available to those afflicted and eliminates a major food staple, bread. Buckwheat offers an alternative that can provide additional health benefits compared to rice flour alone. The aims of this study were (1) to develop a base buckwheat-rice flour containing bread formulation, and (2) to determine effects on baked properties and resistant starch contents of breads made with varying levels of canola oil [(4% or 8% flour weight basis (fwb)], egg white solids (5% or 10% fwb), batter consistency (100 B.U. or 125 B.U.), and mixing time (5 min, 10 min, or 15 min). Breads were baked, stored at ambient temperature under sealed conditions, and evaluated for appearance, specific volume, and hardness on day three, and for resistant starch content and moisture on day seven. A lower batter consistency decreased the appearance of cracking on the baked crust surface with more open cell structure in the crumb. Lower consistency batters produced breads with higher specific volumes (1.84 to 2.58 cm^3/g) compared to those of higher consistency batters (1.69 to 2.27 cm^3/g). Crumb hardness was decreased to below 100 N for all breads made from lower consistency batters. For the same batter consistency level, variation in mixing time produced some significant differences in specific volume and hardness of bread loaves. The resistant starch contents of breads after storage for 7 days were not affected by differences in canola oil or egg white solids contents, but were significantly different ($p<0.05$) between 5% egg/4% oil and 5% egg/8% oil (fwb) counterpart samples made from batters with different consistencies.

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CHAPTER 1:

INTRODUCTION AND OBJECTIVES

Celiac disease greatly limits the dietary options available to those with the disease and leads to the necessary removal of an important staple, bread. The only treatment for the disease is to avoid gluten-type proteins, like those found in wheat bread. Unfortunately, the response-eliciting proteins are responsible for giving the products in which they are used their desirable eating properties. Therefore a challenge exists in recreating a bread-type product without the use of wheat and calls for alternative approaches to create products for those suffering from Celiac Disease.

The purpose of this research was to investigate buckwheat's potential in breadmaking for producing alternative gluten-free products by measuring changes in physiochemical properties by varying the initial batter viscosity, contents of egg white solids and oil in the formulation, and mixing times in the processing stage. Additionally, by focusing on and increasing resistant starch, buckwheat may play an important role in future convenience functional food products. This work intends to lay a foundation for the expansion of buckwheat utilization by improving the nutritional and textural profile of common products, such as yeast leavened bread, using egg white solids as a protein scaffolding. The objectives of this study were to:

1. Develop a base buckwheat-rice bread formulation.
2. Measure differences in batter rheological properties.
3. Conduct quality evaluations of finished loaves.
4. Measure the effect of different ingredient levels in the formulation on the final resistant starch content of the baked bread loaves.

CHAPTER 2:

LITERATURE REVIEW

2.1 Celiac Disease and Gluten

Celiac disease (CD) is an autoimmune disorder of humans that results in inflammation of the upper small intestine triggered by an immune response when “gluten” proteins are ingested. The small, finger-like villi in the small intestine are an important site for essential nutrient absorption, but during the response they become damaged which reduces nutrient uptake in those suffering from CD. The damage occurs as peptides from digested gluten proteins traverse the epithelial cells of the small intestine where genes present in CD patients are expressed in antigen-presenting cells which cascade the reactions that lead to the destruction of the villi (Koehler 2011). Symptoms for CD patients vary in intensity making detection a challenge, but include diarrhea, vomiting, growth problems, and susceptibility to nutrition deficiency diseases (Wieser and Koehler 2009). Continuing research is being conducted for a more thorough understanding of the roles of genetics and environmental influences as genetics seems to be only a part of the equation for CD development. With three million people suffering from CD in the United States and an increasing number of studies showing the long term effect of undiagnosed CD and its relation to osteoporosis, infertility, neurological disorders, and even cancer, a growing demand for gluten-free products is inevitable (University of Chicago 2007). The increasing awareness, detection of celiac disease, and growing media attention has promoted expanded research of non-gluten containing products and processing techniques.

Response-eliciting proteins have been broadly coined as “gluten” and associated with wheat, but this nomenclature does not accurately portray the vast ranges and sources of the peptides. The proteins in cereal grains are divided into two broad categories; metabolic proteins and storage proteins (Shewry and Halford 2002). The metabolic proteins are further divided into water-soluble albumins and salt-soluble globulins, with the general consensus of metabolic proteins not being responsible for induction of celiac disease. The storage proteins include two broad categories including alcohol-soluble prolamines and alcohol-insoluble glutelins. The prolamines (known as gliadins in wheat, hordeins in barley, and secalins in rye) are regarded as the main trigger for the immune response in the digestive tract (Lasztity 1996). Specific patterns of amino acid sequences within the prolamines are thought to elicit immune responses though this is still speculation and further research is needed, but those peptides are high in proline, glycine, and glutamine which create specific secondary protein conformations that are resistant to the proteolytic digestive enzymes (Wieser and Koehler 2008). Traditional cereal grains come from grasses, the Triticeae family, but exploring alternative sources such as buckwheat could avoid the response-eliciting proteins and could be used as a safe alternative (Hager et al. 2012).

2.2. Buckwheat

Buckwheat (*Fagopyrum esculentum*) has its roots in southeast China and grows best in cool moist climates, but is adaptable to a wide range of soil conditions including acidic or low nutrient soils. In the United States, buckwheat is grown across the northern regions. Characteristics of buckwheat include a fast growing nature and relatively high resistance to pests. The plant typically grows to five feet with broad leaves and is closely related to sorrels and rhubarb (Valenzuela and Smith 2002). China, Russia, and Ukraine are the three leading producers with 564,000, 570,000, and 188,000 metric tons annually, respectively. The United States produces 86,000 metric tons annually (FAOSTAT 2009). The crop is not only used for its edible seed, but also for weed control due to the ability to outgrow weeds as a cover crop and its ability to improve soil conditions by freeing phosphorus. The seeds produced by the plant are triangular in shape resembling a pyramid with the approximate length of a grain of wheat. The tough hull is typically removed before consumption and can be used for mulching or buckwheat pillows (Canadian Special Crops Association 1987). The seed is used for production of many traditional cereals, porridges, and traditional noodles. Buckwheat has a rich nutritional profile with higher protein content and amino acid diversity than common cereal crops making it an acceptable alternative in gluten-free products (Guo and Yao 2006).

Buckwheat falls into a broad category of grains known as “pseudocereals” and differentiates from other true cereal crops in being classified as a *dicotyledonae* as are amaranth and quinoa. Traditional cereal crops are classified as *monocotyledonae* and include wheat, barley, rye, and oats (Berghofer and Schoenlechner 2009). Due to the different lineages, proteins found in the prolamin fraction of wheat, barley, rye, and oats are absent in pseudocereals. The prolamin component of buckwheat is much lower in glutamine and proline contents compared to that of other grass family grains and also present as a lower proportion of the total protein, which may be why buckwheat can be successfully incorporated into a CD patient’s diet (Kusano et al. 2001). Furthermore, a rich profile of essential amino acids, fatty acids, minerals, and vitamins makes buckwheat an alluring alternative to traditional nutrient-sparse starch-based gluten-free foods (Takahama et al. 2011). Rice is among the most commonly used replacements in a CD diet. However, buckwheat provides advantages in its nutritional profile when compared to rice. Buckwheat has a higher protein content of 12.2% compared to rice at 7.3%. Buckwheat flour was found to have 2.2% (w/w) dietary fiber compared to rice flour at 0.4% (w/w) dietary fiber and also has a higher fat content which contains high levels of unsaturated fatty acids (Hager et al. 2012). Despite the advantages, buckwheat has seen limited albeit increasing use in products like soba noodles, buckwheat pancakes, and traditional porridges in the United States. However, because of buckwheat's mild and pleasant taste, and previous exposure to a wide consumer base, buckwheat has a strong potential to help fill the gap in gluten-free bread products.

2.3 Gluten-free bread

Unfortunately for celiac patients, the same gluten proteins (specifically gliadins) that are responsible for eliciting a negative reaction are also partially responsible for the unique and desirable qualities of wheat flour for breadmaking. Hydrogen bonds and covalent disulfide cross-linking of two protein fractions, gliadins and glutenins, occur during the hydration of the flour and mixing of the dough to produce an elastic and extensible gluten network that traps carbon dioxide during yeast fermentation, giving a light airy product, and providing chewiness to the baked bread. The alignment of the proteins that occurs during the hydration and mixing creates the network as the smaller gliadin proteins act as lubricants between the long extensible sulfur-rich glutenins. (Reuben 2009). The limitation in elasticity of gluten-free bread formulations does not allow for gasses being produced by the yeast to be captured by a non-existent gluten network, and results in a dense finished product. Therefore gluten-free bread "dough" consistency is more comparable to a cake batter than a traditional wheat-based dough to allow for creation of air cells within the matrix. The challenge is in keeping the air cells intact throughout the proofing and baking steps and not collapsing from the pressure of the batter. Other non-wheat flours may have similar levels of proteins but do not possess the unique intermolecular interactions seen in wheat. Therefore a challenge exists in recreating the quality of wheat-based products from other grains.

Hydrocolloids, fibers, and pregelatinized starches have been incorporated into gluten-free bread formulations producing acceptable results, but the products still do not compare to their wheat counterparts in terms of flavor, texture, and nutrition (Hager et al. 2012, Lazaridou et al. 2007, Mariotti et al. 2009). As mentioned, the primary challenge with gluten-free breadmaking is mimicking the light, open, honeycomb structure formed by gluten proteins which trap gasses during the yeast fermentation and baking stages. Hydrocolloids such as guar gum, carboxymethyl cellulose, pectin, agarose, and beta-glucans, each used at 1% flour weight basis (fwb), have been shown to improve loaf volume by increasing dough viscosity, increasing gas retention, and forming cohesive gel networks during baking (Lazaridou et al. 2007). The addition of soluble fiber fractions of rice bran has also been shown to improve specific volume of rice-based gluten-free breads through increased dough viscosity (Phimolsiripol et al. 2012). Hydrocolloids have also been shown to mitigate the staling of bread by reducing the migration of water within the finished product and creating a softer crumb texture (Sciarini et al. 2010). Guar gum is a polysaccharide consisting of a mannose backbone with galactose side groups and has a high water absorption ability. It works by thickening and helping in the formation of a network to trap gases during proofing and baking (Wielinga and Maehall 2000).

Several studies have investigated the inclusion of egg white powder at varying ratios as a protein scaffolding to improve volume (Crockett et al. 2011, Boswell 2010). It is well established that the whipping of eggs whites partially denatures the proteins and allows incorporation of air, producing foam with increased volume. The partially denatured proteins are able to form a viscoelastic film at the air-water interface and could be exploited in the formation of a gluten-free bread with satisfactory volume (Mine 1995). Besides improving the volume and texture of bread products, the addition of egg white solids also improves the protein quality and of the end product. The challenge in using egg white solids is the associated ingredient cost and additional allergen content.

Currently, a majority of the commercially available gluten-free baked products and flour mixes consist primarily of rice flour and native or pregelatinized starches from tapioca, potato, and corn. These make an acceptable alternative to consumers but present an inferior nutritional profile, especially in protein and fiber, compared to wheat-based baked products. Furthermore, gluten-free products often do not have the mandated fortification and enrichment with micronutrients associated with wheat-based products (Gallagher et al. 2004). The exploration of gluten-free alternatives has brought pseudocereals such as buckwheat to the forefront to address these issues. Some studies have already shown that buckwheat bread may have some consumer acceptance as rice and buckwheat breads had equal liking sensory scores, although both scored significantly lower than wheat bread (Hager et al. 2012). Dietary fiber (DF) is higher in buckwheat flour than in rice flour, but the DF may be further enhanced by increasing the resistant starch content. An opportunity exists to produce products of enhanced nutritional value while still maintaining their gluten-free status. Work is necessary to better understand the textural, visual, and finished product volume from the use of gluten-free ingredients such as buckwheat, and how buckwheat can be used in product formulations for the best overall bread products.

2.4. Resistant Starch Chemistry

Recently, the acceptance of benefits offered by resistant starch (RS) has led researchers to investigate raw ingredients and processing conditions to increase RS content in their final products. Resistant starch is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp 1992). Resistant starch has been divided into four categories; RS1, RS2, RS3, and RS4 (Englyst et al. 1992). RS1 is entrapped within the food matrix and enzymes cannot gain access to it. Physically disrupting the food matrix, such as by milling or chewing, may decrease the level of resistance of RS1. RS2 is resistant due to conformation of the starch granule. RS2's high density and crystalline structure diminish the effects of digestive enzymes. This type of RS is most commonly found in high amounts in raw green bananas and raw potatoes.

RS3 is unique in that it can be increased through processing (Yadav et al. 2009, Mahadevamma et al. 2004, Kim et al. 2006) and is formed through the gelatinization and retrogradation of starch granules. The recrystallization, mainly attributed to the amylose fraction of starch, creates non-digestible structures (Brown et al. 1998). Higher levels of resistant starch formation during specific baking conditions were found in flours with higher initial amylose contents (Akerberg et al. 1997). The process of RS3 formation begins with the gelatinization of the native starch granule. In the presence of heat and water, the starch granule begins to absorb water, lose its original crystalline structure and allow amylose fractions to leach out. As the gelatinized starch cools, retrogradation begins and amylose chains begin to aggregate, forming double

The amount of RS3 formed during retrogradation is dependent on a variety of factors. As RS3 is primarily composed of amylose, naturally a higher initial amylose content of a starch favors increased RS3 production. On the other hand, lipids play an inhibitory role in the formation of RS3 complexes. Naturally occurring as well as added lipids can reduce the formation of RS. The presence of lipids results in amylose-lipid complexes, reducing the formation of amylose-amylose double helices and overall RS3 formation (Kaur and Singh 2000).

