

GLUTEN-FREE BREAD: CHARACTERIZATION AND DEVELOPMENT OF PRE- AND POST- BAKED GLUTEN FREE BREAD

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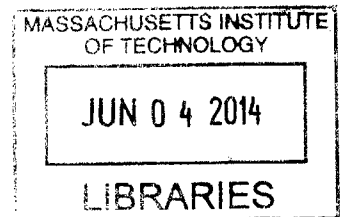
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SUBMITTED TO THE DEPARTMENT OF MATERIAL SCIENCE AND ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELORS OF SCIENCE IN MATERIAL SCIENCE AND ENGINEERING

AT THE

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

ARCHIVES



MAY 2014

[JUNE 2014]

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GLUTEN-FREE BREAD: CHARACTERIZATION AND DEVELOPMENT OF PRE- AND POST- BAKED GLUTEN FREE BREAD

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Submitted to the Department of Material Science and Engineering on May 2, 2014 in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in Material Science and Engineering

Abstract

The study was conducted to characterize the effects of xanthan gum on gluten-free bread formulations. An improved gluten-free flour blend consisting of brown rice flour, quinoa flour, and sorghum flour was used with the aim of developing a gluten-free bread formulation comparable to traditional gluten-based bread and commercial gluten-free bread mix. Rheological measurements were taken to analyze the effects of xanthan gum on pre-baked dough formulations. Higher concentrations of xanthan gum were found to decrease the loss factor thus strengthening the elastic properties of the dough, elongating the linear viscoelastic region and increasing the viscosity of the dough. Furthermore, the xanthan gum samples were not independent of frequency and the loss factor decreased as frequency increased. Porosity of samples was also analyzed using imaging technology to determine the average pore size. Pore size increased as xanthan gum concentration increased indicating the ability for xanthan gum to retain gas during the proofing stage before baking. It was concluded that xanthan gum was necessary for a loaf with nice crumb texture, loaf color, and moisture content though different than gluten-based and commercial brand gluten-free bread mix. 0.3% xanthan gum concentration provided the most desirable post-baked crumb texture, loaf volume, and moisture content

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Objectives

The objective of this study was to understand the effect of xanthum gum on a new gluten-free bread formulation. A special gluten-free flour blend and formulation was developed and compared to both gluten-based bread and a standard commercial gluten-free bread mix. In order to do so, bread was characterized for its moisture content, average pore size, and rheological behavior of dough. In addition, a secondary objective emphasized the use of creating a formulation with less than 15 common ingredients found at local grocery stores due to the high cost of a gluten-free diet. A previous formulation (Boswell 2010) was used as a baseline and was adapted and modified to according to the objectives and other research formulations. Preliminary baking trials were conducted using potato starch, sorghum flour, and brown rice flour, and were later expanded to include quinoa flour and xanthan gum.

Introduction

Bread, a staple food product, is a popular item made and eaten all over the world. Various types of bread-like substances exist and bread has evolved rapidly over the years to achieve creative products for the hungry consumer. The unique chemistry of bread is derived from the properties of the gluten network that is formed during the dough making process. Gluten's ability to form, deform stretch, and trap gas gives bread its unique porous structure, texture, and dough properties (Cauvain 2007).

In the past decade, gluten-free products have rapidly gained popularity in the United States. Celiac disease, a genetic autoimmune disorder, affects 1 in 100 people rendering them unable to properly digest the gluten protein. In those with gluten sensitivities, ingestion of gluten will damage the small-intestinal mucous lining caused by the gliadin, a bread protein used to form gluten, and other similar alcohol-soluble proteins.

Gluten-free bread has been criticized for some of its undesirable characteristics. Recent studies have shown that gluten-free bread has reduced loaf volume, grainy texture, low moisture content, and lighter loaf color (Gallagher et. al. 2004). However over the past five years, many new developments for gluten-free bread have risen in the community. Brands such as Udi's, Kinnikinnik, Ener-G have released whole-sale versions of gluten-free sandwich breads, but further research efforts for an affordable home recipe is still under development. Furthermore, many gluten-free recipes call for complex ingredients such as malodextrin (Witczak et.al. 2010), hydroxypropyl methylcellulose (HPMC) (Crockett 2011), and transglutaminase (Moore et. al 2006) that are known to improve bread quality but are not easily accessible to the home baker. Enzymes such as alpha-amylase and xylanases have also been reported to create improved gluten-free bread recipes (Guarda et. Al 2003) but cannot be found in local grocery stores. Thus, a need for an affordable gluten-free bread recipe has been identified.

