DEVELOPMENT OF GLUTEN-FREE BREAD BAKING METHODS UTILIZING SORGHUM FLOUR

A Thesis

by

SARA ELIZABETH BOSWELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Food Science and Technology

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Approved by:

Chair of Committee,	Lloyd W. Rooney
Committee Members,	Nancy D. Turner
	Joseph M. Awika
Chair of Intercollegiate Faculty,	Alejandro Castillo

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ABSTRACT

Development of Gluten-Free Baking Methods Utilizing Sorghum Flour.

(December 2010)

Sara Elizabeth Boswell, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Lloyd W. Rooney

Increasing diagnosis and awareness of celiac disease and gluten intolerance has created a need for developing improved quality gluten-free sandwich breads. Sorghum is a naturally gluten-free grain with ideal baking qualities that is underutilized in the gluten-free baking industry. Research is needed on developing gluten-free breads utilizing sorghum flour that could be used in future research and commercial production.

Three objectives were tested. Objectives evaluated feasibility of using egg white foam with leavening agents in yeast-free bread, optimum mixing time in a laboratory control bread utilizing sorghum flour, and maximizing the amount of sorghum flour that could be used in the control formulation. Four comparisons were tested for yeast-free breads and 5 were compared for yeast breads. Volume, hardness, and color were measured using 15 replications. Environmental Scanning Electron Microscopy (ESEM) was performed on selected treatments to evaluate crumb structure.

Utilizing egg white foam for gluten-free breads produced acceptable volume, color, crumb structure and hardness compared to commercial gluten-free controls. Using egg white foam eliminates proofing time with increased production speed.

Increasing mixing time in gluten-free yeast breads significantly ($P<0.05$) improved specific volume and overall loaf volume without negatively affecting crumb hardness in 10 and 15 minute mixing treatments. Crumb structure was significantly improved between 5 and 15 minute treatments. Evaluation with ESEM showed reduced clumping of ingredients in the crumb and thinner air cell walls. Specific volume and loaf volume were significantly ($P<0.05$) higher in 15 minute mixing (2.13 cm^3/g; 1845 cm^3) versus the commercial comparison (2.00 cm^3/g; 923 cm^3). Optimum mixing for yeast bread was 15 minutes and optimum percentage of sorghum used in the flour blend was 60%. Increasing the use of commodity grade gluten-free decorticated white sorghum flour will reduce cost of specialty milled ingredients.

In future studies mixing for 15 minutes using the laboratory yeast bread formulation containing 60% sorghum should be used as the research control as it provided consistent optimum results.

DEDICATION

I would like to dedicate this thesis to the mothers of gluten-free children who inspired me to conduct this research. Their quest to educate others on the gluten-free lifestyle and efforts to raise awareness on the quality of gluten-free products on behalf of their children is an inspiration and I can only hope to live up to their accomplishments.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Lloyd Rooney, and my committee members, Dr. Nancy Turner and Dr. Joseph Awika, for their guidance and support throughout the course of this research.

Thanks also go to Cassandra McDonough, Jack Lindsay, and Eliana Pinilla who helped me in accomplishing this project. Thanks go to the department head, Dr. David Baltensperger as well as the department faculty for providing support of this research. I would like to thank my Cereal Quality Lab colleagues and my friends for supporting me throughout this experience. I would like to thank Cynthia Kupper RD, CD and Roben Ryberg for their guidance and support. I also want to extend my gratitude to Corn Products International, Archer Daniels Midland, and the Sorghum Check Off Board for providing supplies and funding for this research.

Finally, thanks to my husband and family for their constant encouragement.

NOMENCLATURE

CD	Celiac Disease
YFO	Yeast-free Original Formulation
YFM	Yeast-free Modified Formulation
YF	Yeast-free
BBA	Breads by Anna
BRM	Bob’s Red Mill

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Celiac disease is a genetic autoimmune disorder found in approximately 1 of 133 people in the United States. Individuals with celiac disease react to prolamin fractions found in wheat, rye, and barley (Niewinski 2008). Increasing diagnoses of celiac disease has created a rise in demand for gluten-free products (Leffler et al. 2008).

Gluten-free bread has undesirable properties such as reduced loaf volume and brittle, hard texture (Aerndt and Moore 2007). Recent studies have reported that quality of gluten-free products was a significant concern to individuals with celiac disease (Leffler et al. 2008)

Researchers as well as cook book authors have attempted to develop acceptable gluten-free breads utilizing starches and hydrocolloids, but an acceptable set of standard methods and a research control bread formulation has yet to be found (Ács et al 1996a; 1996b; Arendt and Moore 2007; Demiate et al. 2000; Gallagher and Arendt 2003; Gambus, Sikora & Ziobro 2007; Hagman 2000; Ryberg 2008; Schober and Bean 2008).

Sorghum is an ideal grain for use in gluten-free breads because it is not currently used in most gluten-free research or commercially sold breads due to lack of commercially available gluten-free flour (Ciacci et al. 2007). Investigation into methods of baking breads with improved quality utizilizing sorghum is needed.

This thesis follows the style of Cereal Chemistry.

CELIAC DISEASE

Celiac disease (CD) is a multisystemic autoimmune disorder that occurs in genetically susceptible individuals as a response to gluten protein fractions found in wheat, rye, and barley. Samuel Gee, M.D. first described celiac disease's classical features in 1887 as failure to thrive, diarrhea, and lassitude, but it was not until 1953 that Willem Karel Dicke identified wheat, rye, and barley as the cause (van Bergeijk et al. 1993). Originally thought to be a rare childhood disorder, recent improvements in serological testing and public screenings have shown the prevalence of CD in the general population to be 1 in 133 (Niewinski 2008).

Pathogenesis

The intestinal epithelium of individuals under normal physiological conditions contains intact intercellular tight junctions that serve as a primary barrier to macromolecules. The protein fractions glutenin, gliadin, hordein, and secalin found in wheat, rye, and barley are classified as "gluten proteins" that are seen as the activating molecules for celiac disease (Niewinski 2008). Portions of these protein fractions are resistant to complete digestion by proteases in the upper portion of the digestive system and remain intact in the intestinal lumen. The long-chain protein fractions pass through tight junctions during times of infection, surgery, an upregulation of zonulin, or in individuals with celiac disease when the epithelial lining has increased permeability (Green & Cellier 2007). In celiac disease, these protein fractions cause an inflammatory response in the upper small intestine mediated by CD4+T cells located in the lamina propria that are reactive to specific fractions of gluten proteins (Green & Cellier 2007).

The transglutaminase enzyme deamidates the gluten protein fractions, which increases the immunogenicity causing an inflammatory cascade from the CD4+T cells. This cascade releases metalloproteinases as well as other tissue-damaging mediators that induce villous injury and crypt hyperplasia. This leads to destruction of the surface epithelium and flattening of the villi (Bethune & Khosla 2008; Green and Cellier 2007). Celiac disease is most closely associated with the genes HLA-DQ2 and HLA-DQ8, however a large portion of the general population carries these genes. Other non-HLA genes may influence susceptibility to CD (Green and Cellier 2007).

Treatment

The only known treatment for CD is a life long diet free of the protein fractions glutenin, gliadin and hordein found in cereals of the family *Triticum* and related grains such as barley, triticale, and rye (Ciacci et al. 2007). Grains, legumes and pseudocereals that are considered gluten-free and safe for celiacs include amaranth, arrowroot, all types of pulses, buckwheat, corn, mesquite, millet, quinoa, rice, sorghum, soy, and teff (Ciacci et al. 2007; Green and Cellier 2007; Niewinski 2008). Upon first diagnosis, celiac patients typically suffer from iron, folate, and calcium deficiencies due to malabsorption in the small bowel. Secondary lactose intolerance is common in celiac patients due to decreased lactase production from damaged villi in the small intestine. Deficiencies of fat soluble vitamins like A, D, E, and K are also common; however, after treatment with a gluten-free diet damage to the small intestine heals and serological values should return to normal (Green and Cellier 2007; Niewinski 2008).

GLUTEN-FREE BREAD

Bread is a staple food in many homes, however upon diagnosis with celiac disease traditional wheat based breads are no longer an option for consumers. One of many challenges to individuals with celiac disease is the increased cost of gluten-free foods. On average gluten-free products are approximately 240% more expensive than their gluten-based counterparts (Lee et al. 2007; Stevens & Rashid 2008). Further studies showed that 56.5% of participants reported it was difficult to find gluten-free food outside the home, while 75.3% of participants reported that quality of gluten-free products was a significant concern (Leffler et al. 2008). Quality and availability of gluten-free foods to adolescents in schools cause adolescent noncompliance to gluten-free diet. Thus appealing gluten-free foods must be available in schools or else Celiacs will be unable to maintain a gluten-free diet (Olsson et al. 2008).

Nutritional Quality

Gluten-free flours and starches used in bread baking lack fortification with micronutrients and, depending on the starch or flour of choice, dietary fiber. In a 2005 survey, Thompson et al. (2005) found that less than 46% of female respondents consumed the daily-recommended amount of dietary fiber and only 44% of female respondents consumed the daily-recommended amount of iron during the 3-day recording period. These low values are problematic because enriched/fortified grain foods are a large contributor to the US adult daily intakes of iron (Subar et al. 1998). Initial investigations of fortifying gluten-free breads with iron have shown potential but no iron fortified breads are currently available in the United States (Kiskini et al. 2007).

Developing gluten-free breads from whole grain and pseudo cereal flours like buckwheat, flax, quinoa, brown rice, teff, legumes, sorghum, and nuts would be an ideal option for improving the nutritional quality and the dietary fiber content of gluten-free breads (Niewinski 2008). Sorghum is an ancient grain and major crop in the United States, which should be significantly less expensive than many of the other ancient grains. It is increasing in availability with some major companies considering utilizing it in products designed for gluten-free consumers.

Sorghum in Gluten-free Bread

Sorghum is an ideal grain to use in gluten-free bread production, as it is safe for consumption by individuals with celiac disease (Ciacci et al. 2007). Though it has not been widely used for food production in the United States, sorghum has been a staple in Africa, India, China and other areas because of its robust production under drought conditions and it is free of aflatoxins that affect maize (Rooney and Waniska 2000). White, tan-plant food-grade sorghums have a mild flavor and are ideal for gluten-free breads (Waniska and Rooney 2002). Sorghum flour is also commonly suggested as an ingredient in gluten-free cookbooks for individuals who prefer to prepare products at home (Ryberg 2008; Fenster 2008). Lack of a supply chain in the United States limits its use in commercial gluten-free products. This is being solved with new hybrids that are white with sweet, bland flavor. Other sorghums are very high in antioxidants with a wide array of unique levels of flavonoids and rare 3-deoxyanthocyanins.

Gluten-free breads made with sorghum require higher water content creating a batter-like system, unlike wheat-based doughs. Formulations attempted with lower water concentrations yield less elasticity and volume, as well as a brittle texture (Taylor et al. 2006). Gluten-free sorghum bread produced without hyrocolloids and stabilizers has small volume and a brittle crumb (Schober et al. 2005). Sour dough fermentation has also been shown to improve crumb and volume in gluten-free breads using sorghum (Schober et al. 2005).

Xanthan Gum in Gluten-free Bread

Lack of a gluten matrix within gluten-free breads requires addition of other functional ingredients to improve batter viscoelastic properties. Hydrocolloids (gums) are considered an essential ingredient in gluten-free breads as they consist of hydrophilic, long-chain, high-molecular-weight molecules that produce gels when combined with water (Aerndt and Moore 2007). Xanthan gum was first reported in the production of gluten-free starch based breads in 1974, and has been commonly used in gluten-free bread production since then (Anton and Artfield. 2008). Derived from the organism *Xanthomonas campestris*, xanthan gum improves viscoelastic properties of gluten-free doughs and batters more than carboxymethylcellulose (CMC), pectin, agarose, and β-glucan (Lazaridou et al. 2007).

Gambus et al. (2007) compared the addition of xanthan gum to guar gum and pectin in gluten-free breads and showed that dough mixed for 10 minutes using xanthan gum had a higher loaf volume. Bread using higher percentages of xanthan gum maintained a softer texture after 72 hours. These findings support the idea that the use of longer mixing periods (10 minutes) with the addition of xanthan gum in gluten-free breads could improve loaf volume. More research is needed because of the low replications seen within that study and the use of multiple hydrocolloids instead of observing the effect of a single hydrocolloid on gluten-free bread (Gambus et al. 2007).

Gluten-free Bread Without Sorghum

Schober (2008) classifies gluten-free breads into two types: starch based and cereal flour based. Starch based breads were initially used in early trials of gluten-free bread (Jongh 1961; Nishita et al. 1976) and again in more recent studies (Ács et al 1996a; 1996b; Demiate et al. 2000; Gallagher and Arendt 2003). Even though there has been investigation into both starch based and flour based gluten-free breads there currently is no standard baking method.

Standardized control formulations are needed for starch based and flour based gluten-free breads that have been thoroughly tested to observe if they have consistent baking properties, specific volume, texture, and color. The use of larger loaf volumes and consistent controls would produce results that could more accurately represent the type of product produced in a small gluten-free bakery as well as promote the development of standard methods of laboratory gluten-free bread baking.