Degree of polymerization of amylose molecules in starch also affects the rate at which retrogradation progresses and under which temperature conditions RS3 formation is most favorable (Annison and Topping 1994). Gidley and Bulpin (1989) found a variety of factors which affected the extent to which amylose precipitated upon retrogradation. Chain length (shorter chains being preferred for increased precipitation), rate of cooling (with slower rates increasing precipitation), agitation (increased precipitation over stationary), and amylose concentration (lower concentration increased RS formation) were all factors in determining the amount of resistant starch produced.

RS4 is the category of starches that have been cross-linked using chemical treatment with propylene oxide or acetic anhydride to incur resistance to enzymatic breakdown (Chung et al 2008). RS4 has resistant functionality through increasing the hydrophobicity of the starch granules and decreasing its exposure to degradation enzymes. RS4 is sold commercially as an ingredient to increase the fiber content of foods to which they have been added and also marketed as offering additional benefits like increasing crispness in baked products and creating a fatty texture.

2.5 Resistant Starch and Human Health

Another shortcoming of a gluten-free diet is the consumption of a high proportion of low fiber products. According to definition of the American Association of Cereal Chemists Internationals (AACCI), dietary fiber includes a wide range of different compounds including cellulose, hemicellulose, lignin, pectin, gums, beta-glucans, some oligosaccharides, and resistant starch (AACCI 2001). Each dietary fiber has its own unique physical and physiological properties in the body. Of particular interest in the present study is resistant starch.

Resistant starch (RS) has been shown to have many benefits on human health. Previous studies have proven resistant starch fermentation by bacterial flora of the large intestine, leading to an overall increase in intestinal microbial growth and total short chain fatty acids (SCFA), byproducts of colonic bacterial fermentation. In a 1994 study, butyrate found in fecal samples increased by 47% and acetate increased by 35% after RS consumption (Van Muster et al. 1994). Other *in vivo* testing with rats and *in vitro* testing both confirm that increased RS consumption increases rates of SCFA production, fecal bulking, and anaerobic bacterial counts. Butyric acid fecal levels increased most significantly, but with acetic and propionic acids also increasing. (Mahadevamma et al. 2004).

Studies have shown that an increase in SCFA concentration, especially of butyrate, can result in physiological health benefits through a variety of mechanisms. SCFA lower the overall pH of the colon which inhibits the growth of potentially pathogenic bacteria that could colonize the colon (Birkett and Brown 2008). Organic acids lower colon pH, reducing the growth of other non-acid-tolerant potentially pathogenic bacteria and reduce secondary bile acids which are cytotoxic to cells (Topping et al. 1993, Van Muster et al. 1994). A lower pH changes the microflora makeup of the large intestine. An increase in percentage was seen in the beneficial bacteria of the group Bacteroidetes after rats were fed a high amylose maize diet (Abbel et al. 2011). Butyrate is a source of energy for colonocytes, colonic epithelial cells that have been shown to reduce risks of colon cancer by increasing apoptosis of cancer cells and reducing the growth of new cancer cells in *in vivo* studies of rats (Hague et al. 1993, Bird et al. 2000, Smith et al. 1998). Micronutrients are also better absorbed at a lower pH due to increased solubility. Moreover, many harmful alkaline toxins are less well absorbed at a low pH (Bird et al. 2000).

Besides fermentation into SCFA in the colon, RS also has other traditional benefits common to other dietary fibers. RS has lower caloric content than easily digestible starch because it is not broken down to glucose and absorbed in the small intestine, but rather is converted into SCFA and gases in the colon. There is also potential for RS to be used as a delivery system for probiotics (microorganisms important for intestinal health) into the lower portion of the small intestine as well as acting as a prebiotic (energy source for microorganisms found in the intestinal tract). RS granules have pits and pockets which could allow for the adherence and safer transportation of probiotics through the human digestive tract (Bird et al. 2000). Earlier studies showed that many probiotics did not survive to colonize the large intestine due to the harsh conditions of the stomach and small intestine. Providing a delivery system could increase the amount of probiotics reaching and colonizing the large intestine (Brown et al. 1998).

Despite the many benefits associated with resistant starch, the average American consumes fewer than 5 grams/day (Murphy et al. 2008). Many other developed countries show similar low levels of consumption (Birkett and Brown 2008). This value is far below the 20 grams/day that have been shown to yield positive health effects. Therefore a strong need and market exist for creating products that contain higher levels of resistant starch and could present an opportunity for marketing new functional foods. Although resistant starch exists naturally in many food sources, the levels at which they are consumed are not adequate to reach the full health benefits (Baghurst et al. 1996). Many people affected by celiac disease may have an especially low dietary fiber and resistant starch intake due to gluten-free foods being produced from highly refined unfortified flours and starches. A need exists to create a staple product like bread that is both gluten-free and high in dietary fiber and, specifically, high in resistant starch which may be particularly important in the case of those with celiac disease.

Besides physiological benefits, RS also can improve the overall perceived quality of a product. Increased resistant starch can increase the fiber content of a product, lower the caloric value, and impart new sensory properties such as improved crispness, color, and mouth feel (Waring 1998). Finding the best conditions for highest yield of resistant starch can be valuable in formulation of new products of potentially functional ingredients. Previous studies have shown that resistant starch can be increased through normal processing conditions. Yadav et al. (2009) increased RS in a variety of crops including beans, peas, wheat, and potatoes after autoclaving. The RS content was further enhanced by sequential heating and cooling treatments. Gonzalez-Soto et al. (2006) used extrusion cooking to increase the RS content of bananas and mangos. Extruded pastry wheat flour had elevated levels of resistant starch after extrusion cooking and cold storage (Kim et al. 2006). The logical conclusion is that the buckwheat RS profile can also be increased through processing and storage conditions.

CHAPTER 3:

MATERIALS AND METHODS

3.1 Experimental Design

In this study, formulations with a buckwheat flour and rice flour base (3:1 blend, w/w) with egg white solids (5% or 10% fwb), canola oil (4% or 8% fwb), water addition for two viscosity levels (100 B.U. or 125 B.U.), and with mixing at three different mixing times (5 minutes, 10 minutes, or 15 minutes) were evaluated for their effects on overall baked bread appearance, moisture levels, bake loss, final baked volume, crumb hardness, and resistant starch content in a gluten-free bread system . The overall experimental design can be found in Figure 1. Table 1 lists the formulations tested in this study. The blended formulations (excluding water) were initially tested using Rapid-Visco-Analyzer (RVA) to measure the pasting properties. The Farinograph was used to determine the levels of water addition necessary for batter samples to reach either 100 B.U. or 125 B.U. in consistency for each formulation studied, to determine the water addition required for each bread sample. The batters were baked, removed from oven and pans, cooled for one hour at room temperature, and then stored in Ziploc bags at room temperature until day three when baked loaves were sliced and hardness and crust and crumb evaluations were performed. Bread slices were returned to the respective Ziploc bag and stored at room temperature until day seven when the non-crust sections of the bread were ground and analyzed for moisture. The resistant starch contents of 15-minute-mixed bread samples were evaluated on day seven for each formulation. The experiment was performed in duplicate.

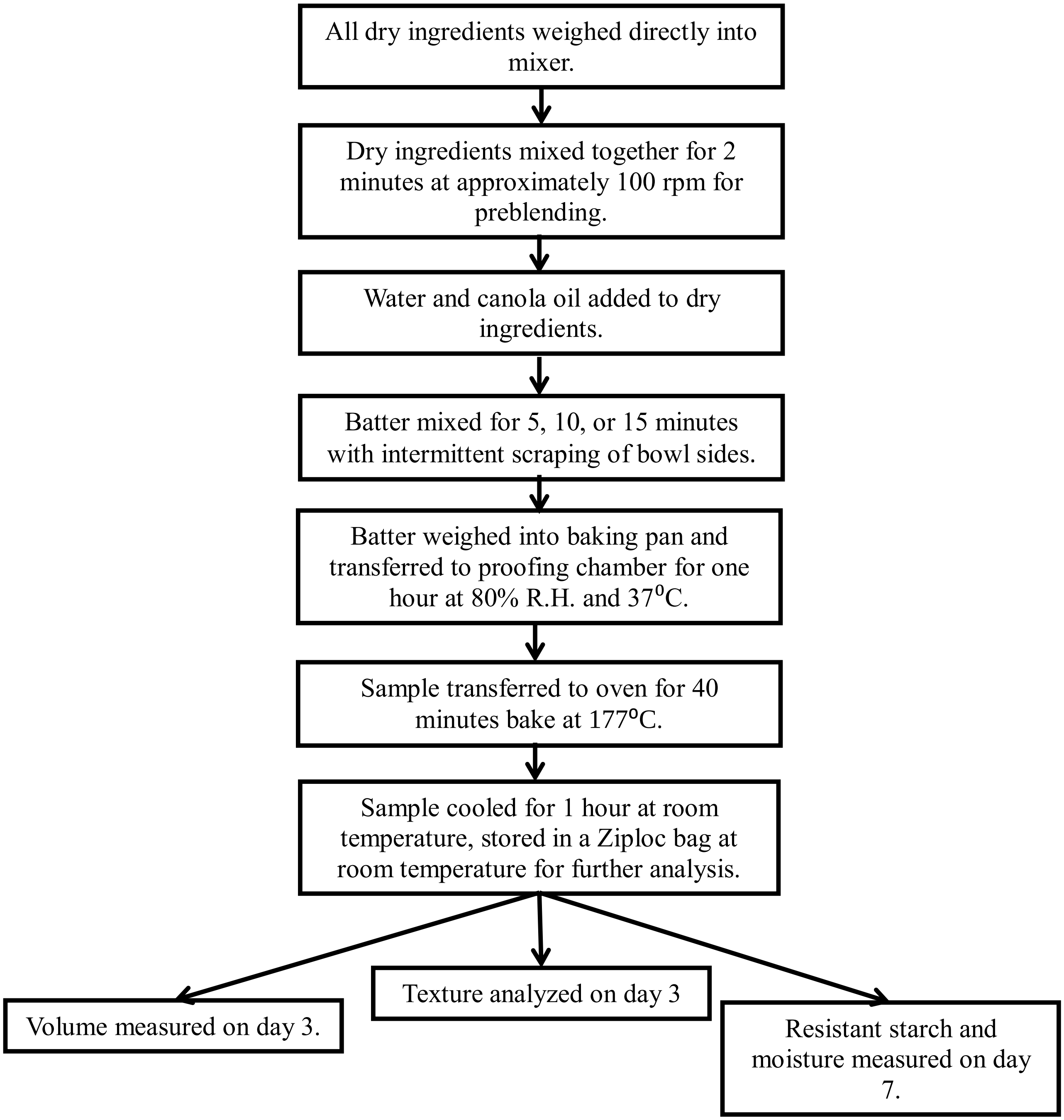


Figure 1**.** Overview of experimental design. Each formulation was made in duplicate. Specific procedures are described in the text.

Table 1. Buckwheat-Rice Bread Batter Combinations for Four Different Egg and Oil Percentages LE/LO, HE/LO, LE/HO, and HE/HO represent 4% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil, 10% egg/8% oil (fwb), respectively. See text for water addition determination based on batter B.U. consistencies. Each formulation was mixed for 5 minutes, 10 minutes, or 15 minutes. All baked breads were made in duplicate.

	Ingredient Formulations (grams)			
	LE/LO	HE/LO	LE/HO	HE/HO
Buckwheat flour	75	75	75	75
Rice Flour	25	25	25	25
Egg White Solids	5	10	5	10
Canola Oil	4	4	8	8
Guar Gum	1	1	1	1
Sugar	2	2	2	2
Salt	2	2	2	2
Yeast	3	3	3	3
Water (100 B.U.)	80.2	87.6	77.8	85.7
Water (125 B.U.)	88.2	96.4	85.6	94.3

3.2 Materials

Rice flour (RL100) was purchased from RivLand (Jonesboro, AR). Buckwheat, which had been purchased from Strobl Naturmuhle (Linz, Austria), was donated by the University of Natural Resources and Life Sciences of Vienna, Austria. The rice flour had a total starch content of 85% (dwb), resistant starch content of 1.23% (dwb), fat content of 0.5-1.2%, and moisture content of 12.9%. Buckwheat flour had a total starch content of 70% (dwb), resistant starch content of 1.04% (dwb), fat content of 2.0%, and moisture content of 13.9%. Guar gum and egg white solids were donated by TIC gums (Belcamp, MD) and Rose Acre Farms (Marshall, MO), respectively. Pioneer sugar (Bay City, MI), Morton iodized salt (Chicago, Il), Meijer canola oil (Grand Rapids, MI), and Meijer instant active yeast (Grand Rapids, MI) were purchased from a local grocery store.

3.3 Moisture

Moisture content of raw ingredients was determined according to AACCI Method 44-15.02 (2001) which consists of heating samples at 130°C for one hour. Aluminum pans were predried and stored in a desiccating chamber before use. Samples were cooled in the desiccating chamber to room temperature before final weighing. The analyses were conducted in triplicate and reported as an average for each ingredient.