Gluten-based Bread vs. Gluten-free Bread

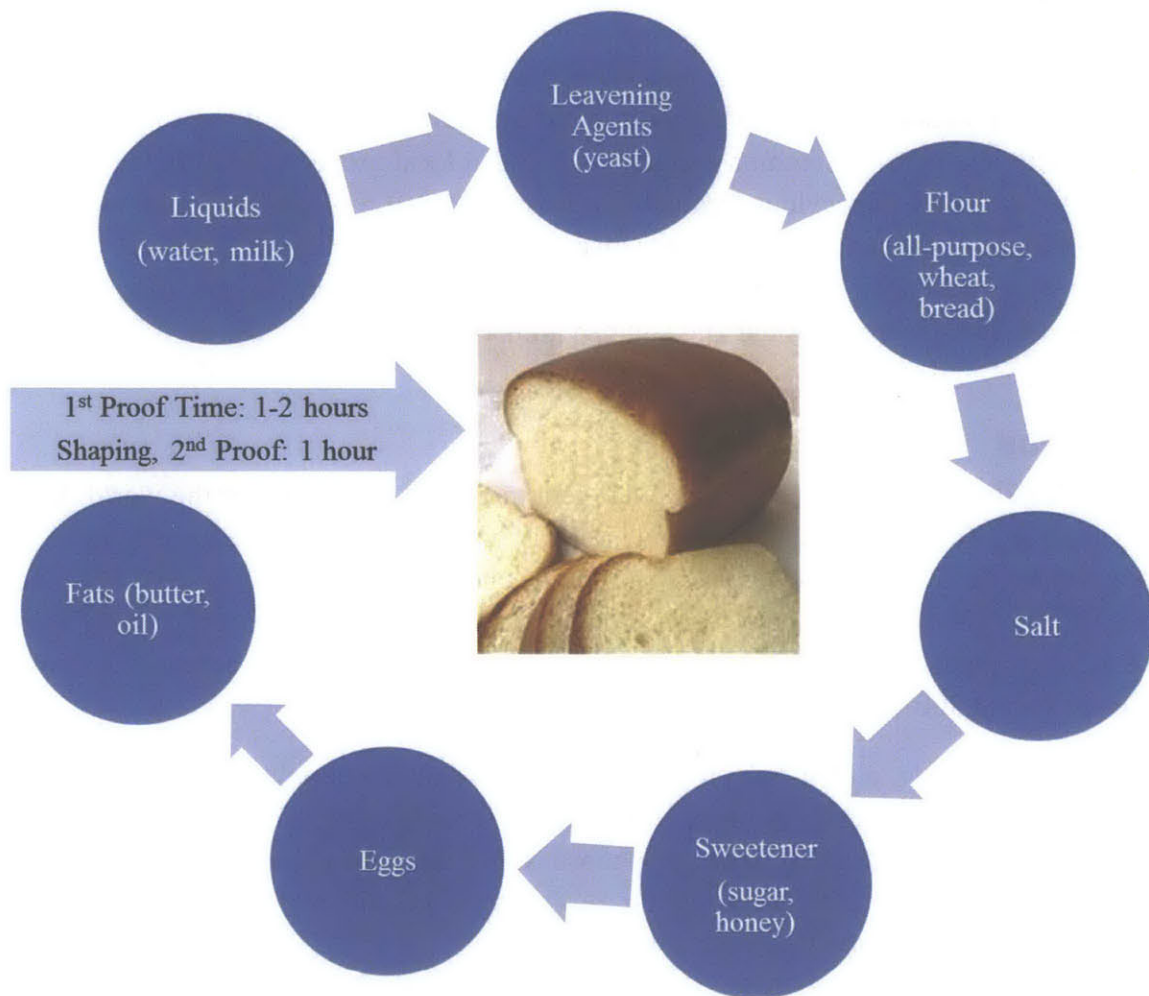


Figure 1: Traditional gluten-based bread ingredients and proof time descriptions

Bread is characterized by its formation of gluten and typically consists of a couple of major ingredients outlined in Figure 1. The liquids, water and milk, are combined with yeast to activate the yeast and begin fermentation. Flour is then added to the yeast mixture along with sweeteners and salt and allowed to combine. Finally, the eggs and fats such as butter, oil, or shortening are introduced into the dough and it is kneaded until the dough comes together and the gluten structure begins to form. Once combined, the dough is kneaded for 5 minutes with a dough hook, and set to proof for 1-2 hours until doubled in size. For a sandwich loaf, the bread is then shaped into a long roll, placed in a bread pan, and set to rise for another hour until doubled in size again (Classic Sandwich Bread). The bread is baked at 350°F until golden brown.