Batter Based System

Gluten-free bread dough is a more fluid system similar to that of cake batter due to the lack of a gluten matrix (Moore et al. 2004), so a different methodology is needed for preparation and baking. Gas holding capacity is more difficult to obtain in this type of batter, but different pregelatinized starches and gums improve gas retention (Cauvain 1998; Satin 1998). An example of the batter like state of certain gluten-free formulations can be observed in Figure 1. This gluten-free formulation mixed for a short period of time (3 min) collapsed during baking due to inadequate gas retention.

Fig. 1. Poor Gas Retention in Gluten-Free Bread. Typical result of a gluten-free recipe that collapsed due to poor gas retention. Loaf was baked in preliminary trials.

Batter preparation has not been thoroughly investigated in gluten-free research. Extending mixing time to promote incorporation of ingredients specifically in formulations containing flours with larger particle size like brown rice flour or sorghum flour could improve crumb development. Figure 2 shows an example of bread mixed according to directions for 3 min. that appears to have unevenly incorporated egg white on the exterior crust.

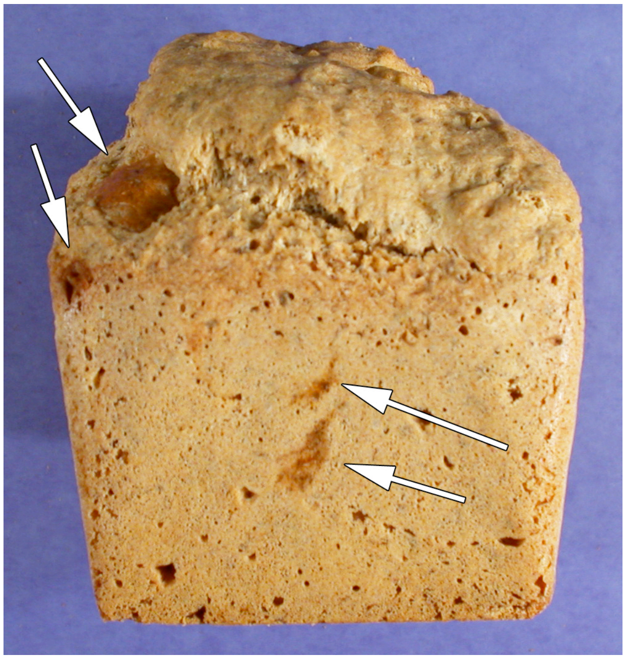

Fig. 2. Uneven Incorporation of Ingredients. Example of uneven incorporation of ingredients from loaf baked in preliminary trials. Arrows indicate unmixed egg in baked loaf.

Alternative Sources of Bread Formulations

Since the late 1980's, gluten-free cook books have been essential to food preparation for individuals living with celiac disease. Most authors use batter based methods (Fenster 2008; Hagman 2000). Ryberg proposed using egg white foam with leavening to achieve a desirable loaf (Ryberg 2008). Utilizing egg white foam and chemical leavening, similar to angel food cake, produces air pockets trapped within the egg white protein matrix. The bread matrix expands during the baking process due to the reactions of chemical leavening. This formulation has not been tested in a large-scale research study and could provide insight into developing a standard quick bake method for gluten-free breads.

CHAPTER II

MATERIALS AND METHODS

OBJECTIVES

1. Evaluate feasibility of using egg white foam with leavening agents in a yeast-free bread using sorghum flour and starches.

2. Evaluate the quality of a yeast bread formulation using sorghum to develop a laboratory control for use in future studies.

3. Maximize the amount of sorghum flour used in the gluten-free yeast bread control formulation without negatively affecting overall quality factors (volume, hardness, color).

RAW MATERIALS

Gluten-free sorghum flour, Brown Rice Flour and corn starch were purchased from Bob's Red Mill (Milwaukee, OR). Expandex™ was donated by Corn Products International (Westchester, IL) and spray dried angel type egg whites were donated by Sonstegard Foods Co. (Sioux Falls, SD). Xanthan Gum was obtained from CPKelco (Okmulgee, OK), and Mott's 100% apple juice (Rye Brook, NY), LouAna Canola Oil (Ventura Foods, LLC, Brea, CA), Arm & Hammer baking soda (Princeton, NJ), Rumford Baking Powder (Terre Haute, IN), Heinz apple cider vinegar (Pittsburg, PA), Red Star Dry Active Yeast (Milwaukee, WI), Feather Crest Farm Eggs (Bryan, TX), Imperial Sugar Pure Cane Granulated Sugar (Sugarland, TX), and Morton salt (Chicago, IL) were purchased at a local grocery store. EnerG Brown Rice Yeast-free and Yeast bread (Seattle, WA), Bob's Red Mill Homemade Wonderful GF Bread Mix (Milwaukee, OR), and Breads by Ana Gluten-Free, Yeast-free, Dairy Free bread mix (Dubuque, IA) were purchased at local grocery stores for comparison. Decorticated white sorghum flour used in Objective 3 was donated by Archer Daniels Midland Milling Division (Overland Park, KS).

YEAST-FREE BREAD TREATMENTS

Formulations and experimental treatment comparisons chosen for the yeast-free section of this project were EnerG Brown Rice Yeast-free Bread, Breads by Ana Gluten-free, Yeast-free, Dairy-free bread mix (BBA YF), Roben Ryberg's Original Yeast-free, Dairy-free, bread formulation (YFO), and a version of Roben Ryberg's Yeast-Free, Dairy-free bread that was modified by the author of this thesis (YFM). EnerG Brown Rice Yeast-free Bread (EnerG YF) was chosen because it is a widely available gluten-free bread that is yeast-free and dairy-free that is pre-baked to a consistent set of volume parameters. One limitation is that EnerG bread is a shelf stable bread and the time lapse between baking and purchase is unknown. The Breads by Ana mix was chosen because it is the only commercially available gluten-free, yeast-free bread mix available in the country. Slight modifications were made to Ryberg's formulation by the researcher in an attempt to improve stability of the final product and show that it can be produced using commercially available egg white powder.

YEAST-FREE BREAD PREPARATION

Tables 1 and 2 contain formulations for Roben Ryberg's Yeast-Free, Dairy Free and the Modified Variation of Roben Ryberg's Yeast-Free, Dairy-Free Bread. Batter was prepared by the method described by Ryberg with modifications to mixing speed for the specific mixer used (Ryberg 2008). Preparation procedures are found in Figure 3. For variation 1, the liquid egg whites were mixed at speed 10 on a Viking Professional 1,000 watt, 7 quart mixer (Viking Range Corporation, Greenwood, MS) using the whisk attachment for 1 minute 30 seconds. Variation 2 required hydration of 20 g powdered egg white with 120 g water at speed 10 for 45 seconds. After initial mixing and foaming of the egg white, remaining dry and wet ingredients were slowly added to the mixture at the "Stir" speed. This is a crucial step and ingredients need to be slowly added to not collapse the foam.. Batter was mixed for an additional 45 seconds on "Stir" speed after all dry and wet ingredients were added. Batter was gently poured into a lined 1 pound Chicago Metallic's bread pan (Chicago, IL).

Loaves were baked in a National Electric Rotating Oven (KS) at 350°F for 45 minutes. After baking loaves were removed immediately from baking pans and allowed to cool until further tests were performed. Samples for texture analysis were prepared in the same method, except 250g of batter was placed into a lined pup loaf pan for each loaf sample and baked for 25 min.

TABLE 1
Roben Ryberg's (2008) Yeast-Free, Dairy-Free Original Formulation

Ingredient	Bakers Percentage (%)	Total Percentage (%)	Weight As Prepared (g)
Corn Starch		35.2	250.0
Sorghum Flour	100.0	8.4	60.0
Baking Powder	4.8	2.1	15.0
Xanthan Gum	2.9	1.3	9.0
Salt	1.9	0.8	6.0
Baking Soda	1.5	0.6	4.5
Apple Juice	57.1	25.0	177.0
Canola Oil	9.0	3.9	28.0
Apple Cider Vinegar	6.8	3.0	21.0
Egg White	45.2	19.7	140.0
Total		100.0	710.5

TABLE 2
Modified Version of Roben Ryberg's Yeast-Free, Dairy-Free Formulation (2008)

Ingredient	**Bakers Percentage (%)**	**Total Percentage (%)**	**Weight As Prepared (g)**
Corn Starch	100	26.8	190.0
Sorghum Flour		8.4	60.0
Expandex™		8.4	60.0
Baking Powder	4.8	2.1	15.0
Xanthan Gum	2.9	1.3	9.0
Salt	1.9	0.8	6.0
Baking Soda	1.5	0.6	4.5
Apple Juice	57.1	25.0	177.0
Canola Oil	9.0	3.9	28.0
Apple Cider Vinegar	6.8	3.0	21.0
Egg White Powder	6.5	3.8	20.0
Water	38.7	16.9	120.0
Total		100.0	710.5

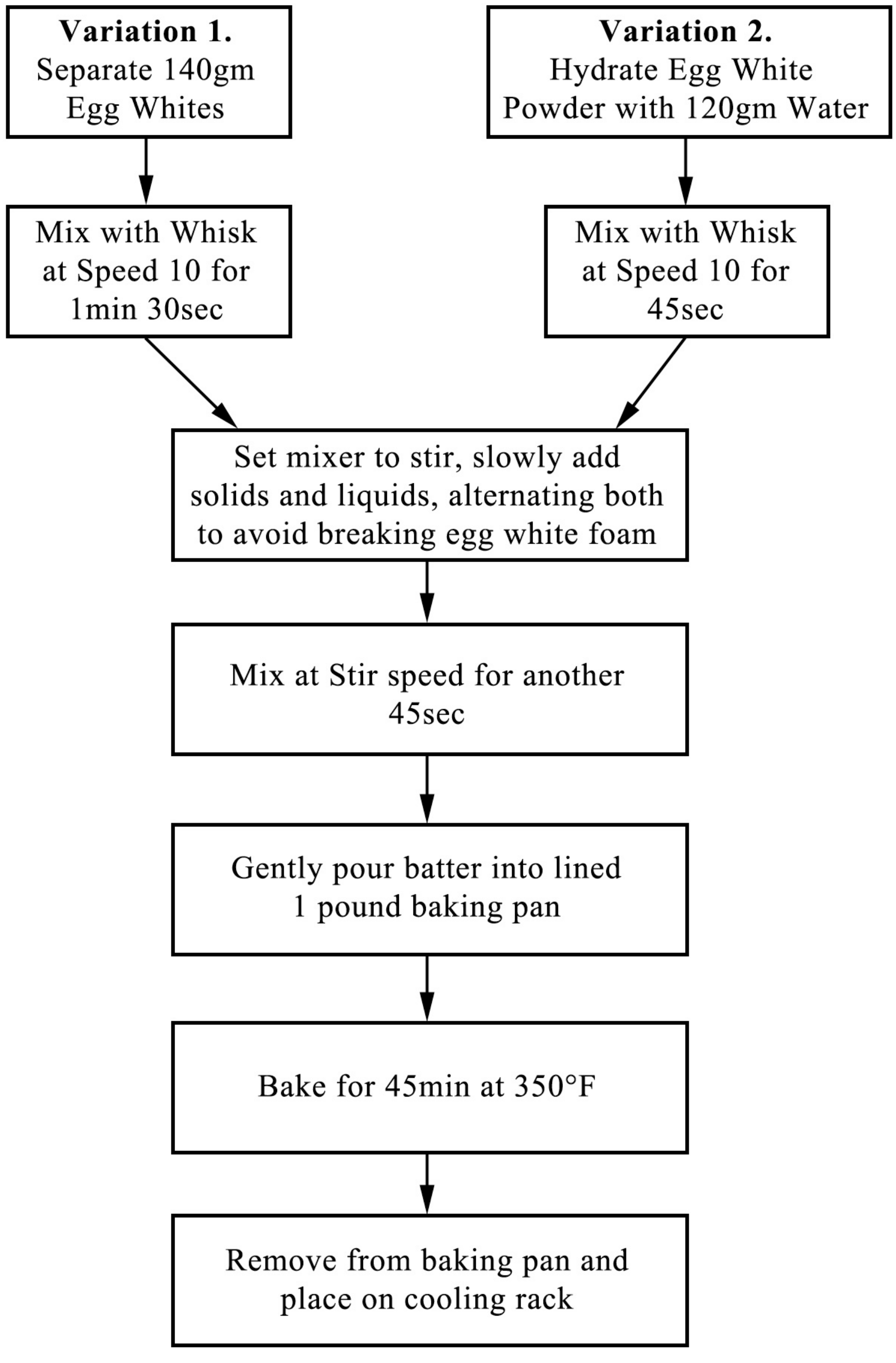

Fig. 3. Preparation Methods for Yeast-Free Breads.