3.4 Total Starch

Total starch content was determined for buckwheat flour and rice flour using a Megazyme Resistant Starch Kit (Wicklow, Ireland). All supernatants produced during ethanol washing steps were collected and pooled together and adjusted to 100 mL with distilled water.

Total supernatant D-glucose was quantified using glucose oxidase and peroxidase, a dye forming system, with spectrophotometer reading at 510 nm according to the Starch Kit procedure. The analyses were performed in triplicate and reported as an average for each flour sample.

3.5 Resistant Starch

Resistant starch content of samples was measured for both raw materials and bread samples at day seven of storage after baking, following the analytical procedure provided in a Megazyme Resistant Starch Kit (Wicklow, Ireland). The procedure followed was the method for samples containing less than 10% resistant starch, except when evaluating the control which was run according to the method for samples with greater than 10% resistant starch, the difference being a dilution step. For bread samples, the center cross section of a slice of bread without crust, used for texture analysis (approximately 60 grams; see section 3.10), was ground using a mortar and pestle and passed through a sieve with 1 mm openings. Analysis was carried out according to manufacturer's instructions with the modification of increasing centrifuge force to 6000 x *g* due to incomplete pellet formation at the recommended force. In short, samples were incubated overnight with the prescribed amounts of α-amylase and amyloglucosidase. Multiple alcohol washing, centrifuging, and decanting steps left resistant starch which was solubilized in 2 M potassium hydroxide. The solution was neutralized with sodium acetate buffer and the remaining starch was broken down to D-glucose with amyloglucosidase. Remaining D-glucose was determined upon treatment with glucose oxidase and peroxidase to form oxidized colored dye which was measured using a spectrophotometer (Spectronic Instruments, U.K) at 510 nm. Triplicate readings from each bread loaf sample, the buckwheat flour, and the rice flour were made and then averaged. The results of bread loaves samples are reported as the an average between the duplicate samples made for each formulation.

3.6 Rapid Viscosity Analyzer (RVA)

Buckwheat flour, rice flour and each formulation containing all its ingredients except for water were evaluated for their pasting viscosity properties using a RVA with a 3.5 g sample size. Twenty-five milliliters of distilled water was added to each sample, then run under a Standard Condition One as described by the RVA Thermocline Manual (Newport Scientific, Maryland). The heating and mixing profiles consisted of an initial temperature and mixing speed of 50°C and 960 rpm, respectively. After 10 seconds of mixing at initial speed, the speed was reduced to 160 rpm for the remainder of the run. The sample was heated at a constant rate to 95°C at 5 minutes after initiation of run. Samples were held for 5 minutes at 95°C and then cooled at a constant rate until the temperature reached 50°C at the end of the trial at 13 minutes.

3.7 Batter Viscosity

Because of varying formulation ingredient ratios (Table 1), the water absorbance also varied among formulations. To standardize viscosity of samples, a Farinograph (Brabender Instruments, South Hackett, NJ) was used to determine the amount of water to be added to achieve a constant initial dough viscosity. AACCI Method 54-21 (2010), which is traditionally used to determine wheat flour water absorption, was modified for use with gluten-free formulations. Based on preliminary results, dough consistency between 100 Brabender Units (B.U.) and 125 B.U. was chosen as being within the range of an acceptable final loaf volume and convex top crust. The consistency of the dough samples at this stage of B.U. behaved more like batter, accordingly, gluten-free “dough consistency” is hereafter referred to as “batter viscosity”. Batters with viscosities below 100 B.U. quickly rose to the lip of the bread pan during the proofing stage, but often collapsed during transfer to the oven or during baking, resulting in a concave top of the baked bread loaf. Samples with an initial viscosity above 125 B.U. tended not to rise and had a flat top crust. To record batter viscosity and the amount of water to be added, the Farinograph was run empty for 5 minutes and calibrated to 0 B.U. Forty-five grams of each formulation (including the necessary portion of canola oil) were weighed into the mixing chamber, and mixing paddles were run for 5 minutes to equilibrate samples at a mixer temperature of 37°C. Thirty milliliters of distilled water were quickly added and then additional water was slowly added until the samples reached the desired B.U. (100 or 125 B.U.), and that total water volume was recorded and scaled up to test formulations. Farinograph determination of water required for each formulation at each viscosity level was tested in duplicate and reported as an average.

3.8 Breadmaking

Breads were made according to the buckwheat breadmaking method described by Hager et al (2012) and Renzetti et al (2007). The same yeast, salt, and sugar levels were used as in the above mentioned formulations, but with the modifications of adding rice flour totaling 25% of the total flour with the remainder being buckwheat flour, modifying water content as described in Section 3.7, and adding guar gum as described by Lazaridou et al (2007). Formulations are outlined in Table 1. The formulations were further modified by addition of egg white solids at either 5% or 10% fwb, canola oil at either 4% or 8% fwb, and water added to achieve either a 100 B.U. or 125 B.U.. All of the dry ingredients including yeast were weighed directly into a mixograph “spindle-type” mixer (National Mfg Co, Lincoln, Nebraska) and blended at maximum speed at approximately 100 rpm, for two minutes to homogenize ingredients. A further modification was to the yeast incorporation method. The general approach of the methods referenced above was to reactivate the yeast using warm water for ten minutes. It was found in preliminary trials that the same final baked bread volume was achieved regardless of prior yeast suspension, and to be more in line with general industry practices, yeast was directly added as a dry ingredient. This practice was also adopted to eliminate any changes in yeast activity over time from a stock source of yeast solution. If compressed yeast had been used instead of instant active dry yeast, directly incorporating the yeast may not have been a feasible option. Next, canola oil and water were weighed and added directly to the premixed dry ingredients in the mixer. Each formulation was mixed at the maximum speed for either 5, 10 or 15 minutes. Mixing times wereselected based on complete visual homogenizing and hydrating of ingredients and limited to 15 minutes due to practicality, throughput, and high energy input necessary on a production scale. At two minute intervals, the sides of the mixer were scraped to ensure proper mixing and inclusion of all materials. As seen in Figure 2, the batter was more like a cake batter than wheat bread dough.

Batters were weighed into 6.0” x 3.5” x 2.0” pans which were greased with a thin layer of vegetable shortening to ease in removal of loaves post baking. The weight of batter added to each baking pan was recorded and used in calculating bake loss. Bake loss was defined as the weight of the finished and cooled bread loaf over the weight of the initial batter. Filled baking pans were directly transferred to a proofing chamber (NationalMfgCo, Nebraska) for 60 minutes at 85% R.H. and 37^{o}C.

Figure 2. Example of 100 B.U. buckwheat-rice flour batter (4% egg/10% oil fwb) mixed for 10 minutes. Batter was scraped from mixing spindles at 2 minute intervals to ensure proper inclusion and mixing of all material. The image illustrates the cake batter like consistency of the formulations necessary to achieve the finished gluten-free baked breads in this study. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

After proofing, samples were carefully transferred to a four-stage carousel oven (NationalMfgCo, Nebraska) and baked for 40 minutes at 177°C. Any excessive mishandling of proofed batter caused samples to collapse, and these samples were then remade to maintain duplicate samples for each formulation. After baking, samples were immediately removed from baking pans and placed on a cooling rack for 1 hour, then transferred to a Ziploc plastic bag which was then sealed tightly. Samples were stored at room temperature until further analysis. The entire baking process is outlined in Table 1.

Bread samples from formulations containing only one flour, either 100 g of buckwheat or 100 g of rice flour, were also made in duplicate as controls for comparison to loaves from blended flour and results are presented in the Appendix section. These bread samples were made using the HE/HO formulations with the 100 B.U. consistency batter and mixing time of 15 minutes and were subject to all the same tests as presented in the Methods section.

3.9 Bake Loss

Bake loss was calculated according to Renzetti et al (2007) and described as percentage of finished loaf weight over the initial batter weighed into the baking pan. Baked samples were weighed one hour after cooling.

3.10 Loaf Volume and Specific Volume

The loaf volume of each sample was measured three days post baking according to AACCI Method 10-05.01 (2000). The apparatus was calibrated using two standardized 400 c.c. wooden blocks and volume was adjusted to 800 c.c. on a measurement window. The volumes of the bread samples were below the minimum measurement threshold of the apparatus, so one wooden block was left in the chamber with the bread loaf during sample measurement and 400 c.c. was subtracted from the measurement total. The weight of the final bread sample was measured at three days post baking and used to calculate specific volume. Specific volume was reported as the volume divided by loaf weight (cm^3/g). One set of measurements was made on each of the duplicate samples.

3.11 Product Hardness

The hardness of samples was measured using the Texture Analyzer TA-HDi (Stable Microsystems Ltd, U.K.) and Texture Expert v1.2 software (Stable Microsystems Ltd, U.K.). The bread samples were measured for hardness three days post-baking as this was seen as the average time it would take the bread to leave a plant and be purchased by consumers. Samples were tested according to Boswell (2010) with modifications in probe and load cell due to equipment availability. A horizontal 1.90 cm thick cross sectional slice from the center of each loaf was taken and two measurements were made as seen in Figure 3. Each bread sample was compressed to 60% of its original height at 2 millimeters/second with a flat round 36 millimeter pyrex probe using a 50 kg load cell. The maximum force (N) during compression was recorded and the duplicate readings were averaged and reported as product hardness.

3.12. Moisture Content of Baked Bread Samples

For each baked samples, the 1.90 cm thick horizontal center slice of bread (approximately 60 grams) that had been used for texture analysis was ground to pass through a 1 mm sieve in preparation for moisture measurement. Crusts were not included in preparation for the moisture measurements. Moisture content analyses were conducted using AACCI Method 44-15.02

(2001) Analyses were carried out on two baked bread samples from each formulation and three measurements were made on each bread sample, for a total of six measurements which were averaged and reported.

3.13 Statistical Analysis

Data analysis was conducted using Statistica software version 9 (Statsoft, Oklahoma). Analysis of variance (ANOVA) was calculated among samples, and least significant difference (LSD) was used to analyze the difference between means with $p<0.05$ considered a significant difference

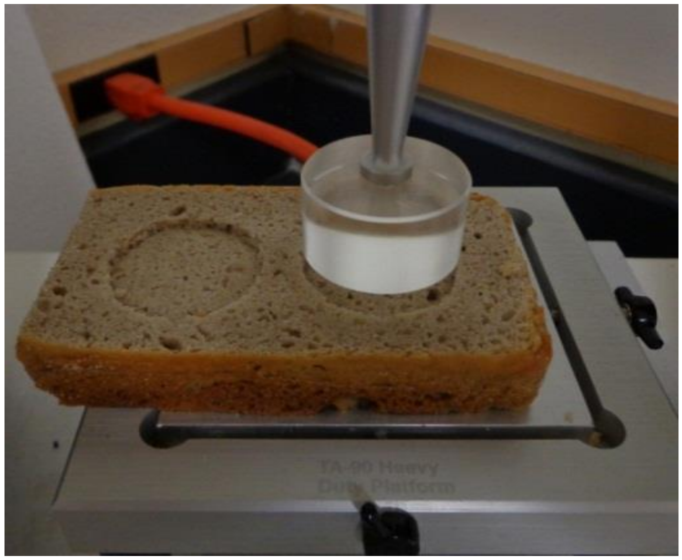


Figure 3. Example of hardness measurement on bread slice (4% egg/10% oil) using Texture Analyzer . Each bread slice was measured in two different locations and the result reported as an average.

CHAPTER 4:

RESULTS AND DISCUSSION

4.1 Raw Flour Composition

The compositions of rice and buckwheat flours are given in Table 2. The samples had similar moisture values, of 12.9% and 13.9%, respectively. The total starch content (dwb) in buckwheat was 70% which was lower than that of rice which was 85%. The lower starch content in buckwheat was a result of higher protein in its grain. The protein content (dwb) in buckwheat has been reported between 8.5% and 19% (Balgeet et al 2010). On the other hand, rice flour has a lower protein content between 5% and 8% (Wang and Wang 2004). The lower protein content, along with lower vitamin and mineral contents makes rice a less nutritious grain than buckwheat. Long grain rice, like the sample used in this study, has approximately 25% amylose which is at similar levels to those reported for buckwheat flour (Zielinska et al 2009, Panlasigui et al 1991). With similar amylose contents in buckwheat flour and rice flour, one could expect similar potential to form resistant starch.

Table 2. Moisture, Total Starch, Resistant Starch, and Fat Content of the Raw Rice and Buckwheat flours

	Moisture (%)	Total Starch (%) dwb	Resistant Starch (%) dwb	Fat Content (%) dwb
Rice Flour	12.9	85.0	1.2	0.5-1.2
Buckwheat Flour	13.9	70.0	1.0	2.0

4.2 Rapid Visco Analyzer (RVA)

In the present study, the general profiles of the tested formulations are in agreement with those reported by Alvarez-Jubete et al. (2010) in which buckwheat flour and rice flour RVA profiles were measured. Although a similar profile was found between buckwheat flour and rice flour, the actual RVA viscosity values for buckwheat flour and rice flour controls in this study were lower than reported values, especially for buckwheat flour (Appendix, Figure 14). The reported value of peak viscosity of rice flour was 5,155 cP compared to 3,540 cP found in the present study. Reported values for trough viscosity and final viscosity were 3,637 cP and 7,200 cP, respectively, for rice flour (Alvarez-Jubete et al 2010) compared to this study's rice flour values of 1,920 and 3,960 cP, respectively. The reported value of peak viscosity of buckwheat flour was 4,092 cP compared to only 984 cP found in the present study. Reported values for trough viscosity and final viscosity were 3,852 cP and 7,272 cP compared to this study's buckwheat flour values of 960 cP and 1,080 cP, respectively. The lower RVA values in this study may have been caused by a higher enzyme activity due to crop conditions at harvest, duration and conditions of storage of the flour, and variety of each grain. The reason for buckwheat flour's lower peak viscosity as compared to that of rice flour is likely a result of buckwheat's lower total starch level. A change in buckwheat flour to rice flour ratio from 75:25, as in the formulation used in this study, to 25:75 would create an evident change in the RVA profile of the formulation.