On the other hand, gluten-free bread consists roughly the same major ingredients of gluten-based bread, but uses gluten-free flours (amaranth, quinoa, brown rice, etc.) and introduces the use of starches and gums to compensate for the lack of the gluten network. Differences in the traditional bread and gluten free bread process are highlighted in purple in Figure 2. Full details on gluten-free bread making process can be seen in Figure 3, but it can be noted that gluten-free bread requires only one proofing time that is significantly shorter than proofing for gluten-based bread.

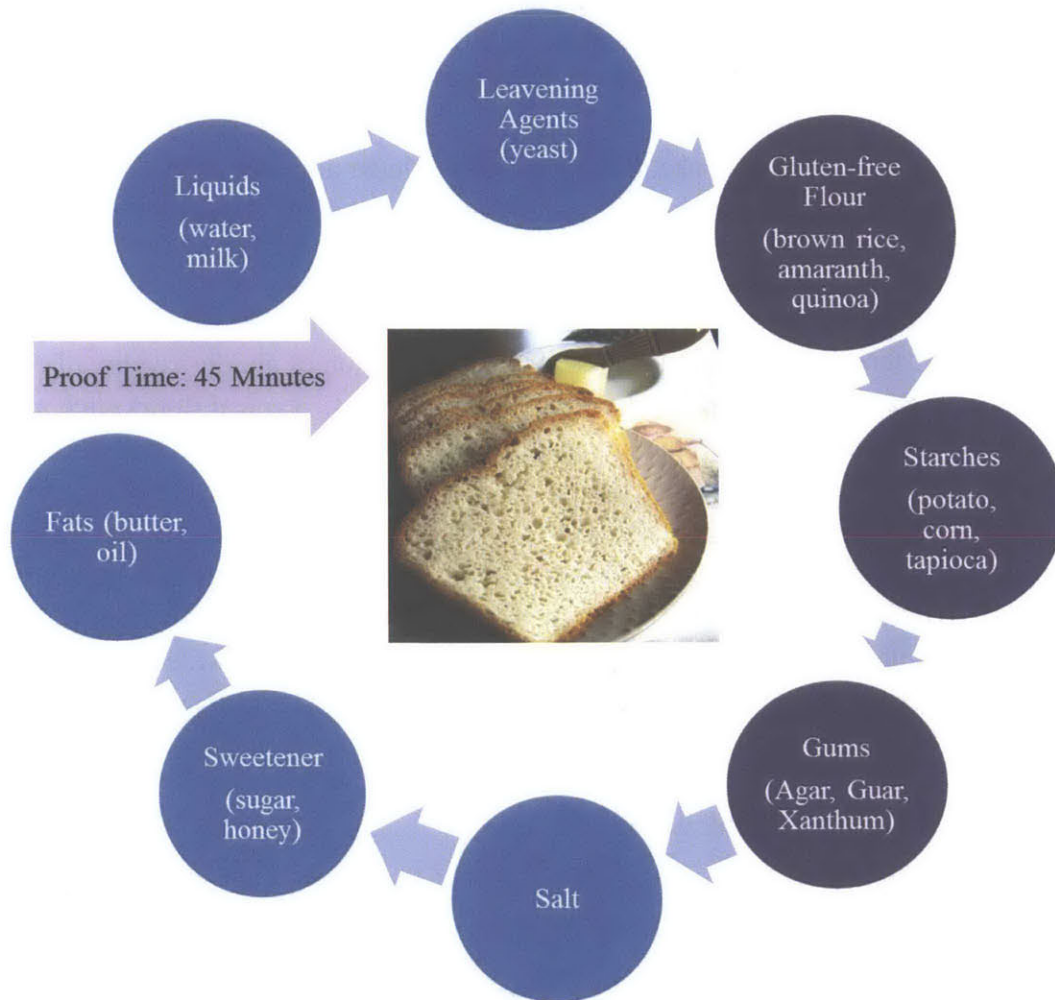


Figure 2: Gluten-free bread ingredients and proof time. Purple color indicates differences from traditional gluten-based bread formulations