OPTIMUM MIXING TIME TREATMENTS

Treatments for the yeast-bread portion of the project included EnerG Brown Rice Yeast Bread, Bob's Red Mill Homemade Wonderful GF Bread Mix, and a formulation (Table 3) developed at Texas A&M University's Cereal Quality Lab that was mixed for 5, 10, and 15 minutes to attempt to find an optimum mixing time for this formulation. The formulation from the Cereal Quality Lab was developed after several months of preliminary trials alternating flour, egg, and water ratios. EnerG Brown Rice Yeast Bread was chosen for the same reasons mentioned for the yeast-free alternative and it will have the same limitations. Bob's Red Mill's bread mix was chosen because it is a widely available gluten-free bread mix.

OPTIMUM MIXING TIME BREAD PREPARATION

Dry ingredients were weighed and premixed according to the formulation given in Table 3. A flow chart of preparation methods used for 5, 10, and 15 minutes is in Figure 4. Yeast (7 g) was activated in 413 g of warm water (~ 90°F) in a separate bowl for approximately 5 min. before mixing. All dry and wet ingredients were added to the mixer bowl and batter mixed for 5, 10, or 15 min at speed 10 depending upon the variation. After mixing batter was poured into lined 1 pound Chicago Metallic baking pans and placed into a proofing chamber (88° F; RH 88%) until batter is ¼" below the top of the pan (~50 minutes). Bob's Red Mill Bread Mix was prepared according to directions on the package.

Loaves were baked in a National Electric rotating oven at 350° F for 50 min. After baking loaves were removed from baking pans and allowed to cool until further

Table III
Laboratory Gluten-Free Yeast Bread Formulation

Ingredient	**Bakers Percentage (%)**	**Total Percentage (%)**	**Weight as Prepared (g)**
Brown Rice Flour		18.6	200.0
Sorghum Flour	100	18.6	200.0
Expandex™		7.9	85.0
Nonfat Dry Milk	4.7	2.1	23.0
Sugar	1.4	0.66	7.0
Salt	1.2	0.56	6.0
Xanthan Gum	0.9	0.42	4.5
Water	85.6	38.6	415
Egg	20.6	9.3	100
Canola Oil	5.8	2.6	28
Yeast	1.4	0.66	7
Total		100.0	1,075.5

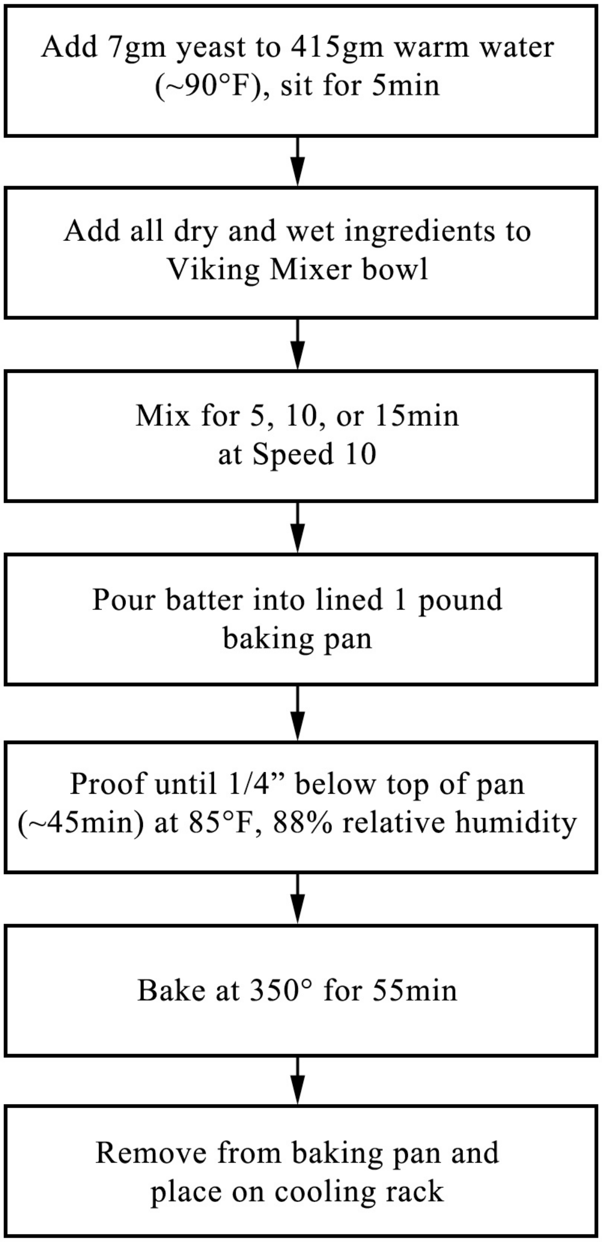

Fig. 4. Preparation Methods for Yeast-Breads.

tests were performed. Samples for texture analysis were prepared using the same method, except 300g of batter was placed into a lined pup loaf pan and baked for 25 min.

SORGHUM BREAD PREPARATION

Samples were prepared using the same method used for the laboratory control formulation except ADM decorticated white sorghum flour was used instead of Bob's Red Mill Sorghum flour (Fig. 4) The baker's blend percentage of flour was altered to increase the amount of sorghum used. Sorghum was tested at 40, 60, and 80% of the total baker's flour blend used (Table 4). As sorghum flour increased brown rice flour was decreased. Water content was not altered.

Table IV
Sorghum Substitution in Baker's Flour Blend

Ingredient	**Control (%)**	**60% Substitution (%)**	**80% Substitution (%)**
Brown Rice Flour	40	20	0
Sorghum Flour	40	60	80
Expandex™	20	20	20
Total	100	100	100

EVALUATION OF LOAF VOLUME

Loaf volume was measured using rapeseed displacement according to AACC Method (AACC 10-05.01). Volume was measured 20 minutes and 6 hours after removal from the oven to observe volume loss after baking. Volume was measured on fifteen one pound loaves for Objective 1 and 2. Three one pound loaves for each treatment in Objective 3.

EVALUATIONOF CRUMB HARDNESS

Hardness was measured 2 hours after baking using a TA.XT2i 36 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) (AACC 74-09). Bread was sliced into 15 mm thick slices, cut into a 2" square using a metal biscuit cutter for standardization, and analyzed with a flat, round 20 mm probe. Three replications per loaf were measured with a total of 45 samples measured for each treatment for Objectives 1 and 2. Nine samples were measured for each treatment in Objective 3. Slices were compressed to 60% of their initial thickness using a 25 Kg load cell.

EVALUATION OF CRUMB COLOR

Crumb color was determined using a Chroma Meter II Minolta Colorimeter (Osaka, Japan). Color of slices was measured after slicing for hardness measurements, and immediately before each slice was cut into 2" squares.

MICROSCOPY EVALUATION OF CRUMB

The continuous phase and cell wall structure after baking was investigated in select bread formulations using an Environmental Scanning Electron Microscope-ESEM (Electroscan Model E-3, Electron Corp., Wilmington, MA) with an accelerating voltage of 20 Kv. The samples for ESEM analysis were prepared by mounting the samples on aluminum stubs, with adhesive, and viewed with no further preparation.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed on all data using SPSS v16.0 for Macintosh (SPSS Inc.). Differences between means were analyzed with Tukey's Honest Square Difference Test. A confidence level of 95% was used.

CHAPTER III

RESULTS AND DISCUSSION

YEAST FREE BREAD

Subjective Evaluation of Crumb

Modifying the original formulation of yeast-free bread did not significantly harm the volume, texture, or color. YFO and YFM had large round appealing domes with consistent crumb formation (Fig 5) EnerG Yeast-free (EnerG YF) bread had a very light color with a dense coarse crumb (Fig 5) Breads By Ana yeast-free mix had a soft crumb with large air cells and significant tunneling. Breads by Ana appeared to have settling of the crumb as described by Schober (2009) where the air cells are not evenly distributed and cell size is decreased towards the crust. Other samples did not appear to have this tendency. Breads by Ana also had a brown unpleasant coloring and a flat dome with a brick like shape (Fig 5).

Fig. 5. Yeast-Free Loaf and Slice Profiles. Loaf profiles and slice profiles of gluten-free yeast-free breads.

Hardness

Modification of the original yeast-free formulation (YFO) did not significantly affect hardness (P value<0.05; Fig. 6). YFO needed 4,923 g of force on average to compress to 60% of initial volume compared to 4,785 g needed for the yeast-free modified (YFM) formulation. These results show that YFO and YFM have similar hardness measurements compared to fresh baked wheat bread control (reported range was 5-6 thousand grams of force needed for 60% compression) used in a previous study that performed the same protocol for measuring hardness (Moore et. al. 2004). Gluten-free yeast bread formulations utilizing brown rice flour as well as freshly baked white wheat pan style breads have similar or softer hardness values to YFO and YFM (Kiskini et al. 2010; Moore et. al. 2004; Renzetti and Arendt 2009). Commercial comparisons were significantly different from experimental treatments. EnerG Yeast-free Bread was not a good comparison because it was a shelf stable bread and date of baking was not known. It was chosen for this project as a comparison for acceptable commercial volume, rather than hardness. It was extremely hard (avg Force for 60% compression 16,399 g) and more brittle than other samples prepared, as expected due to unknown storage time and preparation methods. Breads by Ana Yeast-free bread were the most useful comparison for hardness measurements because it was freshly baked. It had significantly softer crumb (1,799g force needed for 60% compression) with a lighter stickier crumb than the experimental yeast-free variations.

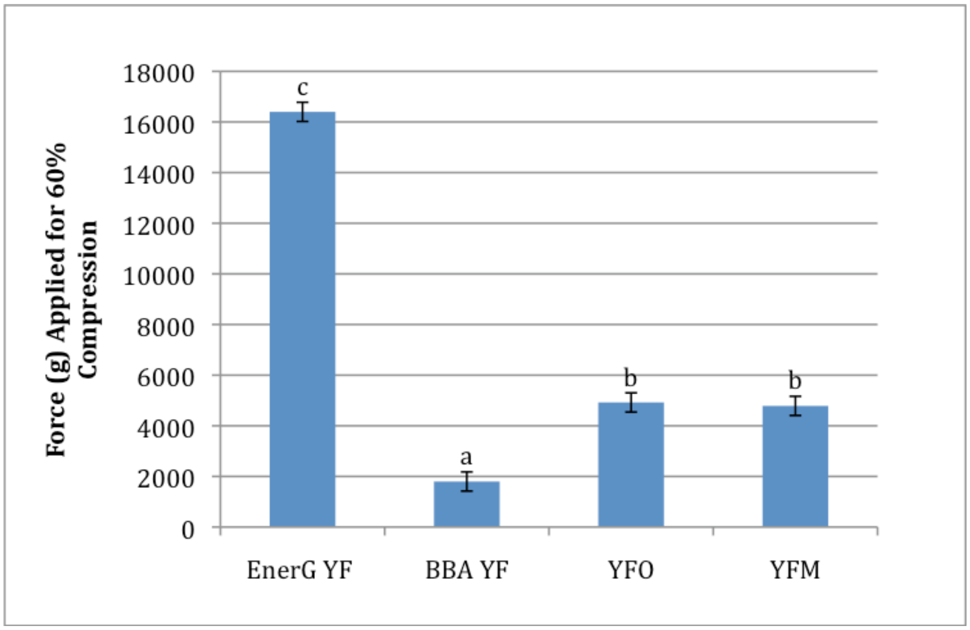

Fig. 6. Yeast-Free Hardness. Slice hardness of yeast-free breads after cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, $P<0.05$).

Volume Measurements

Initial loaf volume was significantly larger (1,248 cm^3, $P<0.05$) in the YFM treatment (Fig. 7). All treatments were significantly larger than EnerG Yeast-free Bread (873 cm^3). YFO (1,165 cm^3) did not have a significantly different volume from that of Breads By Ana Yeast-free pre-mix bread (1,193 cm^3). Initial specific volume measurements showed that the Breads by Ana Yeast-free pre-mix was significantly (2.21 cm^3/g, $P<0.05$) larger than other treatments (Fig. 8) and EnerG Yeast-free Bread had the lowest specific volume. The high specific volume observed in the Breads by Ana mix was most likely due to the crust formation. This formulation produced a loaf with a thick hard crust that supported a thin crumb network that would have collapsed without the crust reinforcement. There was no significant difference between YFO (1.96 cm^3/g) and

YFM (2.02 cm^3/g).

Loaf volume was measured 6 hours after baking to assess the final volume of the loaves. The YFM treatment (1,187 cm^3) had a higher total volume ($P<0.05$) than other treatments (Fig. 9). No statistical difference was seen between YFO (1,137 cm^3) and Breads by Ana Yeast-free pre-mixed bread (1,158 cm^3), and EnerG Yeast-free Bread (873 cm^3) had the smallest total volume. Final specific volume after 6 hours of cooling was significantly ($P<0.05$) higher in the Breads By Ana Yeast-free pre mixed bread (2.13 cm^3/g) than all other treatments (Fig. 10). No statistical difference was seen between the specific volumes of EnerG YF (1.86 cm^3/g), YFO (1.89 cm^3/g), and YFM (1.93 cm^3/g) treatments.

Specific volume for YFO and YFM were lower than reported wheat bread specific volume (4.18-3.18 cm^3/g) but similar to that of the gluten-free yeast bread treatments within that study (Kiskini et al. 2010). Specific volume was equal to higher than reported gluten-free yeast bread treatments using brown rice flour in the formulation (1.50-1.89 cm^3/g; Renzetti and Arendt 2009). These results indicate that the use of egg white foam in gluten-free breads can create acceptable loaf volumes when compared to commercial gluten-free alternatives and reported results of gluten-free yeast bread in previous studies.