The initial viscosities were similar among all formulations at nearly 100 cP for all RVA measurements (Figure 4). Peak, trough, and final viscosities varied among the formulations. Peak viscosity (PV) is the highest viscosity the sample reaches during the heating and holding stage and generally is the first peak seen during RVA. During the PV stage, the starch granules are at their highest hydration capacity before a breakdown of the granular starch structure occurs. The trough viscosity (TV) is the viscosity at the lowest point after the first peak (PV). The final viscosity (FV) is the highest final viscosity reached by the sample and is an indication of the "set-back" or retrogradation of the sample.

The HE/HO (high egg/high oil) formulation had the highest PV at 3,296 cP compared to the lowest peak viscosity of a formulation in the LE/HO (low egg/high oil) sample at 2,097 cP (Table 3). LE/LO had the second highest peak viscosity at 3,154 cP followed by the HE/LO (high egg/low oil) formulation with a PV of 2,652 cP. The same trend was seen for TV. The FVs followed in the same order as both PV and TV with HE/HO samples reaching a FV of 4,381 cP followed by LE/LO (3,996 cP), HE/LO (3,357 cP) and LE/HO (2,741 cP).

Other studies on cake batters are relevant to the present study due to the cake-like batter necessary to produce a gluten-free bread. In a study by Shelke et al. (1990), it was found that cake batters with egg white solids had a greater viscosity than those without egg white solids (1,600 cP vs. 2,200 cP) which is in agreement with the results found in the present study. In the same study, increasing levels of fat (shortening) increased the viscosity of the batter, but reduced final baked product volume at the levels used. The relationship between batter viscosity and final volume are discussed in Section 4.5. Results found in this study, in which the formulation with the greatest egg and oil contents had the highest viscosity, are in alignment with the reported results (Shelke et al 1990). The same effect of increasing batter viscosity with increasing oil and egg content did not appear in all the formulations in the present study. The LE/HO sample had a higher final viscosity than the HE/LO, so in some cases increasing the egg white solids did not increase the viscosity.

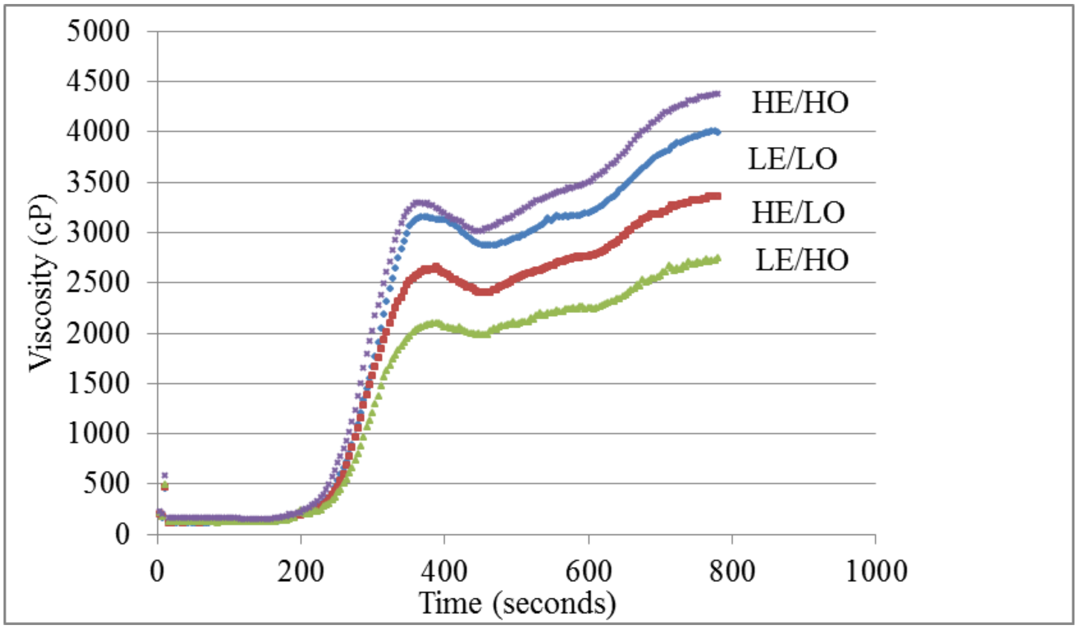


Figure 4. Rapid-Visco-Analyzer profile of formulations. Samples heated from 50°C to 95°C and held for 5 minutes, and then cooled back to 50°C. HE/HO, LE/LO, HE/LO, and LE/HO represent 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Samples were run in duplicate and averaged. RVA profiles of the raw materials can be found in the Appendix.

Table 3. Pasting Properties of Gluten-free Bread Formulations, Buckwheat Flour and Rice Flour

	PV (cP)	TV (cP)	FV (cP)
HE/HO	3296	3009	4381
LE/LO	3154	2872	3996
HE/LO	2652	2396	3357
LE/HO	2096	1985	2741
Buckwheat flour	984	960	1080
Rice flour	3540	1920	3960

PV= Peak Viscosity; TV= Trough Viscosity; FV= Final Viscosity

HE/HO, LE/LO, HE/LO, and LE/HO represent 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively

The physical chemistry in the unfolding of the proteins, denaturing/coagulation of proteins, and hydration of starch and fiber molecules all play a role in the viscosity of the formulations during the RVA cycle and are affected by changes in ratios of ingredients. The level of air incorporation created by egg white proteins' foaming ability may also be another factor affecting the final batter viscosity (Lang and Rha 2007). The differing RVA results may be an effect of the emulsification properties of the egg white solids in an oil-in-water emulsion and also their ability to form foams.

RVA is traditionally used to measure the effects of heat and shear on the viscosity of starches and flour. Running an RVA on different product formulations can predict the performance of a formulation during the baking process and is used as a method to determine the final quality of a finished food. A sample that increases in viscosity during mixing and heating would have less oven spring during baking as the gas produced by yeast fermentation cannot overcome the outside pressure created by the batter or dough. A lower viscosity sample may allow for greater batter expansion but may not have the required strength to trap the gasses within the sample.

4.3 Batter Viscosity

Dough consistency plays an important role in final product quality. For wheat bread baking, the amount of water added to the formulation can have drastic effects on bread quality. In a study on wheat bread, low moisture, high viscosity dough yielded lower specific loaf volume while high moisture, low viscosity dough showed an increased specific loaf volume. However, the higher level of moisture increased water activity, increasing the susceptibility of the bread to mold and bacterial growth (Czucjajowka et al 1989). Dough consistency appears to have an even greater effect in gluten-free baking as the "dough" viscosity is nearer to that of cake batter. Cake batter is traditionally measured using a viscograph (Gaines and Donelson 1982), but the principles of the Farinograph can also be applied to gluten-free batters for breadmaking (Lazaridou et al 2007). In the following discussion, the term "batter" is used instead of "dough" for gluten-free baked products.

The Farinograph is traditionally used to establish the water addition necessary to reach a consistency of 500 B.U. for wheat flour. This level is seen as being optimal for creating a loaf of bread with acceptable quality parameters and is used as the benchmark in AACCI breadmaking Method (10-10.3). The Farinograph was used in a similar manner for application in gluten-free baking by Curic et al. (2007) and is used in the present study as a method to standardize performance.

In the present study, the necessary water addition using the Farinograph was based on 45 grams of each formulation (all ingredients except for water). The resulting water addition value obtained using the Farinograph was scaled according to the total weight of each formulation. The amount of water added to 45 grams of each formulation is presented in Table 4. It was found in preliminary trials that if the water addition was too low, limited rise occurred during the proofing time due to a viscous and inextensible "dough" system that did not allow yeast fermentation to improve loaf volume. Batters that had high moisture content, greater than 100 B.U., rose quickly, but often collapsed during the proofing time or during baking resulting in a convex crust on the baked bread loaf. The addition of hydrocolloids is used in gluten-free breads to increase batter viscosity and increase mixing tolerance (Curic et al 2007), but it decreases sensory properties when used at higher levels by causing wet crumbles, as noticed in samples stored longer than two weeks.

Table 4. Batter Viscosity Water Addition: Amount of Water Added to Achieve 125 B.U. or 100 B.U Batter Consistencies for Each Formulation.

	Water Added (mL)			
	LE/LO	HE/LO	LE/HO	HE/HO
125 B.U.	30.9	32.4	28.9	30.6
100 B.U.	35.7	37.3	33.4	35.2

HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Each formulation was run in duplicate and reported as the average.

The actual water addition for the Farinograph testing for each formulation and batter viscosity is reported in Table 4, but for ease in discussion and comparison with other literature, the Farinograph water addition values (Table 1) are presented on a flour weight basis for the remainder of the section for each formulation. The water addition values were consistent with the levels found by Alvarez-Jubete et al (2010) in which water was used at 87.0% fwb. For low viscosity samples (100 B.U.), the water addition varied from 77.8% in the LE/HO formulation to 87.6% in the HE/LO formulation. The increase in water addition was primarily affected by the level of egg present in the formulation. Samples with the same level of oil, but differing level of eggs had the most drastic differences in water necessary to achieve the desired batter consistency, with high egg samples requiring greater water addition (LE/LO at 80.2% fwb vs. HE/LO at 87.6%, and LE/HO at 77.8% vs. HE/HO at 85.7%). The difference in oil content did not have a drastic an effect on batter consistency.

The same trends in water addition were seen for the 125 B.U. consistency batters as had been seen in the 100 B.U. consistency samples. An increase in water addition of less than 10% across all formulations was seen to achieve the difference in consistency from 100 B.U. to 125 B.U.

No clear trends exist between RVA profiles and water addition necessary to achieve desired batter consistency. LE/HO had the lowest peak viscosity and required the least water compared to the other formulations. The same trend was not seen for the highest peak viscosity sample, HE/HO, as it had only required the second highest level of water addition. The RVA profile therefore cannot be used as a prediction tool for batter consistency based on this study.

4.4. Quality Evaluation of Bread

The visual appearance of a bread is the first cue to consumers of the overall quality of a bread product. In depth and scientific studies to identify the drivers of consumer liking in gluten-free bread have not been extensively reported in the literature. The visual quality of wheat bread has been divided into several categories including; loaf size/shape, crust color, symmetry of form, grain/crumb appearance (AACCI 2010), and these terms will be used for describing the qualities of the gluten-free breads. In the present study, the quality of the breads were described in comparison to each other and, in a general way, compared to commercial wheat breads. No sensory analyses were conducted for this study.

The size and shape of the baked breads could be broadly divided into two groups based on the initial batter viscosity. Lower viscosity batters (100 B.U.) produced larger loaves with more convex top crusts and rounded edges between the upper and side crusts, more typical of a commercial wheat bread. Higher viscosity batters (125 B.U.) tended to produce smaller loaves with flatter top crusts with less rounded edges between upper and side crusts, resembling the shape of a brick (Figures 5 and 6). Valleys and small holes were created on the crusts of bread samples from higher viscosity batters as cell structures collapsed during baking (Figure 5).

Crust color was only visually inspected for this study. Samples with increased egg levels produced a darker brown color than those with lower egg levels. The increased protein level increased the browning Maillard reaction caused by protein-sugar interactions (Kent-Jones 1967). Within each formulation, the lower consistency batters had duller appearance than their higher consistency counterparts. This was likely caused by the increased water content which diluted the color components in the crusts. The oil and mixing times were not judged to affect the color of the crusts.