Bread Proteins

A unique property of wheat proteins is the ability to, once hydrated, form a sticky and rubbery mass known as gluten. Gluten is derived from 4 major wheat proteins: albumins, globulins, gliadins, and glutenins each of which contribute uniquely to the protein network in the dough. Gliadin provides the dough with its adhesive properties and can be very sticky when

hydrated. On the other hand, glutenin provides elasticity and strength due to its large complex protein structure (Cauvain 2012).

During the kneading and mixing process of the dough, gliadins and glutenins absorb water in the dough and are capable of combining with lipids to form lipoproteins. The gluten proteins together form a strong network through disulfide and hydrogen bonding along with hydrophobic interactions which provides the backbone for dough structure (Cauvain 2007). Gluten’s ability to trap gas, deform, stretch, and recover gives dough its unique properties and structure. The gluten network is responsible for the dough’s ability to absorb double its weight in water ensuring the final product is moist and tender rather than dry and flaky (Gluten). Furthermore, gluten is responsible for the dough’s elastic characteristics and also shapes the color and crumb texture of the baked bread. Because gluten does not exist in gluten-free bread, the protein structure usually provided by gluten must be substituted by other ingredients such as other grain flows, starches, and gums.

Gluten-free Flours

Many different flours have been used in the formation of gluten-free bread each consisting of their own unique properties and protein content. Typical flours such as all-purpose or white flour and wheat flour contain approximately 10-14% protein content whereas rice-flour only contains 6-8% protein content. Thus, even though white rice flour is popular for gluten-free baking due its white color, neutral taste and easy digestibility, many studies have begun to employ the use of more unique flours to increase the protein content in the dough. Sorghum flour (Schober et. Al 2005), quinoa flour (Gallagher and Arendt 2004), soybean flour (Ribotta 2004), etc. have been added to gluten-free bread formulations in order to provide structure and elasticity to the batter. The percentage of protein content based on mass percentage for different flours is displayed in Table 1.

Flour Type	Protein Content
Gluten Flour	
All Purpose Flour	11-13%
Wheat Flour	12-14%
Barley Flour	10%
Gluten-Free Flours	
White Rice Flour	6%
Brown Rice Flour	8%
Quinoa Flour	14%
Sorghum Flour	12%

Table 1: Protein content of various gluten-based and gluten-free flours based on mass percentages from Bob’s Red Mills

Quinoa Flour

Given the higher protein content and recent popularity in food trends, there was a strong interest in incorporating quinoa in its flour form into a gluten-free bread formulation. In addition, quinoa is allergy sensitive and can be easily used in vegan and paleo diets. However, quinoa flour also has a very distinct, grainy taste and because of its high protein content has a tendency to make products crumbly and dense. In addition, quinoa flour produces loaves with significantly higher volume in comparison to gluten-free rice bread (Alvarez-jubete et. Al 2010). Furthermore, quinoa flour is mostly composed of globulins and albumins rather than gliadins, glutenins, or prolamin. Globulins and albumins contain less glutamic acid, and also have a more balanced blend of essential amino acids.

Sorghum Flour

Sorghum flour has become a favorite amongst the gluten-free bread community and is a staple ingredient all around the world in other countries such as Africa, India, and China (Rooney and Waniska 2000). Sweet sorghum flour purchased in this experiment is a newer hybrid of the original white plant that has a sweeter, yet bland flavor. Furthermore, different sorghum hybrids contain various levels of antioxidants, and can all lend to unique tastes in and quality of the final bread product. Research combining sorghum flour with various starches to make gluten-free dough has been examined (Schober, et al 2005), but not many have been conducted in combination with other types of gluten-free flour. In all gluten-free bread formulations, especially those containing sorghum, higher water content is needed to create a batter-like system rather than wheat-based elastic dough flour (Taylor et al 2006).