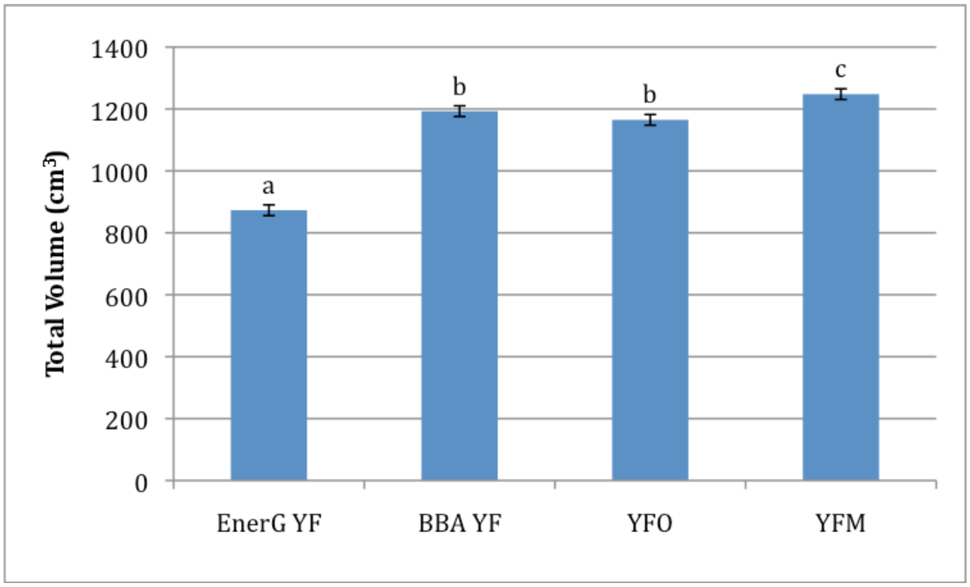

Fig. 7. Yeast-Free Initial Volume. Initial total volume of yeast-free breads after cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

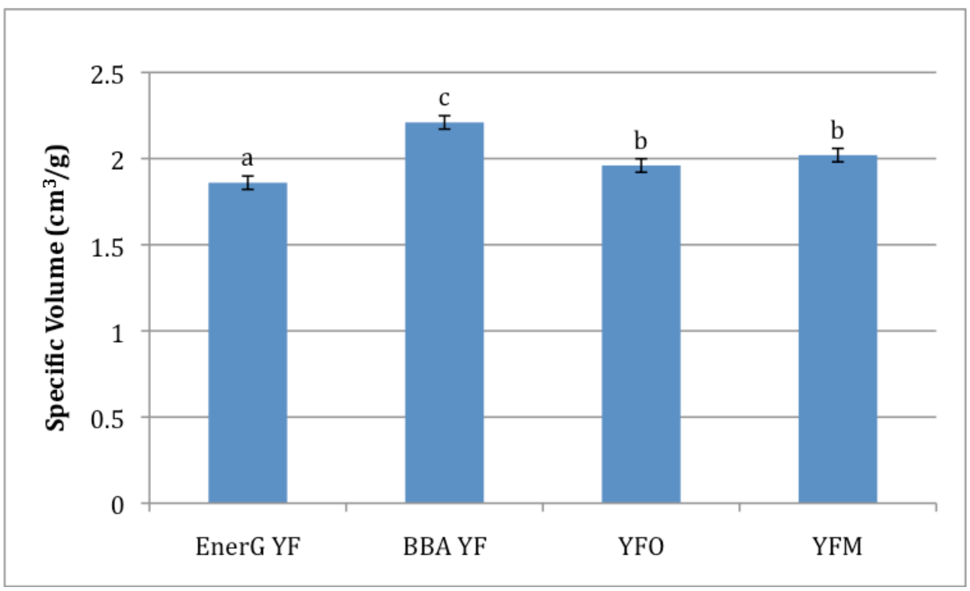

Fig. 8. Yeast-Free Initial Specific Volume. Initial specific volume of yeast-free breads after cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

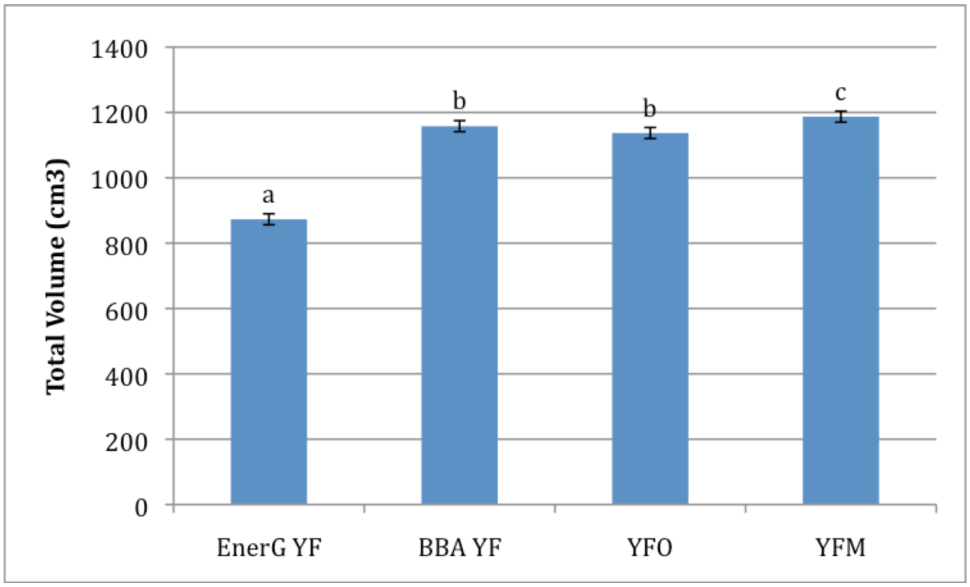

Fig. 9. Yeast-Free Final Volume. Final yeast-free total volume after 6 hours of cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

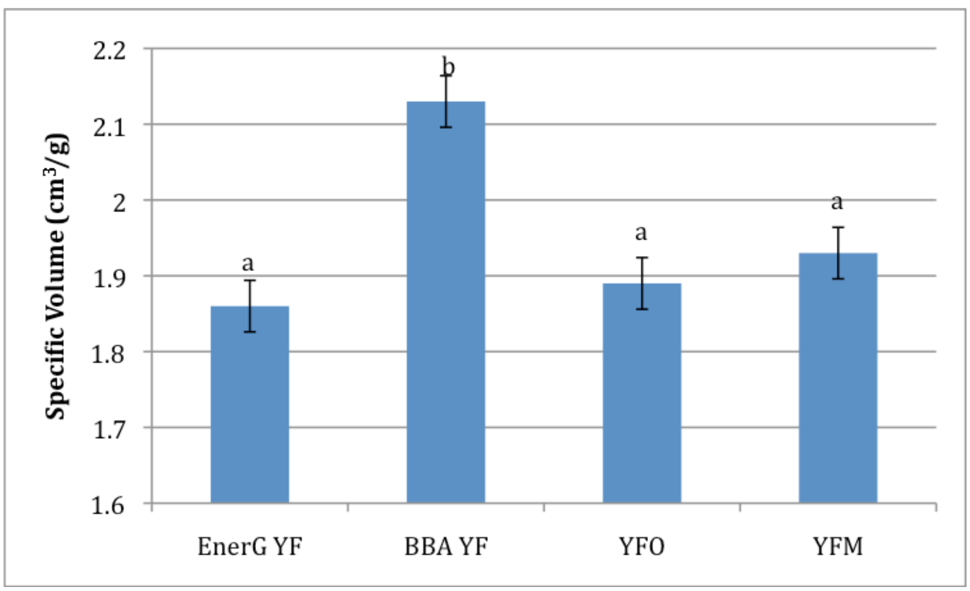

Fig. 10. Yeast-Free Final Specific Volume. Average specific volume of yeast-free breads after 6 hours of cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05)

Color

Average L* values did not statistically differ between YFO (65.6) and YFM (64.8, Fig. 11, P<0.05) and were lower than L* values reported in purely starch based gluten-free breads (Pagliarini et al. 2010). EnerG Yeast-free Bread had the highest average L* value of 86.4 and Breads by Ana yeast-free pre-mix had the lowest average value of 52.7 indicating that it was the darkest in color. Average a* values for Breads By Ana Yeast-free pre-mixed bread (5.98), YFO (4.30), and YFM (4.06) did not statistically differ (Fig. 12 P<0.05). EnerG Yeast-free Bread had the lowest a* value (0.17). Average b* values statistically differed in all samples (P<0.05) with YFO having the highest (25.9) and Energy Yeast-free Bread having the lowest (12.2, Fig. 13). Breads by Ana Yeast-free pre-mix bread and YFM had average values of 15.1 and 25.4 respectively. The color results indicate that the EnerG Yeast-free bread has light, white color tendencies. YFO and YFM have light yellow color tendencies and the Breads by Ana Yeast-free pre-mix bread is much darker than the other samples. The differences in color characteristics of commercial control breads indicate that the yeast-free formulations fall within the range of acceptability of color for gluten-free breads when compared to commercial products, however they are darker than pure starch based breads used in gluten-free research.

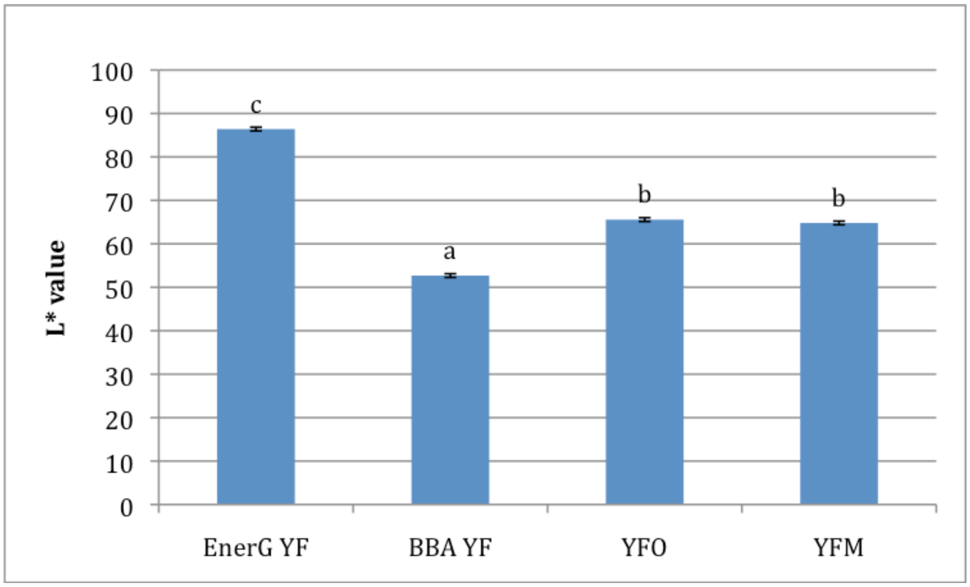

Fig. 11. Yeast-Free L* Values. Average L* values observed for yeast-free breads. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05)

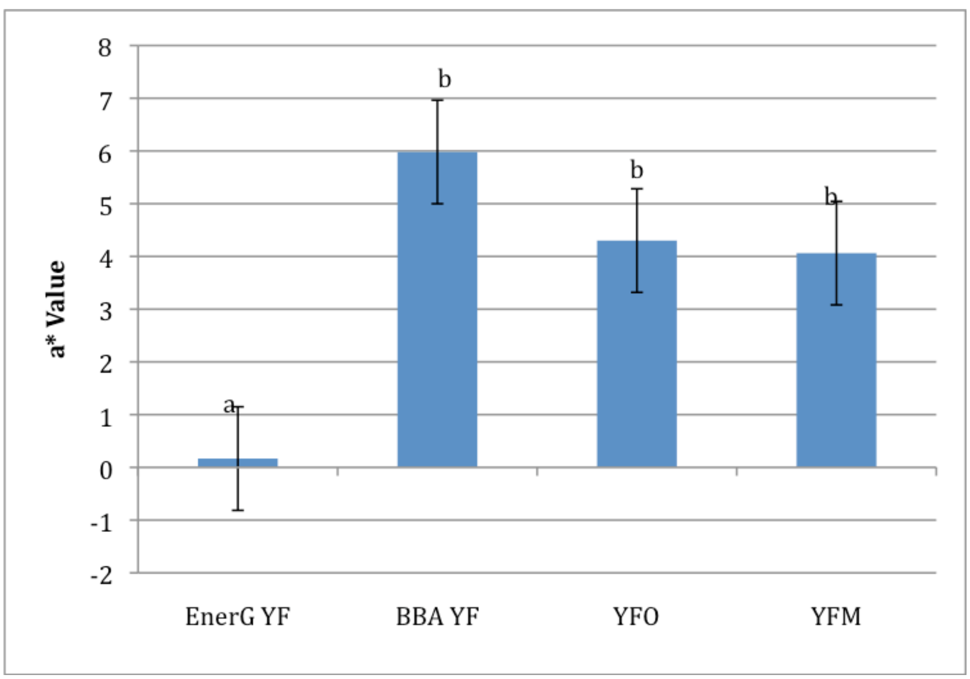

Fig. 12. Yeast-Free a* Values. Average a* values observed for yeast-free breads. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