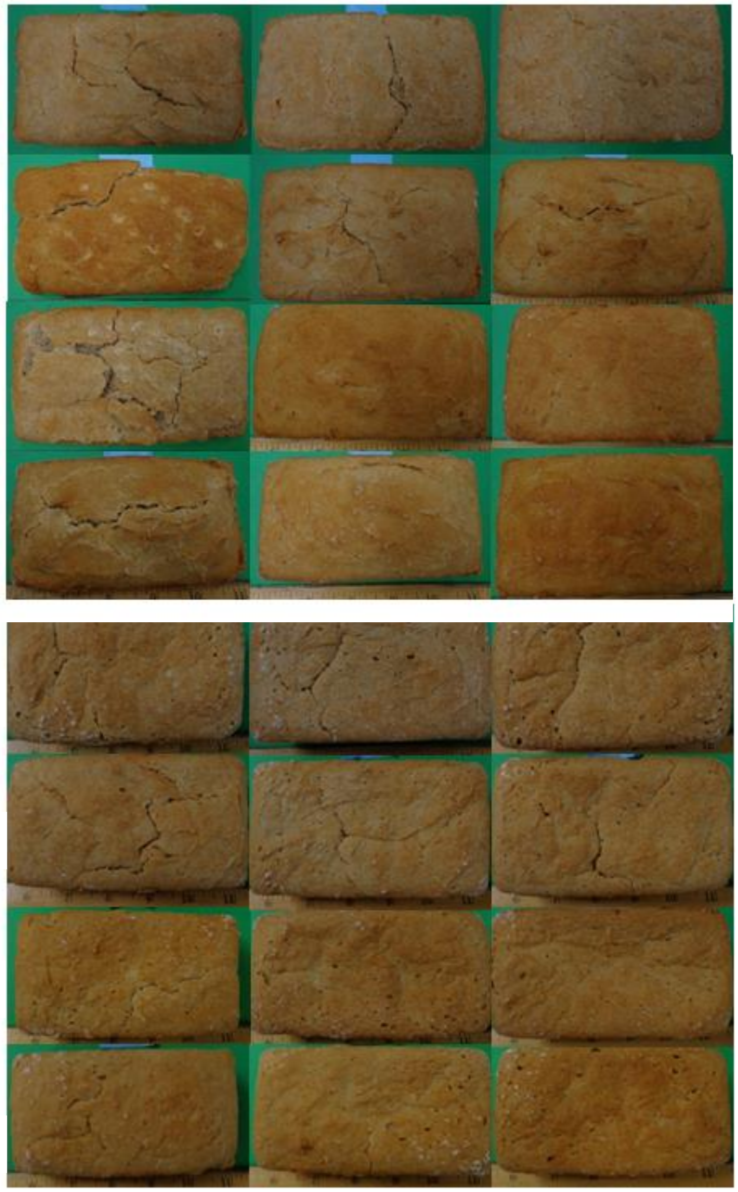


Figure 5. Crust appearance: Figure 5a and Figure 5b samples 15 min from batters with water added to achieve 125 B.U. and 100 B.U. consistencies, respectively. See text for details of water addition determination based on B.U. measurements. Samples are arranged left to right based on mixing time and top to bottom based on formulation. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively.

The symmetry of form was generally low across all baked samples. Samples mixed at five minutes had the greatest levels of deformations in baked crust appearance and were not uniform compared to the longer time mixed samples (Figure 5). Random crack formations were most evident at the shortest mixing time and became less evident at longer mixing times. Samples baked from higher consistency batters (125 B.U.) that had been mixed for 15 minutes were close to uniform, with cracks and other deformations becoming less evident. An increased mixing time may have allowed for better protein hydration which created a more extensible protein network. The raw material formulation had an effect on the uniformity of the crusts as HE/HO bread loaves produced the most symmetrical loaves with almost no cracks or other visible deformations at the 10- and 15- minute mixing times for both batter consistencies (Figure 5). The LE/HO formula bread samples had uniform crusts, similar to those of HE/HO samples, leading to the conclusion that increased oil content in the formulation increases symmetry of the crust. This finding is reinforced by the image taken of an initial trial formulation produced with no added canola oil (Appendix, Figure 13). The oil likely eases the tension that forms during the "oven spring" that occurs as the yeast increases the rate of carbon dioxide production during the quick rise in temperature as the bread enters the oven. These findings were confirmed as bread made without lipids had about a 25% lower specific volume and less uniform texture and structure than formulations with lipid (Czuchajowska 1989).

The crumb/grain appearance was visually inspected and images can be found in Figure 6. In all formulations, large air cells tended to form below the surface of the baked crust. This is an indication that air cells were not successfully trapped within the batter matrix during the rapid release of carbon dioxide upon entry into the oven. The elasticity of the air cells needs improvement to decrease the rupture of air cells throughout the bread. The appearance of air cells

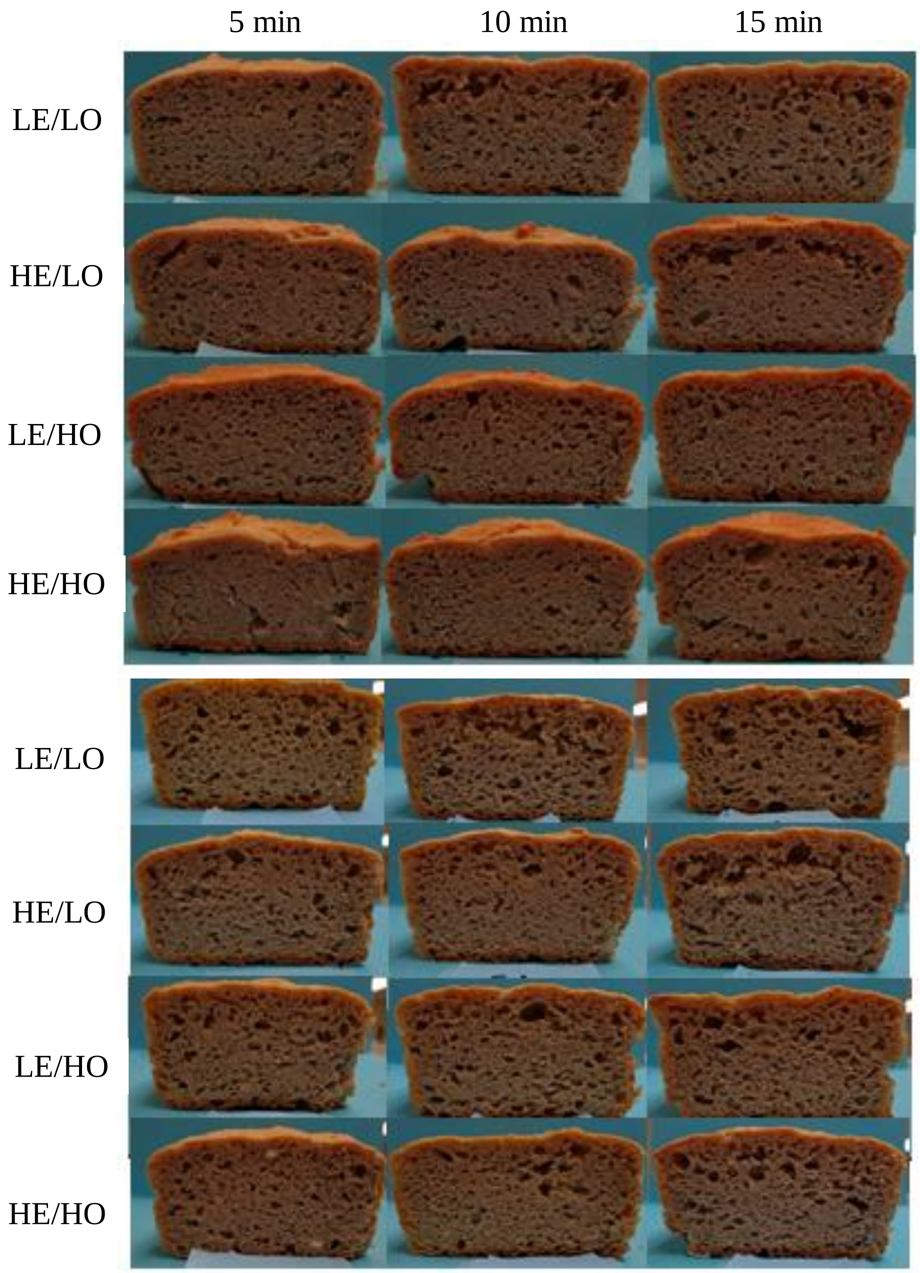


Figure. 6a

Figure. 6b

Figure 6. Bread crumb appearance: Figure 6a and Figure 6b samples prepared from batters with water added to achieve 125 B.U. and 100 B.U. consistencies, respectively. See text for details of water addition determination based on B.U. measurements. Samples are arranged left to right based on mixing time and top to bottom based on formulation. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively.

5 minutes batter mixing time | 15 minutes batter mixing time

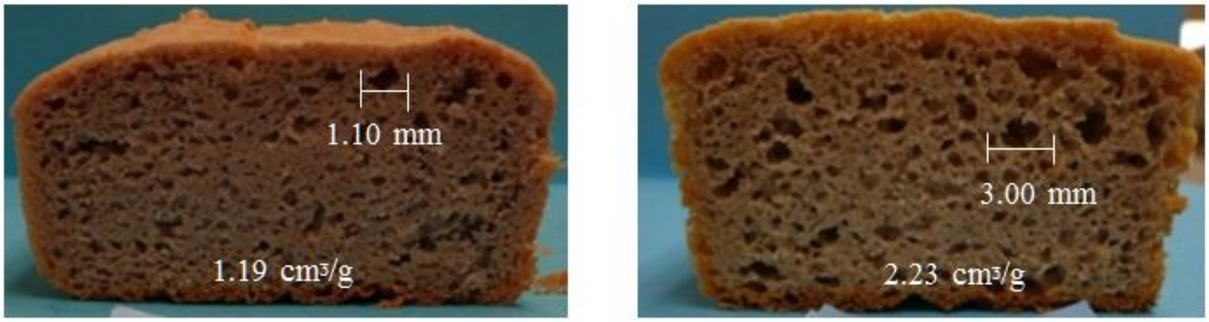


Figure 7. Cell size differences in samples made with the same formulation (5% egg white solids and 4% oil) but with 5 minute versus 15 minute batter mixing times.

near the top crust are amplified in the bread loaves from the lower consistency batters. This appearance of these larger air cells may be a result of the smaller proportion of solid material available to trap the carbon dioxide gas and increased steam created from higher moisture content. Samples with longer mixing times had higher proportions of large air cells (Figure 7).

4.5. Specific Volume

The specific volume of baked bread is an indication of final eating quality and helps dictate texture and overall liking of bread. Breads with a lower volume will have a more dense texture which will affect the sensory perception of the product. In a study by Torbica et al (2010), similar formulation buckwheat breads were evaluated using a sensory panel. Loaves with higher heights, and overall higher specific volumes scored higher on appearance than more dense loaves. In the present study, mixing time, batter consistency, and formulation all affected the specific volume of the baked loaf.

Within each formulation, a significant increase in baked bread specific volume was found from batters with the 5- minute to the 15-minute mixing time (Figure 8). In all formulations except for LE/LO, a significant increase in baked bread volume was found for batters mixed for 10 minutes versus 5 minutes. The increase in specific volume with increasing mixing time was most pronounced for bread samples from lower consistency batters (i.e., 100 B.U.). The largest increase in specific volume was found for the lower consistency batter (100 B.U.) of the LE/LO formulation with increased mixing time from 5 minutes (2.17 cm^3/g) to 15 minutes (2.56 cm^3/g), which represented an increase in volume of approximately 18%. Bowell (2011) also demonstrated that increased mixing time up to 15 minutes increased baked loaf volume in sorghum-based bread formulations. It is thought that increased mixing time increases the interactions between sulfur-containing amino acids in the proteins which form disulfide bonds enhancing the network strength. These bonds are crucial in yeast-leavened wheat breads as gluten network has the extensibility and strength to trap carbon dioxide and increase the loaf volume (Gerrard 2002).

Decreased batter consistency was associated with improved specific loaf volume. The average increase in specific volume of baked loaves was approximately 10% for samples at 100 B.U consistencies compared to the 125 B.U. batters. The average loaf volume increased by 15% for bread samples from 100 B.U. consistency batters with an increase from 5-minute to 15-minute mixing time. The higher viscosity batter (125 B.U.) did not allow the yeast cells to expand the loaf to the same increased level as 100 B.U. batters, because the batter matrix was too dense and the air cell structure was not able to fully expand against the pressure exerted by the batter. The larger cell structure discussed in the previous section was found in higher specific volume loaves.

Formulation affected specific volume but was also dependent on mixing time. Among the higher consistency batters (125 B.U.) with the 5-minute mixing time, LE/LO and LE/HO samples had the highest baked loaf specific volumes of 1.94 cm^3/g and 1.97 cm^3/g, respectively. For the same consistency batters at the 10-minute mixing time, LE/LO and LE/HO samples again had the highest specific volumes of 2.10 cm^3/g and 2.18 cm^3/g, respectively. The same trend was found among the 15-minute mixing time samples, with the highest specific loaf volumes for LE/LO and LE/HO samples, 2.26 cm^3/g and 2.18 cm^3/g, respectively. The lower egg white solids addition level tended to produce baked breads with a higher specific loaf volume regardless of the level of canola oil added to the formulation compared to formulations using a higher egg white solids level. The HE/HO baked samples had the lowest specific volumes for

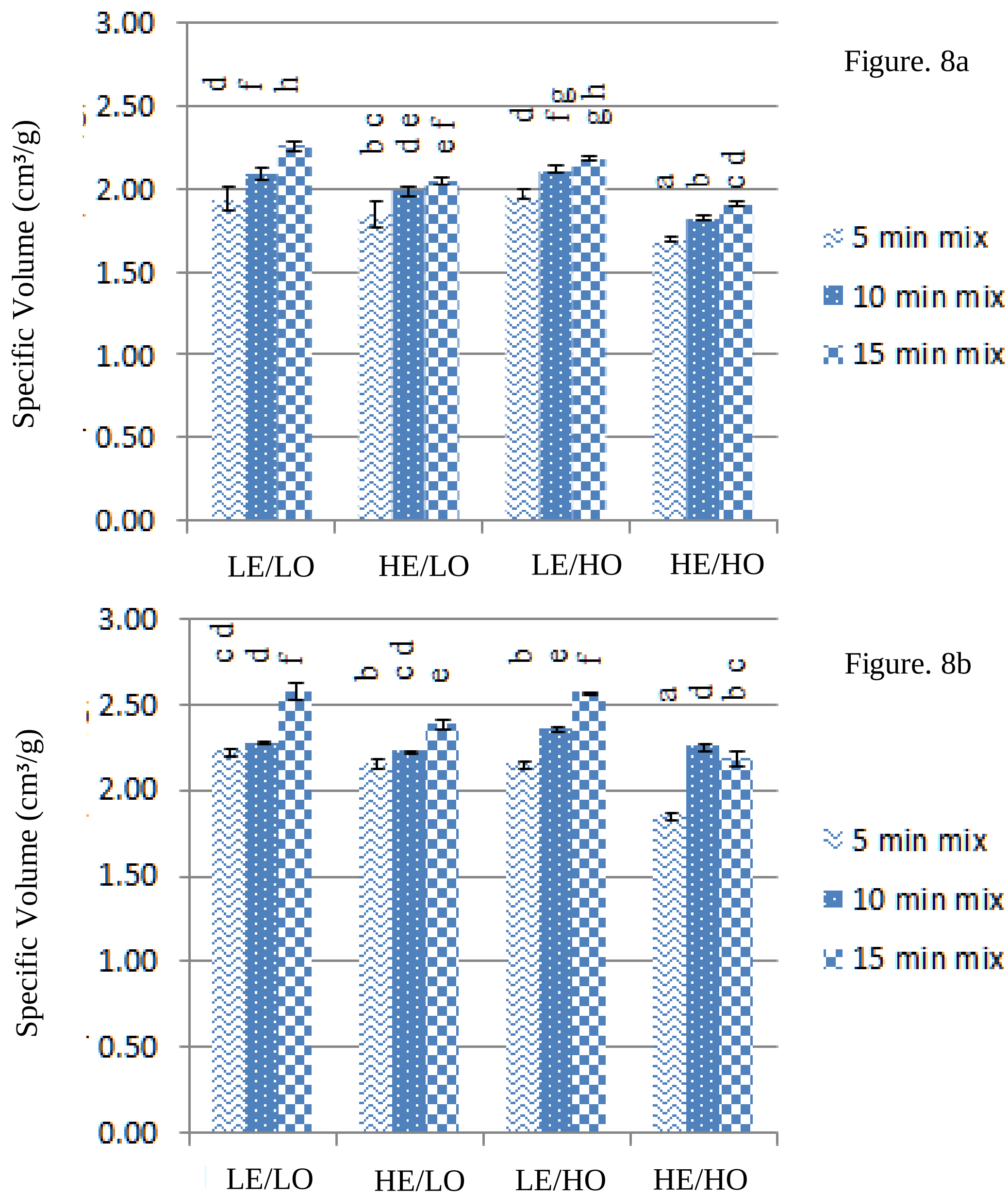


Figure 8. Specific volume of buckwheat-rice flour bread loaves made from 125 B.U. (Figure 8a) and 100 B.U. (Figure 8b) consistency batters. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Different letters indicate a significant difference at $p<0.05$ using LSD.

each mixing time (1.70, 1.82, and 1.91 cm^3/g for 5-, 10-, and 15-minute mixing times, respectively) among the higher consistency batters.

Among the lower consistency samples, LE/LO, HE/LO, and LE/HO all performed similarly across the three mixing times with a few exceptions. The LE/HO produced a significantly larger loaf (2.36 cm^3/g) from the 10-minute mixing time batter compared to the other formulations. Again HE/HO had the lowest specific volume of bread samples for all batter mixing times (1.85, 2.25, and 2.19 cm^3/g at 5-, 10-, and 15-minute mixing times, respectively).

In the present study, baked breads from formulations with high oil had the highest specific volume by approximately 15% when egg white solids were added at the lower level in 125 B.U. samples, and by 17% percent in 100 B.U. samples, compared to their counterpart samples with high egg white solid content. Previous studies have shown that oil addition between 5-6% (fwb) increased the volume of wheat bread between 15-25% compared to loaves baked from dough with no oil (Stauffer 1998). In their study, oil played a critical role in increasing specific loaf volume; however, in the present study when egg white solids were increased, the final baked loaf volume decreased, indicating the interaction of egg whites minimizes the effects of oil in increasing the volume. Oil is known to reduce egg white solids' foaming ability, and it may be the binding of the oil to the egg white proteins which reduces their effectiveness in increasing loaf volume by decreasing the role they have in gas retaining ability. Several theories exist to explain the action of oil in wheat-based, gluten-containing dough, but include the lipoprotein structure of gluten forming gluten-lipid complexes with oil which improves the plasticity of the dough and improves gas retention. Another theory suggests that oils help block the pores formed in dough during baking by sealing holes, preventing loss of carbon dioxide (Baker and Mize 1942).

Based on preliminary results, it is known that egg white solids improved the volume of the gluten-free loaves when compared to samples with no egg whites. These results have also been confirmed by Crockett et al. (2011) where egg white solids at 15% (fwb) improved the loaf volume in a rice flour- and HPMC-based formulation. Boswell (2010) showed similar results in sorghum-based breads. However, in this current study, increasing the egg white solids to 10% decreased the subsequent loaf volume when compared to the formulation with 5% egg white solids (fwb). These results show that increased egg white solids content level is not the only factor for increased loaf volume, but that loaf volume is also affected by the oil content of the formulation.

4.6 Bake Loss and Moisture

Bake loss is an important measurement for bakers, and is used to determine if a product is reaching correct predicted final moistures which will affect production rates and shelf life. In this study, it was not possible to compare among sample formulations as each formulation had a different amount of water added, and therefore would lose amounts of water during the baking process at dissimilar levels. Variation in water addition is likely the biggest contributor to bake loss differences among sample formulations. This finding is confirmed in a paper by Renzetti et al. (2010) in which there were no significant differences in bake loss among a variety of control gluten-free formulations consisting of altered levels of quinoa, buckwheat, corn, teff, and oats with the same water addition. Despite the lack of ability to draw conclusions among formulation differences in the present study, a trend does exist in some samples in which mixing time does significantly affect the bake loss in the sample.

In five of the formulations, there was a significant difference in bake loss between the samples mixed for 5 minutes and their counterpart samples mixed for 15 minutes. Among the higher consistency batters (125 B.U.), the biggest difference was seen for the HE/HO samples where the bake loss percentage increased from 10% to about 18% from a 5-minute to 15-minute mixing time (Figure 9). Both high oil formulations, LE/HO and HE/HO, showed significant increases in bake loss with an increase in mixing time from 5 minutes to 15 minutes, for both the higher and lower consistency batters. This may be an important finding for bakers trying to reduce their final loaf moisture content and improve the shelf life of their products.

The reason for the changes in bake loss may be partially accounted for by an increased specific volume of baked bread samples with higher mixing times. The larger cells formed in the longer-mixed batters may also have allowed for increased heat penetration into the center portions of the bread, increasing the moisture loss during baking (Figure 7). The significant changes among higher oil formulations may indicate that the baked samples with increased mixing time formed a more homogenized batter that allowed for increased bread volume that therefore increased bake loss.

Again, like bake loss, final product moisture among the samples could not be directly compared as the water quantity for each formulation varied. The obvious difference existed in that bread samples with higher initial water contents had higher final moistures than bread samples with lower initial water contents (Figure 10). The final moisture contents of the gluten-free breads produced in the present study were comparable to those of wheat breads made using the AACCI straight dough method which are around 44% (Czuchajowska et al 1989). Similar to bake loss, mixing time may also have some effects on product final moisture if the mixture is exposed to air longer. Accordingly, a test was conducted to investigate if the loss in moisture was

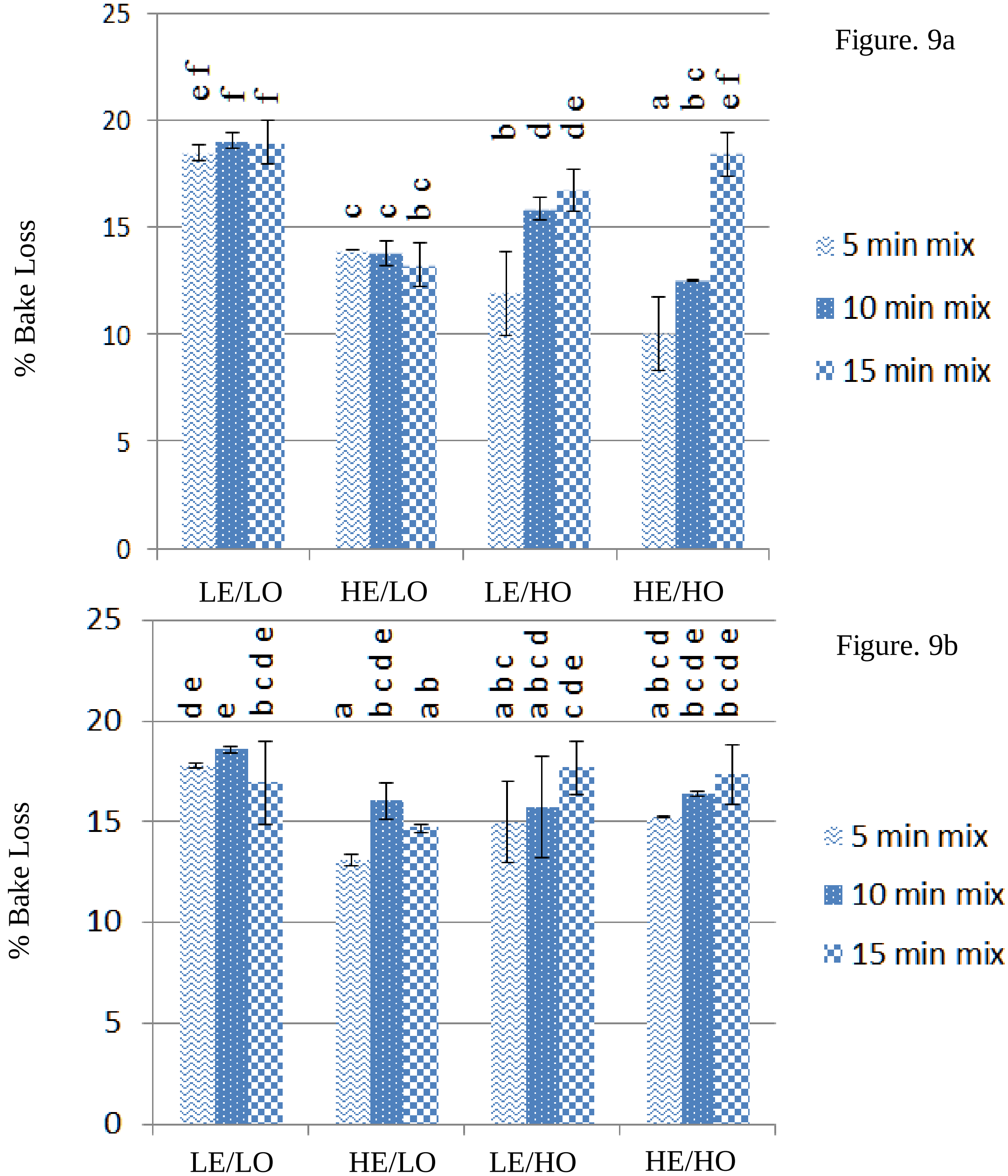


Figure 9. Bake loss of bread loaves made from 125 B.U. (Figure 9a) and 100 B.U. (Figure 9b) consistency batters. See text for details of water addition determination based on B.U. measurements. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Different letters indicate a significant difference at $p<0.05$ using LSD.