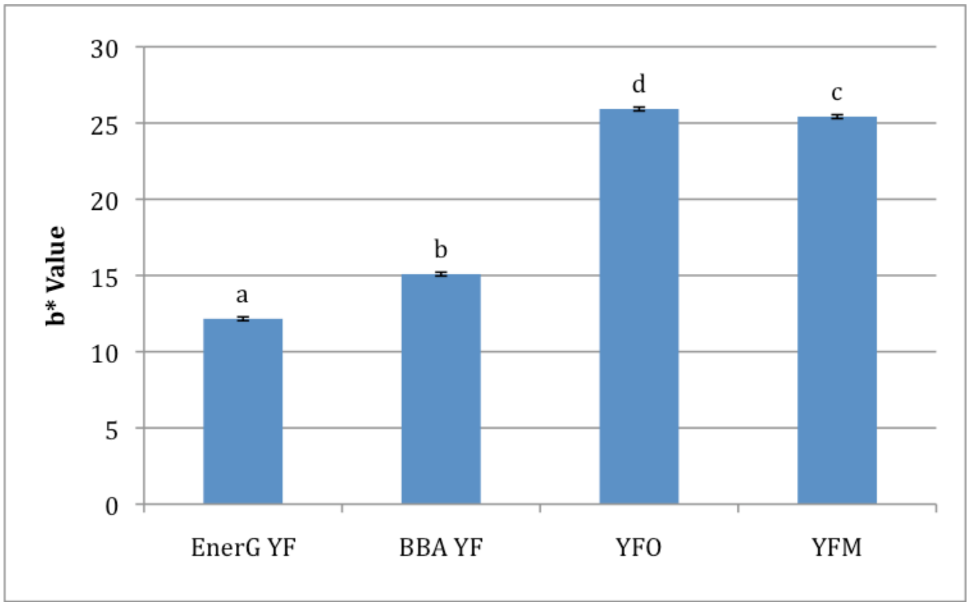

Fig. 13. Yeast-Free b* Values. Average b* values observed for yeast-free breads. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, $P<0.05$).

Microscopy

Microscopy showed that EnerG had large potato starch granules within the matrix that were not gelatinized (Fig. 14a). They appear to serve as structural components of the crumb. The donut shape observed in the microscopy photographs is a characteristic of a gelatinized starch and is labeled accordingly. YFO had thick air cell walls that were thicker than YFM (Fig. 14b,c). The portion in YFO that broke apart in the cell had a thick cell wall that was still airy, thus the structure of the crumb came from thick structural elements (Fig. 14b). Starches were gelatinized to the same levels in YFO and YFM.

YFM had thin air cell walls and gelatinized starch that was thick enough to form strand shaped air cells that mimic the formation of gluten in traditional bread (Fig. 14c).

It had a continuous phase that formed a solid structure that was set, providing an explanation for less volume loss in this formulation. The air cell walls were thinner in YFM, yet still had strength and stability due to setting in place. Stretching and breaking within the cell wall observed in both YFO and YFM can also be seen in traditional wheat bread (Fig. 15). The stretching and breaking of the cell walls was not seen in EnerG's yeast-free bread. The ability to stretch and tear cell walls shows that YFO and YFM's batter was able to partially mimic characteristics of a wheat gluten matrix without gluten. This stretching is indicative of elasticity that is desirable in dough.

The gluten-free breads observed had different cell wall characteristics. YFO and YFM had thicker cell walls with more irregular air cell shape than wheat bread, however air cells were similar in size. EnerG's yeast-free bread had smaller air cells with thinner cell walls. Larger starch granules were intermittently mixed throughout the EnerG yeast-free bread unlike YFO, YFM, and the wheat bread had starch granules spread throughout the entirety of the crumb.

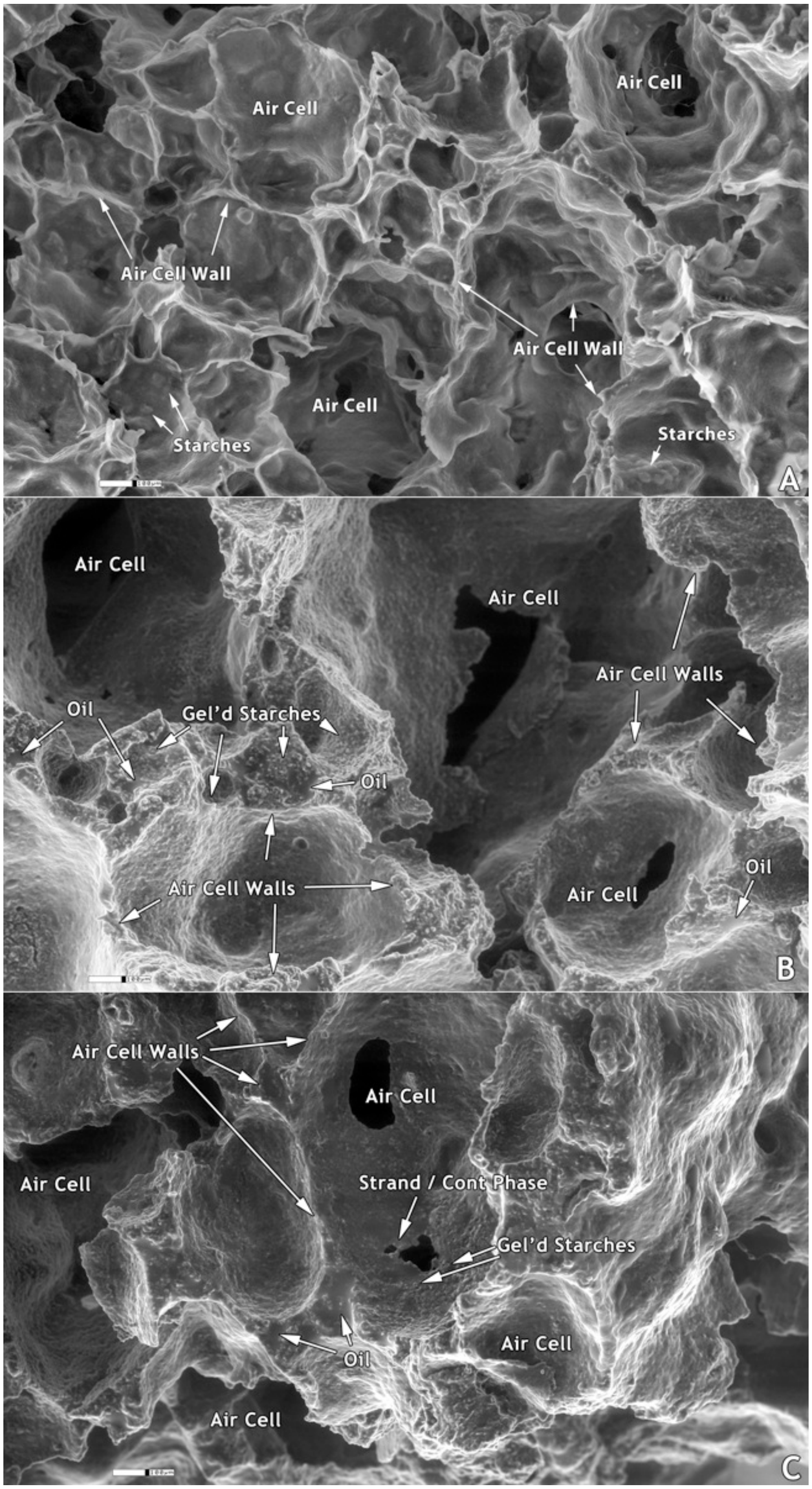

Fig. 14. Yeast-Free Microscopy. Environmental scanning electron microscopy views of yeast-free breads A) EnerG YF; B) YFO; C) YFM.

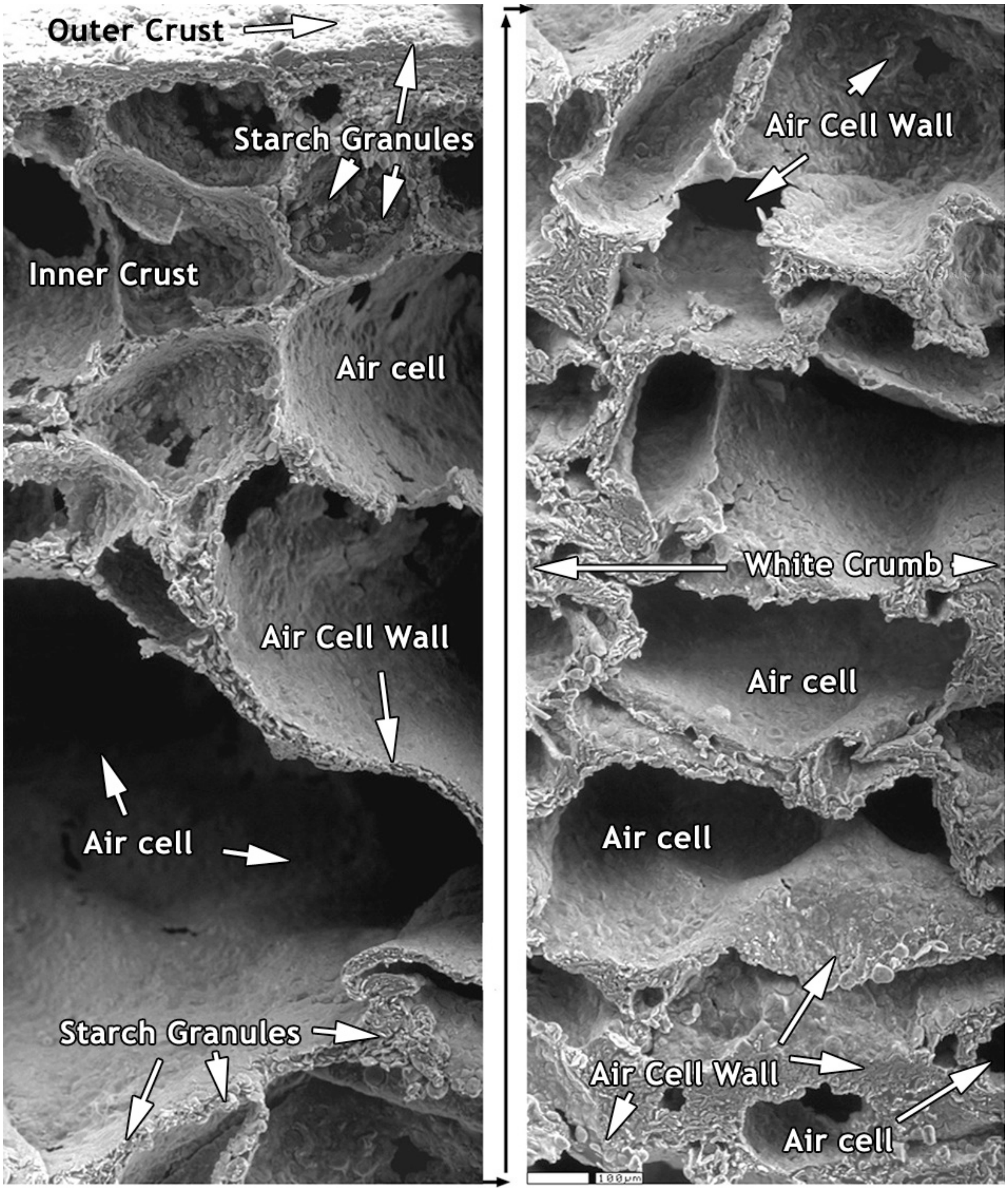

Fig. 15. Wheat Bread Microscopy. Crust and crumb environmental scanning electron microscopy views taken by researchers at Texas A&M University's Cereal Quality Lab.

OPTIMUM MIXING TIME

Subjective Evaluation of Crumb

An overall increase in volume was observed as mixing time increased in the test loaves. Loaf profiles (Fig. 16) show the general physical characteristics. High rounded domes were not observed in any of the test breads (C-E), which is typical for this formulation, and a savory golden color was noted after baking. The slices of bread (Fig. 16) show that as mixing time increased (C-D), air cells within the slices became more evenly dispersed and tunneling decreased.