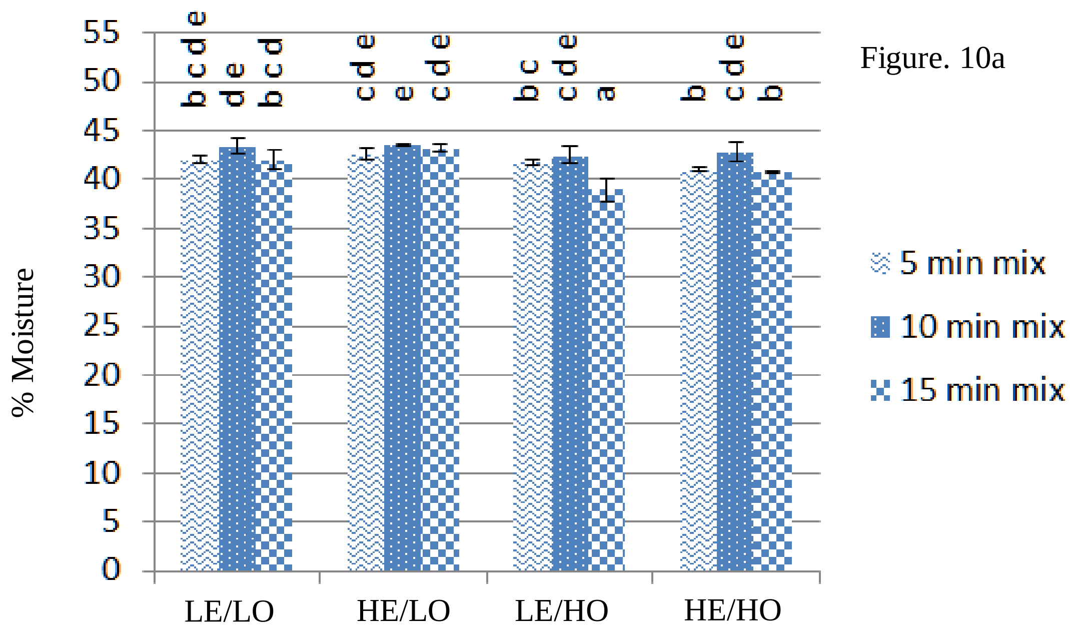


Figure. 10a

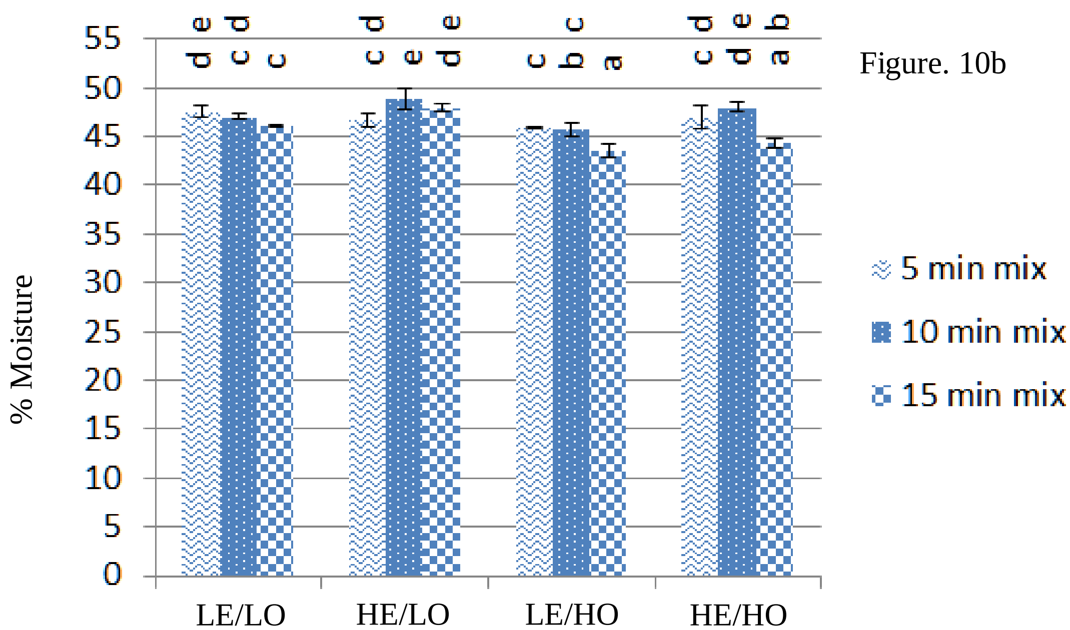


Figure. 10b

Figure 10. Moisture content of bread loaves made from 125 B.U. (Figure 10a) and 100 B.U. (Figure 10b) consistency batters. See text for details of water addition determination based on B.U. measurements. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Different letters indicate a significant difference at p<0.05 using LSD.

due to the batter having a longer time of exposure to air during mixing and possibly losing more water to the environment prior to baking. Results from this study revealed that there was less than 0.3% difference in weights between counterpart batters that were mixed for 5 minutes and batters that were mixed for 15 minutes, indicating that the major portion of the moisture loss was in fact occurring during the baking process.

4.7 Product Hardness Measurements

The hardness of samples was measured 3 days after baking, which was selected as an appropriate time point based on when consumer's would purchase the product while allowing several days of storage before microbial growth. As the hardness of bread increases over time, the consumer acceptance of that bread begins to decrease leading manufacturers to create breads that maintain their softness over extended periods of time. In the present study, batter consistency had the most influence on product hardness with lower consistency batters producing less hard bread. Mixing time significantly reduced product hardness in some formulations (i.e. longer mixing times yielded softer bread loaves). Among bread samples from batters of the same consistency, formulation generally had a significant effect on 7-day product hardness.

Batter consistency was the major influence over product hardness at 7 days. Higher consistency batters (125 B.U.) produced breads with hardness levels above 100 N for all formulations, expect for the LE/LO samples produced at 10- and 15- minute mixing times (Figure 11). All lower consistency batters (100 B.U.) resulted in breads with hardness levels below 100 N. A large range of hardness was seen in the present study. The sample with the highest force measurement was the HE/HO formulation with the 5-minute mixing time of the 125 B.U. consistency batter (363 N) and the lowest force measurement was for LE/LO with the

10-minute mixing time of the 100 B.U. batter (43 N). The difference in 7-day bread hardness between the higher and the lower consistency batter samples may be explained by several factors. The increased water addition may allow for increased protein hydration and interactions, enhancing the batter matrix with some expansion and some entrapment of the gas produced by the yeast. The yeast proliferation may also be enhanced by higher water levels through increased sugar mobility, allowing for relatively more gas production and creating a less dense bread loaf with a decreased overall hardness. Breads with a higher specific volume had decreased crumb hardness levels. The level of moisture in the batter may also decrease the rate of staling, as He and Hoseney (1990) demonstrated: wheat breads with higher moisture had lower crumb hardness.

Increasing or decreasing mixing time significantly reduced product hardness in some formulations. In all bread samples from batters with higher consistency, except for LE/LO, the hardness at 7 days after baking significantly decreased from the 5-minute to the 15-minute mixing time. The largest decrease in this group was seen from the 5- to 15-minute mixing time for the HE/HO formulation (363 N vs. 122 N). A significant decrease in product hardness from 5-minute to 15-minute mixing time for the lower consistency batters was only seen for the HE/LO and HE/HO formulations (73 N to 52 N and 80 N to 53 N, respectively). Mixing time seems to play an important role in product hardness, but only in lower consistency batters (i.e. 100 B.U.). Formulations with higher levels of water addition did not benefit to the same extent as those with lower water addition levels. This may be a result of the high water content sufficiently allowing for dispersion of all ingredients into a cohesive matrix without the necessity of increased mixing time.

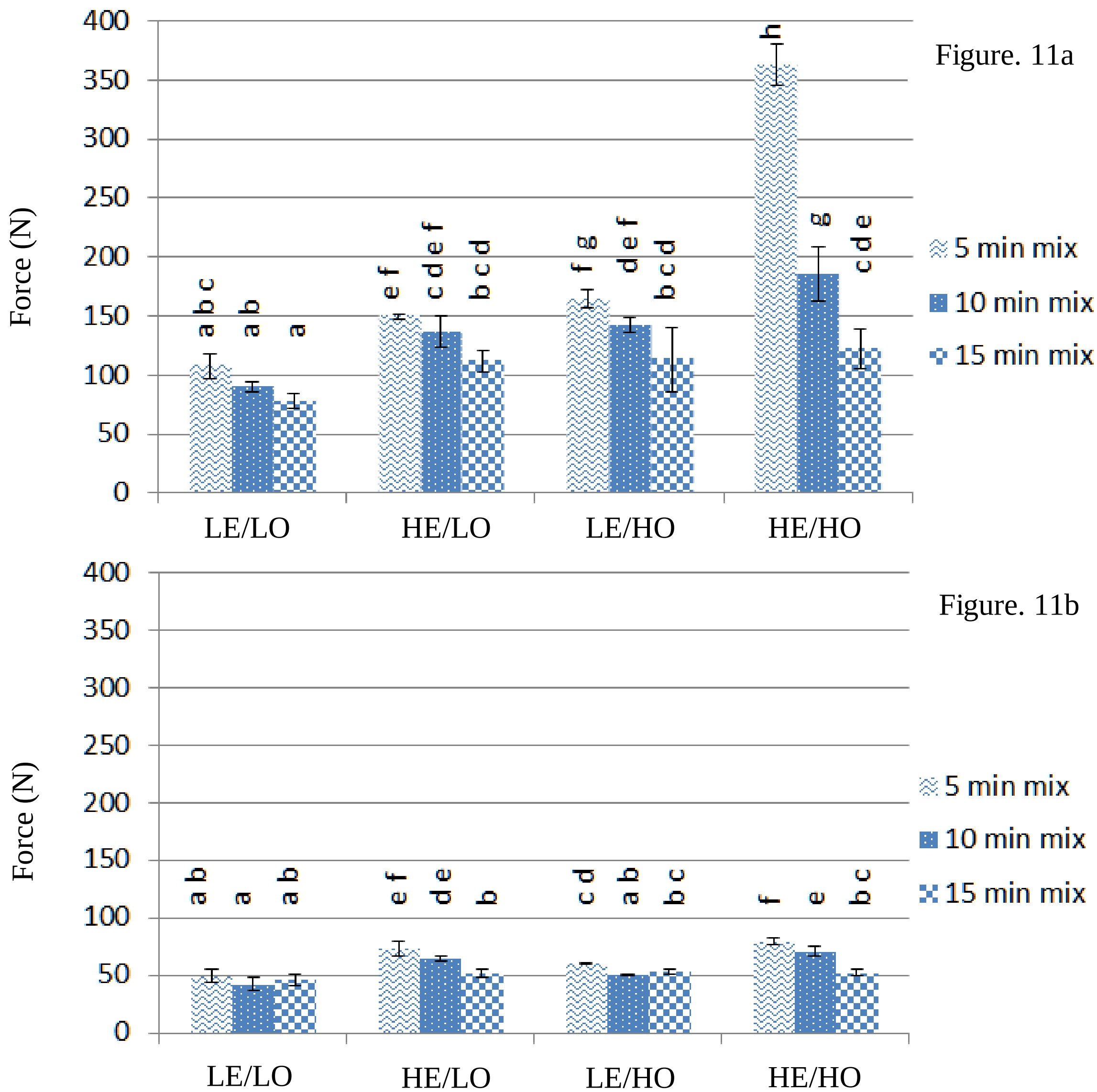


Figure 11. Hardness of bread slice at day 3 from buckwheat-rice flour bread loaves made from 125 B.U. (Figure 11a) and 100 B.U. (Figure 11b) consistency batters. See text for details of water addition determination based on B.U. measurements. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Different letters indicate a significant difference at $p<0.05$ using LSD.

The effect of formulation on day 7 hardness was most pronounced for the lower consistency batters (100 B.U.) compared to higher consistency batters (125 B.U.) when counterpart formulations were compared at the same mixing time. Among the lower consistency batters, LE/LO had significantly lower product hardness than HE/HO at each counterpart batter mixing time. No significant bread hardness differences existed between the HE/LO and LE/LO formulations at the lower batter consistency. For the higher consistency samples (125 B.U.), no clear trends exist between formulation and bread hardness. Higher consistency batter formulations had significant differences at some mixing times, but not at others. Formulation had a much more pronounced effect on hardness values of baked loaves from higher consistency batters than from the lower consistency batters.

4.8 Resistant Starch

Resistant starch (RS) levels in raw buckwheat flour and rice flour were measured before baking, and the resistant starch levels in all bread formulations from samples with 15-minute batter mixing times were measured 7 days after baking. Starch in raw materials (seeds and flour) naturally contains some levels of resistant starch which can be increased through processing. The levels of resistant starch in the raw flours were 1.42% and 1.04% (RS/total starch dwb) for rice flour and buckwheat flour, respectively. The resistant starch content in the breads increased from the levels found in the raw material in all measured formulations (Figure 12). Some significant differences among bread resistant starch content were found between counterpart batter consistency formulations, but no significant differences were found between formulations at the same batter consistency.

The levels of resistant starch on day 7 after baking ranged from 2.13% to 2.42% (HE/HO and LE/HO, respectively) in breads with a higher initial batter consistency (125 B.U.), although there were no significant differences. In the breads baked from lower consistency batters (100 B.U.), the resistant starch content ranged from 1.86% to 1.90% (HE/LO and LE/HO, respectively) with no significant differences across formulations. A significant difference in RS content existed between breads baked from higher and lower consistency batters in LE/LO and LE/HO formulations where the oil content seem to play a role in resistant starch formation.

Bread staling is attributed to starch regaining its crystalline structure which increases the hardness of the crumb (Coultate 2009). He and Hoseney (1990) found that higher moisture breads had a softer crumb than control moisture samples. The higher moisture breads were baked for a shorter time than the control breads but had a softer bread crumb over a 90-day storage period and, although not measured, likely had lower levels of resistant starch than control samples as the starch granules were only partially gelatinized. Similar results were seen in the present study: samples baked with a higher initial moisture content had lower crumb hardness as seen in Figure 8 and also a lower resistant starch content. The hardness of the samples was likely more the effect of cell size volume rather than crystallizing of the starch microstructure. In breads with increased cell size, the hardness value measured by the texture analyzer likely decreased because of the increase in air present in the defined space the texture analyzer probe was compressing.

The similar resistant starch content found among bread samples from formulations of the same batter consistency was not as expected. Amylose and lipids form complexes during retrogradation of starch which reduce the number of amylose-amylose interactions and therefore reduce resistant starch formation through competitive formation of amylose-lipid complexes

(Szczodrak et al 1992). One would predict that increased lipid content provided by the canola oil in the bread formulation would reduce the overall resistant starch content, but no significant differences were seen in bread samples with increased canola oil content. The levels of canola oil used in the bread samples were possibly not high enough to affect the total resistant starch as predicted. Monoglycerides are used in the baking industry to retard staling, and it may be that in this study, the amount and/or type of free fatty acids provided by the canola oil was relatively low, and not at the level necessary to form enough amylose-lipid to significantly change the resistant starch content of the bread samples. Evidence of the effect of different commercial food grade oils and levels they are added at on resistant starch formation in bakery products is limited.