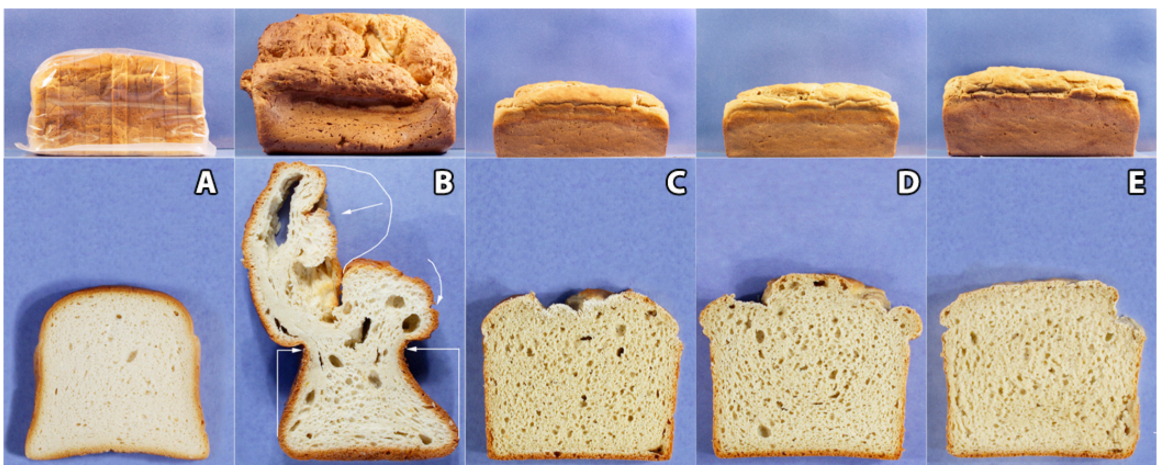

Fig. 16. Yeast Loaf and Slice Profiles. A) EnerG Brown Rice Yeast Bread; B) Bob's Red Mill bread mix; C) 5 Min Mixing; D) 10 Min Mixing; E) 15 Min Mixing. Lines and arrows in B indicate the original contours of the loaf when it came out of the oven and the direction of shrinkage.

The laboratory gluten-free yeast bread formulation at all mixing times was very stable after baking and did not collapse after cooling (Fig. 16 C-E), indicating that the continuous phase network was strong and well developed. EnerG Brown Rice Yeast Bread (store bought prepared loaf) had the appearance of an ideal loaf of bread with a rounded dome and golden color. Bob's Red Mill bread mix, resulted in a deformed loaf of bread that collapsed within 3 min. of removal from the oven, arrows show how the loaf collapsed the moment it was removed from the oven. Large tunnels can be seen throughout the slice. This bread mix was prepared as directed, however there were additional directions for use in a bread machine. In a bread machine the dough would be allowed to expand and then collapse on inward when it reached the outer walls reducing the large air cell formation.

Hardness

Increasing mixing time did not significantly affect hardness in the 5, 10, or 15 min mixing treatments with respective average force for 60% compression of 2,456 g, 2,321 g, and 2,459 g (P value<0.05; Fig. 17). These results show a softer crumb than wheat bread controls used in a previous study using the same hardness protocol (Moore et al. 2004). Hardness for the white wheat pan style bread controls in previous study using the same protocol showed traditional white wheat bread had 5,000 to 6,000 g of force needed to compress to 60% on the initial day of baking (Kiskini et al. 2010). The experimental treatments for this study are much softer than this. The crumb hardness was as soft or softer than gluten-free yeast bread formulas tested in previous research using the same protocol (Kiskini et al. 2010; Moore et al. 2004; Renzetti and Arendt 2009).

Both commercial comparisons were significantly different from experimental treatments. EnerG Brown Rice Yeast Bread was not a good comparison because it was shelf stable bread and the date of baking was not known, it was chosen as a comparison for acceptable commercial volume, rather than hardness. It was extremely hard (Avg Force for 60% compression 11,936 g) and more brittle than other samples prepared, as expected due probably to prolonged storage time. Bob's Red Mill bread mix (Avg force for 60% compression 1,589 g) was the main commercial comparison for hardness measurements because it was fresh baked. It was extremely soft but highly inconsistent in texture. This was most likely due to tunneling and variation of air pockets within the crumb.

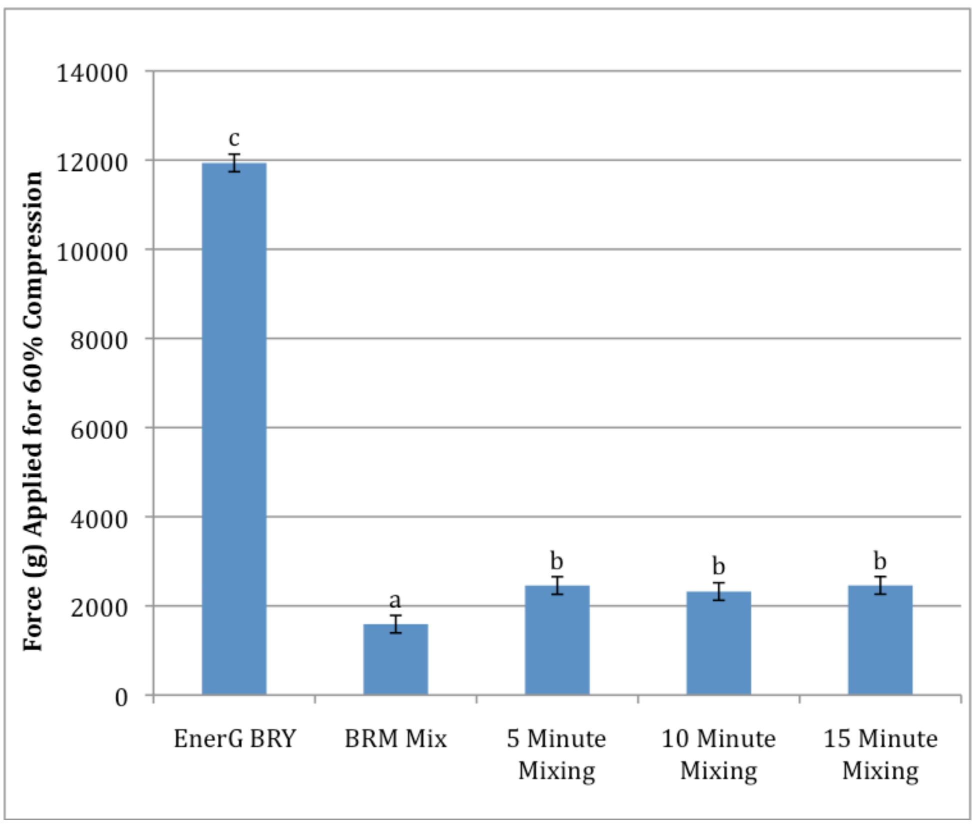

Fig. 17. Average Slice Hardness of Yeast Breads. Measurements taken after cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

Volume Measurements

Volume measurements could not be taken on Bob's Red Mill bread due to its irregular shape. Loaf volume significantly increased ($P<0.05$) as mixing time increased for the three treatments (Fig. 18.) All treatments were significantly larger than EnerG Brown Rice Yeast Bread (923 cm^3). Loaf volume measurement 6 hours after baking showed no volume loss in the mixing time treatments. Specific volume significantly increased ($P<0.05$) in samples mixed for both 10 (2.04 cm^3/g) and 15 min (2.13 cm^3/g). EnerG Brown Rice Yeast Bread (2.00 cm^3/g) did not statistically differ from mixing for 10 min. (Fig. 19.) however it was significantly higher than mixing for 5 min (1.81 cm^3/g). All samples were smaller than reported specific volume for wheat bread that ranged from 4.18 to 3.01 cm^3/g depending on iron fortification treatment (Kiskini et al. 2010).

These data suggest that mixing for 5 min. is not adequate to achieve a similar specific volume to the commercially available comparison even though it has a larger loaf volume and confirms Gambus et al.'s findings that mixing for at least 10 min. is necessary for improved loaf volume (2007). Mixing for 15 min. significantly increased the loaf volume and specific volume relative to the samples mixed for 5 and 10 min. and supports the theory that increased mixing time could improve ingredient incorporation and gas retention. Thus improving specific volume in some formulations of gluten-free breads.

These findings differ from baking characteristics of wheat breads. Mixing time in wheat bread is dependent on the quality and amount of protein within the flour. If the hydrated gluten matrix is mixed for too long the proteins will begin to tear apart and will not have strong viscoelastic properties (Alva et al. 2001; Gras et al. 1990; Mani et al. 1992; Roel et al. 1993). Further investigation is needed to determine if mixing gluten-free breads for longer than 15 minutes will begin to break down the proteins within the egg white that help to emulsify the batter.

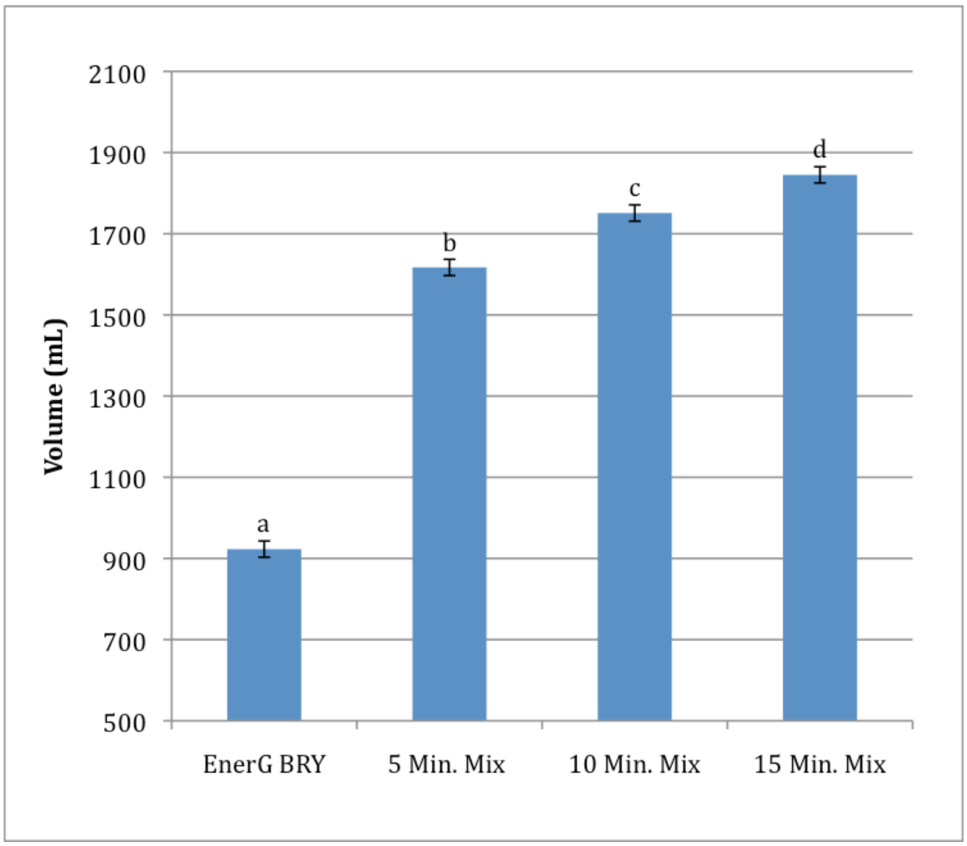

Fig. 18. Yeast Bread Volume. Average total volume for yeast breads after 20 min cooling. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, $P<0.05$).

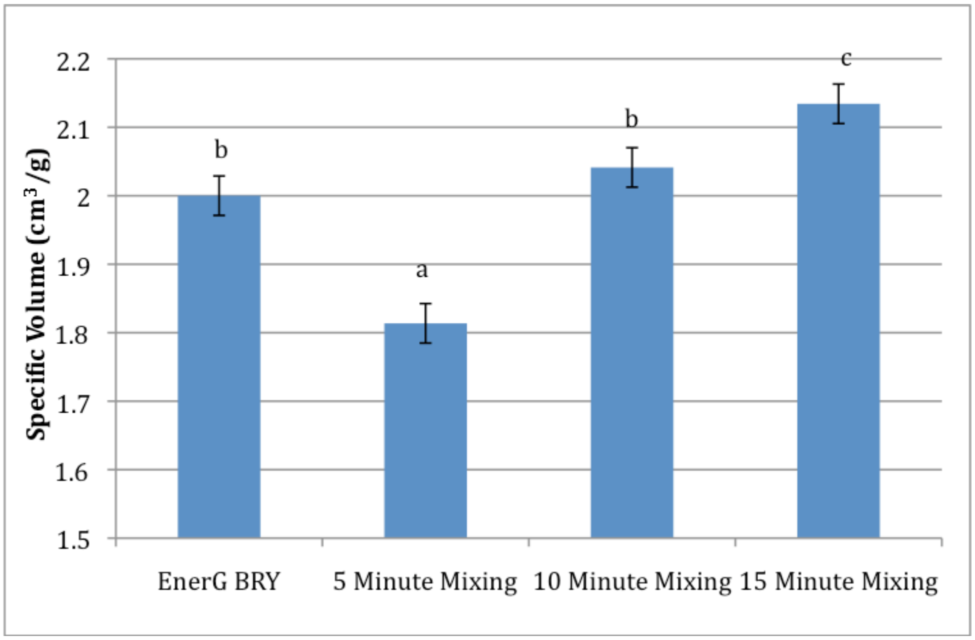

Fig. 19. Yeast Bread Specific Volume. Average specific volume for yeast breads measured 20 min after baking. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, $P<0.05$).

Ingredient Incorporation

The visible appearance of the loaves indicated that there was sufficient distribution of the ingredients during all mixing times, when compared to both controls. Anecdotal evidence of other mixes, an example of which was shown in Fig 2, indicates that the more complicated the recipe, the more important mixing time might be.