The higher batter consistency significantly increased the resistant starch content in the baked breads of both the LE/LO and HE/HO formulations compared to breads made from their lower consistency batter counterparts. One possible theory for the significant increase in resistant starch content of the LE/LO and LE/HO bread samples may be that the increased water addition which could decrease the rate of interactions in the more diluted, lower consistency batters. However, this theory is not supported by the non-significant differences in RS levels among the remaining formulations between their two batter consistencies. Internal baking bread temperature may also be an issue in RS formation. One study found that wheat bread baked for a longer time and lower temperature (24 hours at 120°C) had nearly double the resistant starch as bread baked for a shorter time and at a higher temperature (35 minutes at 200°C) in an increase from 2 to 4%. (Yadav 2011). In the present study, it is likely that there were differences in the heat penetration into the bread samples between the higher and lower consistency batters due to differences in the amount of water added, with chigher amounts of added water resulting in lower heat penetration.

The increased water in the lower consistency batters may have reduced the degree of gelatinization of the starch reduced the amount of the amylose leaching out of the starch granules to form amylose-amylose structures during storage.

The resistant starch values for the gluten-free breads in the present study are greater than those reported for commercial white bread which was determined to be half a percent (Akerberg et al 1997), but lower than the values of lab-made wheat breads reported by Yadav 2011 (2-4%). It was found in a preliminary study that gluten-free bread with just rice flour and high egg white solids and high canola oil with lower batter consistency had a resistant starch content of 0.82% (starch weight basis). The resistant starch content was improved to 4.87% (starch weight basis) in breads made under the same conditions but with only buckwheat flour (Appendix, Table 5). Buckwheat flour therefore is a way to improve the resistant starch content gluten-free bread.

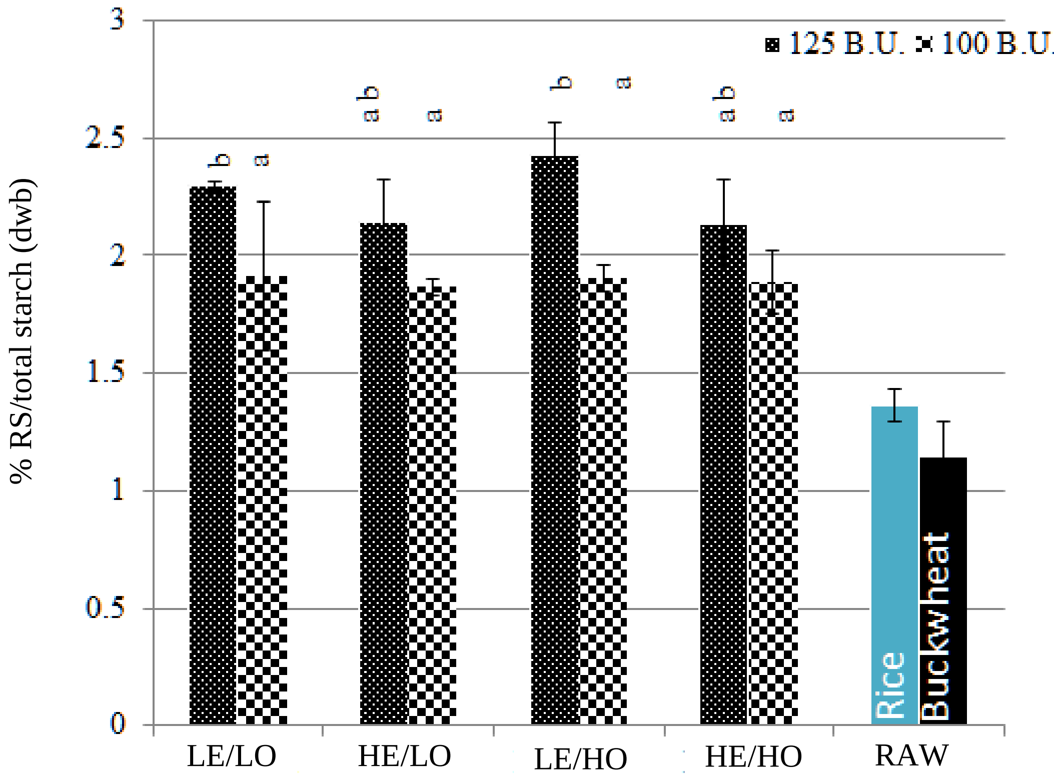


Figure 12. Resistant starch content in baked breads made from 15 minute mixed batters of each formulation at 125 and 100 B.U. batter viscosities. See text for details of water addition determination based on B.U. measurements. HE/HO, LE/LO, HE/LO, and LE/HO represent formulations made with 10% egg/8% oil, 5% egg/4% oil, 10% egg/4% oil, 5% egg/8% oil fwb, respectively. Different letters indicate a significant difference at p<0.05 using LSD. Samples on right are raw flours from rice and buckwheat.

CHAPTER 5:

CONCLUSIONS AND RECOMMENDATIONS

To create gluten-free bread, different processing approaches need to be considered as compared to wheat-based breads to create products with similar physical characteristics. Optimizing the processing conditions is essential in creating products with the desired finished properties. Although this study did not define what finished product properties are considered acceptable based on consumer expectations, it did help explore what conditions drive the changes to the finished bread loaf properties. It will be the work of future researchers to understand the drivers of consumer liking for gluten-free products and use this work to obtain those products. This study demonstrated that the measured quality and physical attributes of gluten-free bread made from buckwheat flour and rice flour were influenced primarily by batter consistency and secondarily by initial batter formulation and batter mixing time.

The batter consistency influenced the crust and crumb appearance, specific volume, hardness, and resistant starch content of the finished baked loaf. Bread samples made from the higher consistency batters had more severe cracks on the upper crust surface, although this effect decreased with increased batter mixing times. Small circular voids similar in appearance to air bubbles were found on the crust surface of breads made from the lower consistency batter samples, but were not present in breads made from the higher consistency batters. In addition, breads made from lower consistency batters had a more flattened top crust while breads made from higher consistency batters had a more convex top crust. The crumb appearance showed that larger air cells formed in the lower consistency batters, but in both batter consistencies more large air cells formed near the surface of the top crust. Although consumer sensory analysis was not included in this study, a more uniform, domed, and blemish-free crust appearance would likely score higher based on currently available market bread products.

Bread samples baked from lower consistency batters had significantly higher specific volumes than those baked from higher consistency batters. This is consistent with published reports showing that gluten-free breads, from a wide variety of grain sources including buckwheat, are typically produced from a cake-like batter to achieve quality properties similar to those of wheat-based breads. Although a mechanism for the significant differences found in the specific volumes of bread loaves from high and low consistency batter formulations was not defined, the lack of a continuous and extensible protein matrix as found in gluten-containing breads makes the consistency in gluten-free bread batter a critical factor to achieve the desired loaf volume.

The batter consistency also influenced the crumb hardness of baked breads. The increased water content in the lower consistency batters significantly lowered the hardness of the measured bread slice at day 3. The larger air cells found in the crumb of the breads made from the lower consistency batters likely provided less resistance to force as the probe from the texture analyzer pushed downwards on the bread slice during analysis. The increased water content in the lower consistency samples may also have acted as a tenderizer to soften the crumb of the finished bread.

The higher consistency batters produced significantly higher levels of resistant starch than their lower consistency counterparts in two of the tested bread formulations, LE/LO and LE/HO. The resistant starch content in all baked bread was increased from the levels found in the raw flours by nearly double. The effect of processing on increasing resistant starch content is consistent with the results found in the literature.

Increasing mixing time reduced the crack severity or completely removed the appearance of cracks from the crust surface of the studied bread loaves, across the 5- to 15-minute mixing times. The increase in mixing time appeared to strengthen the structure of the batter matrix and allow for ease in expansion without the creation of stress fractures shown as cracking. Mixing time influenced the crumb structure of the cells, with longer mixing time creating a more open crumb structure. Increased mixing times tended to increase the specific volume and decrease the hardness of baked bread samples.

The changes in formulations had an influence on the level of water necessary to achieve the target batter consistency, but did not influence the appearance of the baked crust or crumb composition and therefore was likely not outside the ranges necessary to visually judge the differences. LE/LO and LE/HO formulations produced bread loaves that had significantly greater specific volumes when compared with counterpart formulations of the same mixing time and batter consistency. The lower egg white solids addition level tended to produce baked breads with a higher specific loaf volume regardless of the level of canola oil added to the formulation, compared to formulations using a higher egg white solids level. The influence of formulation on hardness was most evident in the higher consistency batters where LE/LO samples had the significantly lowest hardness at day 3. HE/HO formulations tended to produce the hardest bread slices at day 3 regardless of batter consistency and mixing time. Resistant starch content was not significant by affected by changes in formulations.

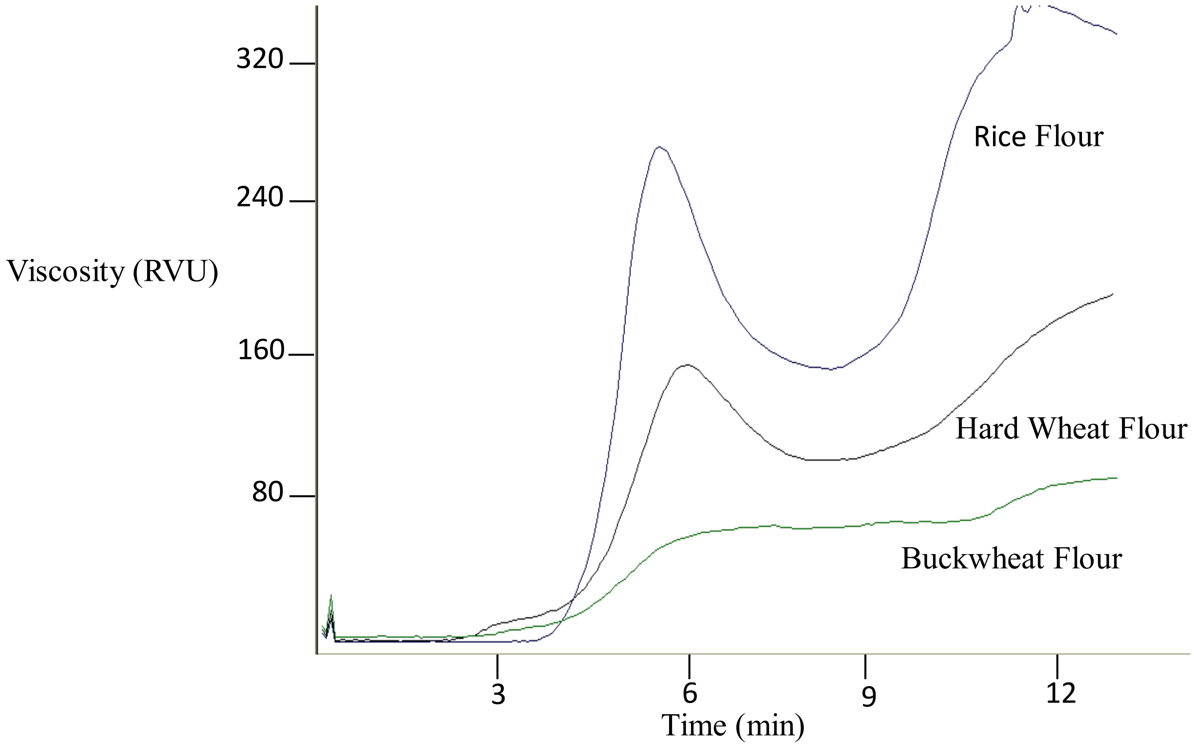
The results from the present study illustrate the importance of not only formulation and water addition, but also of the critical effect of processing steps like mixing time on the overall characteristics of the finished baked product**.** Accordingly, a few recommendations can be proposed to better understand how these factors affect gluten-free bread products: (1) Further research is necessary to understand the effect of processing conditions including mixing temperature, force in mixing, and proofing conditions on the finished quality of the bread to better understand all the tools necessary for creating consumer-acceptable gluten-free bread products. (2) Research is necessary to understand the effect of each individual ingredient on the quality of baked gluten-free bread. (3) Exploration should continue on how resistant starch levels of raw materials (i.e. buckwheat flour and rice flour) can be increased in baked products via different processing conditions.

APPENDIX

Appendix Table 1. Water Addition to Achieve 100 B.U., Bake Loss, Moisture Content, Specific Volume, Hardness, and Resistant Starch Content of Breads Made From 100 B.U. Consistency Batters using HE/HO Formulations.

	Water Added to Reach 100 B.U. (fwb/grams)	Bake Loss (%)	Moisture (%)	Specific Volume (cm^3/gram)	Hardness (N)	% Resistant Starch (dwb)
Only Rice Flour HE/HO	104.3	18.1	48.7	1.71	160.7	0.82
Only Buckwheat Flour HE/HO	97.6	11.1	46.0	2.77	36.9	4.87

Appendix Figure 1. Example of baked buckwheat-rice bread loaf using no added canola oil and low level of egg white solids (5% fwb) made from an estimated 125 B.U.-145 B.U. consistency batter. Crust edges are darkened from oil absorption from oiled pan during baking.



Appendix Figure 2. RVA profiles of rice flour, hard wheat flour, and buckwheat flour. Samples were analyzed according to the same method as described in the text.

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