Color

Color values indicate that as mixing time increased the L* value associated with lightness of the crumb color also significantly increased. EnerG Brown Rice Bread and Bob's Red Mill pre-mix bread were significantly lighter in color than all other treatments, with a bright white color similar to bleached wheat bread (Fig. 20). Average a* values (Fig. 21) did not follow a consistent trend between treatments with the highest value belonging to the 10 minute mixing treatment. Average b* values (Fig. 22) did not statistically differ among laboratory treatments and were statistically lower than the two commercial controls.

When compared to the L* values of the yeast free breads (Fig. 11), it appears that the breads that contained sorghum have an L* value between 60 and 70. These values are lower than L* values observed on gluten-free breads composed of pure starch (Pagliarini et al. 2010). This could be a characteristic of all breads utilizing sorghum flour, however previous research has not thoroughly discussed color values of sorghum based breads (Schober and Bean 2008; Schober 2009; Taylor et al. 2006).

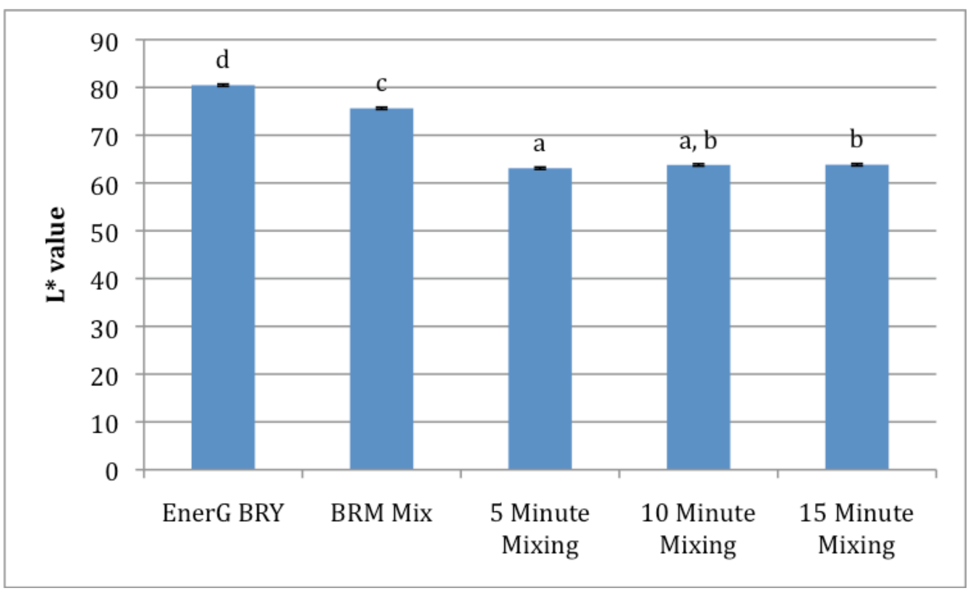

Fig. 20. Yeast Bread L* Values. Average crumb L* values of yeast breads measured. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

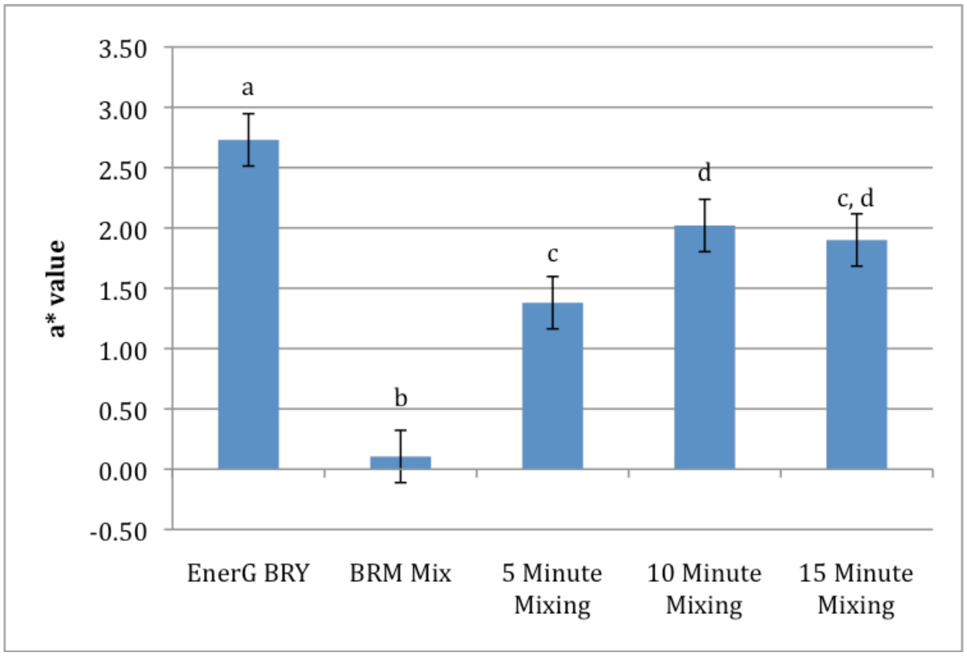

Fig. 21. Yeast Bread a* Values. Average crumb a* values for yeast breads. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, $P<0.05$).

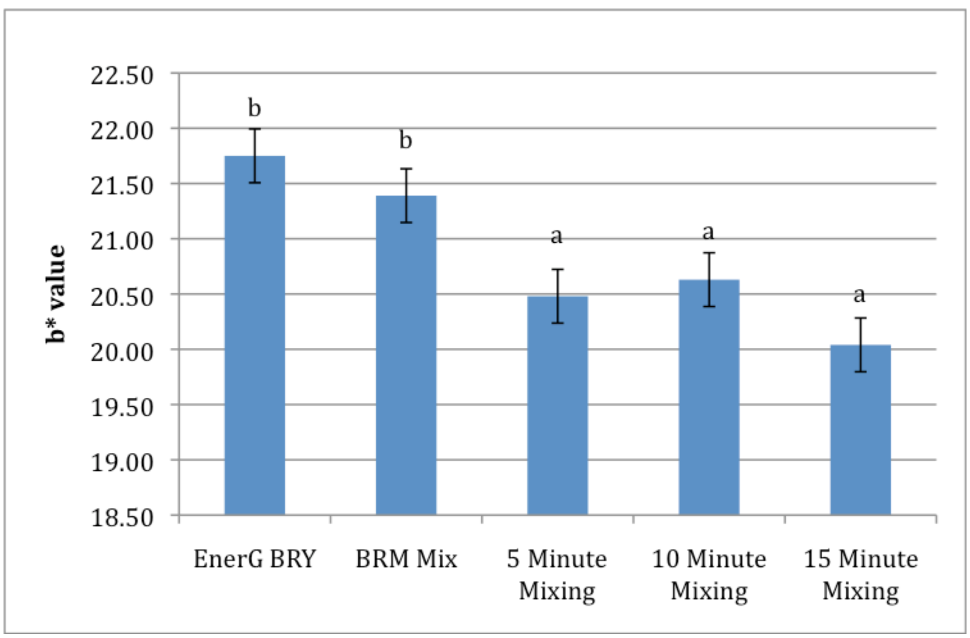

Fig. 22. Yeast Bread b* Values. Average crumb b* values for yeast breads. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, $P<0.05$).

Microscopy

The bread mixed for 5 min had large air cells with thick and thin cell walls that were irregular in shape containing sorghum and rice endosperm particles (Fig. 23a). After mixing for 15 min., air cells were smaller and more regular in size (Fig. 23b). This created more regular air cell distribution throughout the loaf and more evenly dispersed the sorghum and rice endosperm particles present in the crumb. This created a more developed continuous phase, with smaller air cells, which decreased shrinkage, and revealed more evenly dispersed ingredients. Compared to wheat bread (Fig. 15) the 5 and 15 min. mixing breads had thicker cell walls but air cell sizes were similar. The thicker cell walls could account for reduced specific volume when compared to a wheat bread.

EnerG Brown Rice Yeast Yeast Bread (Fig. 23c) exhibited irregular air cell distribution and thin air cell walls with pockets containing rice endosperm particles. Large potato starch granules were identified within the matrix. Potato starch tends to make more of a smooth continuous phase when gelatinized. Bob's Red Mill pre-mixed bread (Fig. 23d) exhibited air cell walls that were irregular in thickness and shape with apparent tunneling. Air cells collapsed under the electron beam, which is a characteristic that is very typical of pure starches. Both EnerG and Bob's Red Mill bread mix had thinner cell walls and smaller air cell sizes than that found in wheat bread (Fig. 15).

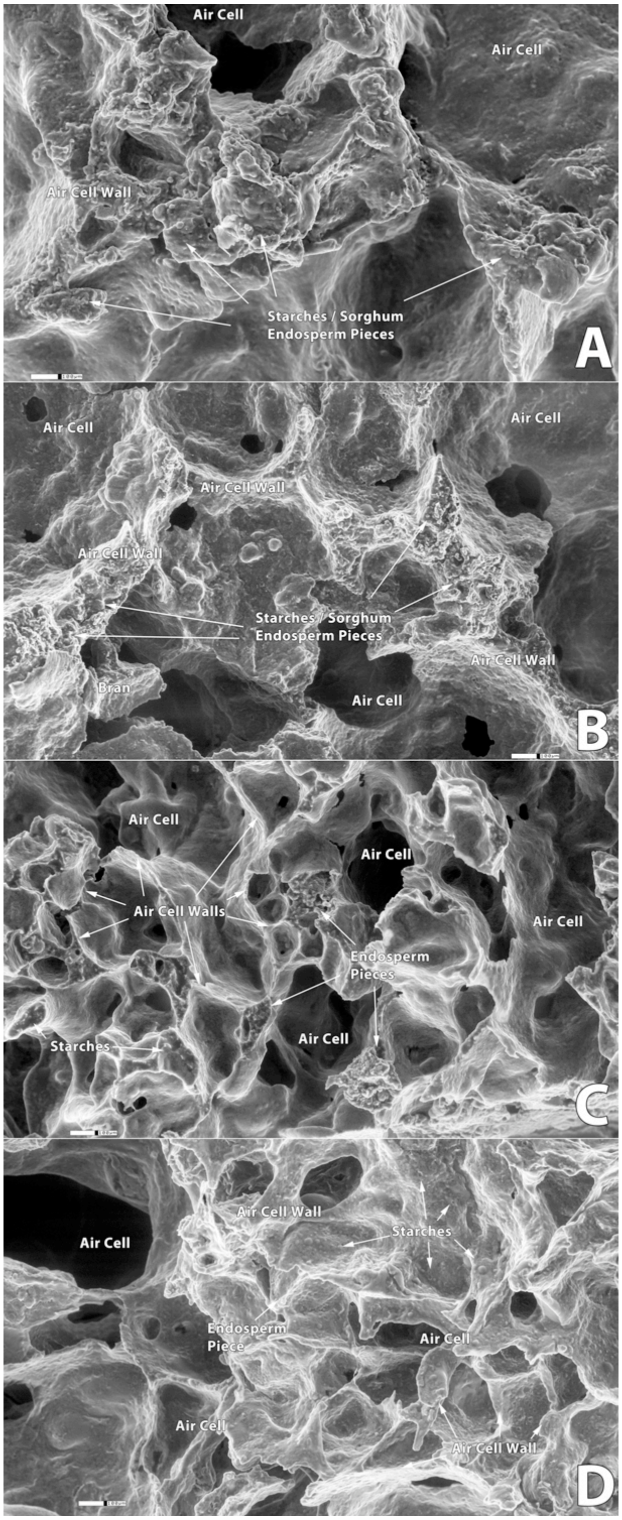

Fig. 23. Yeast Bread Microscopy. Environmental scanning electron microscopy views of bread mixed for A) 5 min. B) 15 min. C) EnerG Brown Rice Yeast Bread; D) Bob's Red Mill Bread Mix

SORGHUM BREAD

Results and Discussion

Slice profiles show that increasing sorghum to 80% of the flour blend increases the cracking on the dome of the loaf and creates a more compact crumb than 40% or 60% substitutions (Fig. 24). This could be due to lack of water addition with the increasing percentage of sorghum flour. Taylor et al. (2006) noted that sorghum flour has a high water absorption, thus when increasing sorghum flour concentration a more brittle loaf structure will occur without water addition. Increasing to 60% does not appear to negatively affect the shape of the loaf. Volume was not significantly affected ($P<0.05$) by increasing the percentage of sorghum to 60% of the flour blend, however it was significantly reduced ($P<0.05$) to an average of 1475 cm^3 when increased to 80% of the baker's flour blend (Fig. 25).

Increasing to 80% substitution significnatly ($P<0.05$) increased hardness while increasing to 60% substitution did not have a significant affect (Fig. 26.) Color data shows that there were significant differences between all three treatments for L* and a* values. Sixty percent substitution and 80% substitution did not have significantly different b* values (Fig. 27).

When compared to the breads measured in Objective 2 that used Bob's Red Mill sorghum flour, the breads using the ADM decoriticated soghum flour were lighter in color (Fig. 20). This could be due to milling practices or different varieties of sorghum being used. Bob's Red Mill does not claim to sell a specific color of sorghum flour so there is potential that other colors of sorghum grain were mixed in decreasing the L* value obtained in Objective 2 results.

Fig. 24. Sorghum Bread Slice Profiles.

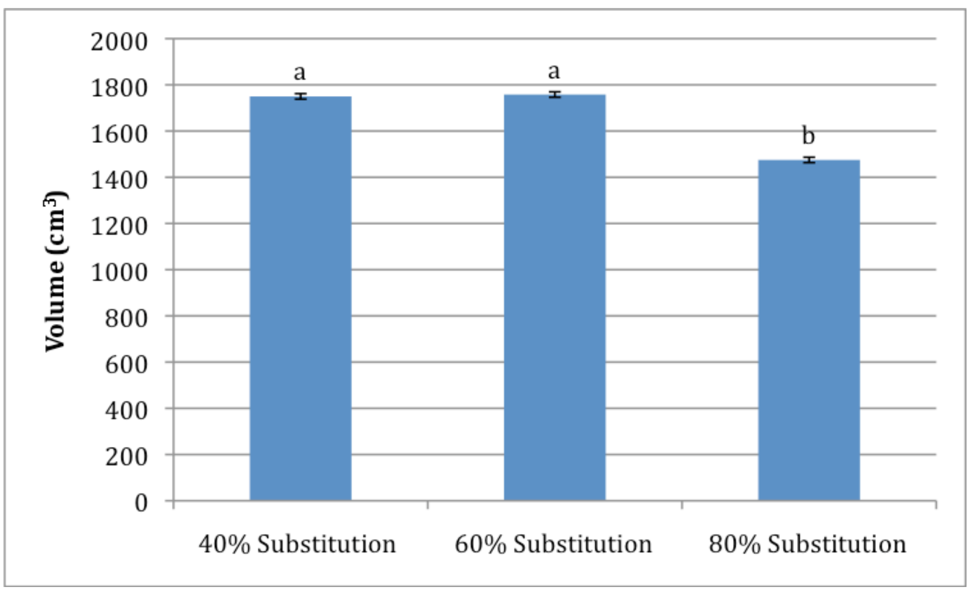

Fig. 25. Sorghum Bread Volume. Total Volume for sorghum flour optimiztion breads. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

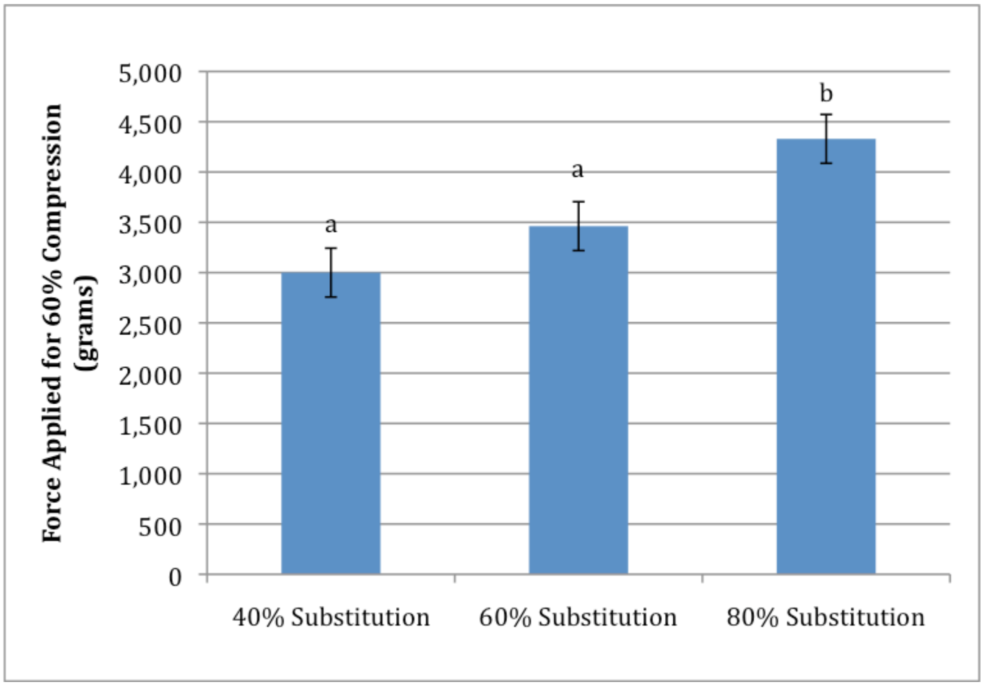

Fig. 26. Sorghum Bread Hardness. Average hardness values for breads with varrying levels or sorghum flour. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

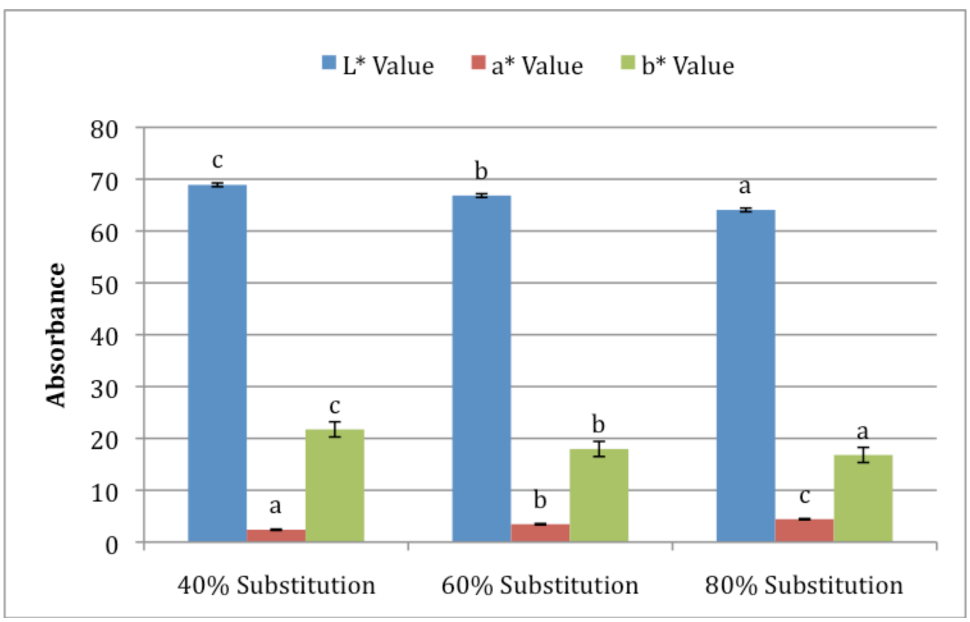

Fig. 27. Sorghum Bread Color. Average color values for sorghum optimization breads. Letters are representative of significant differences within each treatment. Samples with same letter do not statistically differ (ANOVA, Tukey's HSD, P<0.05).

CHAPTER IV

CONCLUSIONS

QUALITY TESTING

Sensory testing was not performed in this study for a variety of reasons. A major problem with designing this type of study is determining the target market and finding a large enough number of subjects to complete the study. The general population could be used for a gluten-free consumer study because there is potential for any typical consumer to be diagnosed with celiac disease and gluten intolerance, however bias related to the color and appearance of the samples could heavily skew results.

Utilizing a large group of individuals living on a gluten-free diet would be ideal for a consumer sensory study, but it would require a large local population that is not affiliated with those conducting the research. Offsite location of gluten-free consumers at multiple locations throughout the country would allow for the most repeatable and valuable consumer sensory results, accommodating for regional preferences for specific sensory attributes.

Shelf stability was not measured for either portion of this project due to lack of appropriate controls to compare with the experimental treatments. After opening the package, EnerG breads molded within 3 days and Breads By Ana yeast-free bread mix molded within 2-3 days of baking. The commercial bread mix bread was deformed after 6 hours due to the rate of retrogradation in the crumb and also molded within 3-4 days, so it is not an appropriate control for shelf stability. Experimental formulations for yeast-free and yeast breads developed a penicillin type mold around 5 days but this was not consistently tested and no anti-staling compounds were added to the formulations.

YEAST-FREE BREAD

Results indicate that the utilization of egg white foam can create acceptable gluten-free, yeast-free bread. Roben Ryberg's formulation (2008) gave loaves that had acceptable loaf volume, specific volume, hardness, and color when compared to the commercial breads and had similar hardness to wheat bread (Moore et al. 2004) and could be more acceptable to newly diagnosed gluten-free consumers. The modified version of this formulation (YFM) utilized commercial ingredients like egg white foam and a modified tapioca starch that could be scaled up to produce multiple loaves at a time while improving total volume and improving the crumb structure observed microscopically. Color and texture were not significantly altered by changes to the formulation and continued to produce lighter colored bread with yellow undertones and a soft crumb.

Commercial comparisons for this experiment exhibited two separate extremes observed in gluten-free bread. EnerG Yeast-free Bread had a bright white, brittle crumb while Breads By Ana had a soft airy crumb with a dark brown coloring. YFO and YFM had properties in between both of these breads with a soft crumb that was harder than Breads By Ana but softer than EnerG, so this formulation had the best of both controls. It looked light and savory, yet retained its volume over several days, and was easily sliced for sandwich style bread, unlike either control.

Utilizing egg white foam instead of traditional proofing methods has potential for the small bakery market. Producing bread with egg white foam drastically cuts down the time of production while requiring less equipment to produce the product. The YFM formulation can be scaled up allowing for a large number of loaves to be produced at once. This allows for efficient production of market acceptable gluten-free bread.

Egg white foam should be investigated further to observe if combining it with yeast and proofing would further improve bread quality. Results have shown that an airy sponge was created before baking, so there is potential for larger air cells to be produced, further improving loaf volume.

OPTIMIZATION OF MIXING TIME AND SORGHUM FLOUR

The results indicate that increased mixing time could improve gas retention, the overall loaf volume, specific volume, and crumb structure of gluten-free breads. These results could be due to improved incorporation of ingredients throughout the mixing process. The particle size of the brown rice and sorghum flour are higher than that typically found in a starch based gluten-free bread. Increasing the mixing time promotes hydration and prevents clumping of ingredients creating a more even dispersion within the matrix as seen in the microscopy results.

Hardness results show that mixing for extended periods did not negatively affect crumb hardness and was softer than traditional wheat breads (Moore et al. 2004). The soft crumb has improved acceptability for newly diagnosed consumers who are looking for a gluten-free bread that mimics wheat bread. Shelf stability of the soft crumb still needs to be tested, but as a fresh baked bread the laboratory control formulation mixed for 15 minutes was softer than breads found in the grocery stores (EnerG bread) and it is not filled with holes and tunneling like the softer commercial bread mix. The commercial bread mix could be more appropriate for use as a comparison in developing bread mixes for bread machines. It expands easily and could potentially have a more stable crumb when prepared using a bread machine.

The significant increase in loaf and specific volume of the 15 min. mixing treatment when compared to commercial EnerG Brown Rice Yeast Bread and 10 min. mixing, indicates that this method and formulation would be ideal for use as a control in future studies investigating the optimum effects of functional ingredients and processing of gluten-free breads. Investigation of optimum mixing times for other formulations is recommended.

Not all gluten-free bread formulations have optimum mixing times of 10 or 15 minutes, however this investigation was based on a formulation predominantly using whole grain flours. Yeast activation time should also be taken into account. Fast rising yeast will not remain active through longer mixing times. Further evaluation on the effect of even longer mixing times using this particular bread formulation is needed to observe if a breakdown of the gluten-free batter will occur. However mixing for longer than 15 min. could be considered an inefficient use of time, and could break down yeast development of air cells. Rheological characteristics of the batter during the mixing process should also be investigated to understand the trends seen and to determine when the ingredients in the batter break down and begin to cause negative effects in the final product.

Increasing the percentage of sorghum in the flour blend of the control gluten-free bread formulation does not appear to negatively affect the overall characteristics of the bread and will decrease costs of replication in future studies. ADM decorticated white sorghum flour is a commodity flour produced at high quantities for a lower price unlike the specialty Brown rice flour used. Color was slightly darkened in the 60% sorghum substitution bread, but it was still lighter than the original formulation (40% sorghum substitution) that used Bob's Red Mill sorghum flour tested in Objective 2. Hardness and volume are not significantly affected by increasing sorghum percentage to 60% of the bakers flour blend indicating that increasing the percentage of sorghum in the bakers flour blend to 60% would be acceptable.

This study provides evidence that extending mixing times in certain gluten-free bread formulations could improve bread quality. Unlike wheat based dough, over mixing gluten-free batters is not necessarily critical. Due to differences in moisture content and ingredient levels in other gluten-free bread formulations it is possible that optimum mixing times would vary among formulations. The results indicate that a longer mixing time would provide improved incorporation of ingredients and air that improves crumb formation in formulations using whole grain flour. In future studies involving gluten-free bread research, the laboratory control formulation using 60% sorghum flour in the bakers flour blend, mixed for 15 min. should be used as the research control. This formulation and method provided consistent optimum results when compared to other treatments and results from other research studies. It would serve as an excellent research control for testing new ingredients and bread baking methods.

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