Brown Rice Flour

Rice flour has been the most popular gluten-free flour substitute due to its hypoallergenic properties, neutral taste, and abundance (Kadan et. Al 2001). However, rice flour is typically blended with other flours because it has low amounts of protein and therefore does not contribute to the elastic-plastic properties found in gluten-based dough (Toufeili et. Al 1994). Many commercial all-purpose gluten-free flours like King Arthur Flour (Gluten-free Multi-Purpose Flour), Cup 4 Cup (Product Info), use white rice and brown rice flour as their baseline ingredients for all-purpose flour substitutions displaying its wide diversity of usage.

Starches and Gums

Starches

Aside from the functional flours, starches and gums also play a very important role in gluten-free bread formulation and are the main difference between gluten-based bread and gluten-free bread (Figure 1). Starches can be used to lighten the flour blend, achieve higher volume, and provide a more airy texture to dense breads. However, too much starch may also lead to faster staling (Moore 2004). Cassava starch (Crockett 2011), corn starch (Sanchez et al 2002.), tapioca starch (Ahlborn et al. 2005), and potato starch (Gallagher et al. 2004) are among the most popular starches used in gluten-free baking. As part of the objective was to limit

ingredients to those that were accessible in most local grocery stores, potato and corn starch identified as most accessible and thus were focused upon in this experiment.

Gums

Without a gluten-free network, gluten-free dough formulations need additional ingredients to improve the batter's viscoelastic. Gums (hydrocolloids) have been found to increase viscoelastic properties of dough and are generally used as thickeners and emulsifying agents. Xanthan, guar, and agar gum are among the most popular hydrocolloids that have been tested in gluten-free batters to improve the viscoelastic properties, increase gas retention, and develop post-baked texture. Furthermore, xanthan gum has been found to have a strong effect on dough viscoelasticity yielding stronger dough (Lazaridou 2007).

Other Functional Ingredients

As seen in Figure 1, aside from flour and water, many other ingredients play a key role in the formation of bread. Yeast provides the fermentation process and the proofing effect in dough, where salt (sodium chloride) regulates yeast fermentation and monitors for a steady rate of dough expansion. Liquids hydrate the yeast molecules and are used to blend all of the ingredients together. Sweeteners act as food for the yeast, contribute to the overall flavor, and lead to a golden brown crust color. Fats are also used to retain CO₂ gas in dough, improve taste, and increase volume and texture. Fats give dough its post-baked qualities such as tenderness, richness, and moisture whereas eggs also contribute to dough tenderness and richness, and provide color to the finale bread product (Common Baking Ingredients).

Materials and Methods

Materials

Gluten-free quinoa flour, brown rice flour, and sorghum flour were purchased from Bob's Red Mill (Milwaukee, OR). Organic cane sugar was purchased from Trader Joe's (Morovia, CA), corn starch was obtained from Sigma Aldrich (St. Louis, MO). Active Dry Yeast, potato starch, and xanthan gum were also purchased from Bob's Red Mills (Milwaukee, OR). Wesson's vegetable Oil (ConAgra Foods, Omaha, NE), Morton Salt (Chicago, IL), eggs, and milk were all purchased from a local grocery store.

Bread Preparation

Batter was prepared according to the methodology shown in Figure 2. Dry ingredients (flours, starches, gums, sugar, and salt) were whisked together to incorporate all of the dry ingredients together before added to the yeast mixture. In addition, all ingredients were brought to room temperature before being used to make dough. Ingredients were combined in an Artisan Kitchen Aid Mixer (St. Joseph, Michigan). 650g of batter was poured into a PAM sprayed and parchment paper lined 1 pound Chicago's Metallic's bread pan (Chicago, IL). The remaining approximately 20g of the batter was used in testing on the rheometer, but was left to proof for at

least 30 minutes before doing so. Proofing time varied around 30-45 minutes as proofing was based up on the doubling of batter rather than by time to achieve more accurate representation of the bread, typically up to the top of the baking pan. Baking occurred in a Maytag Performa oven (Benton Harbor, MI) at 350°F. After baking, the bread was allowed to sit for at least 15 minutes before removed and transferred to a cooling rack to cool thoroughly. Breads were later double wrapped tightly in plastic wrap and stored frozen until analysis.

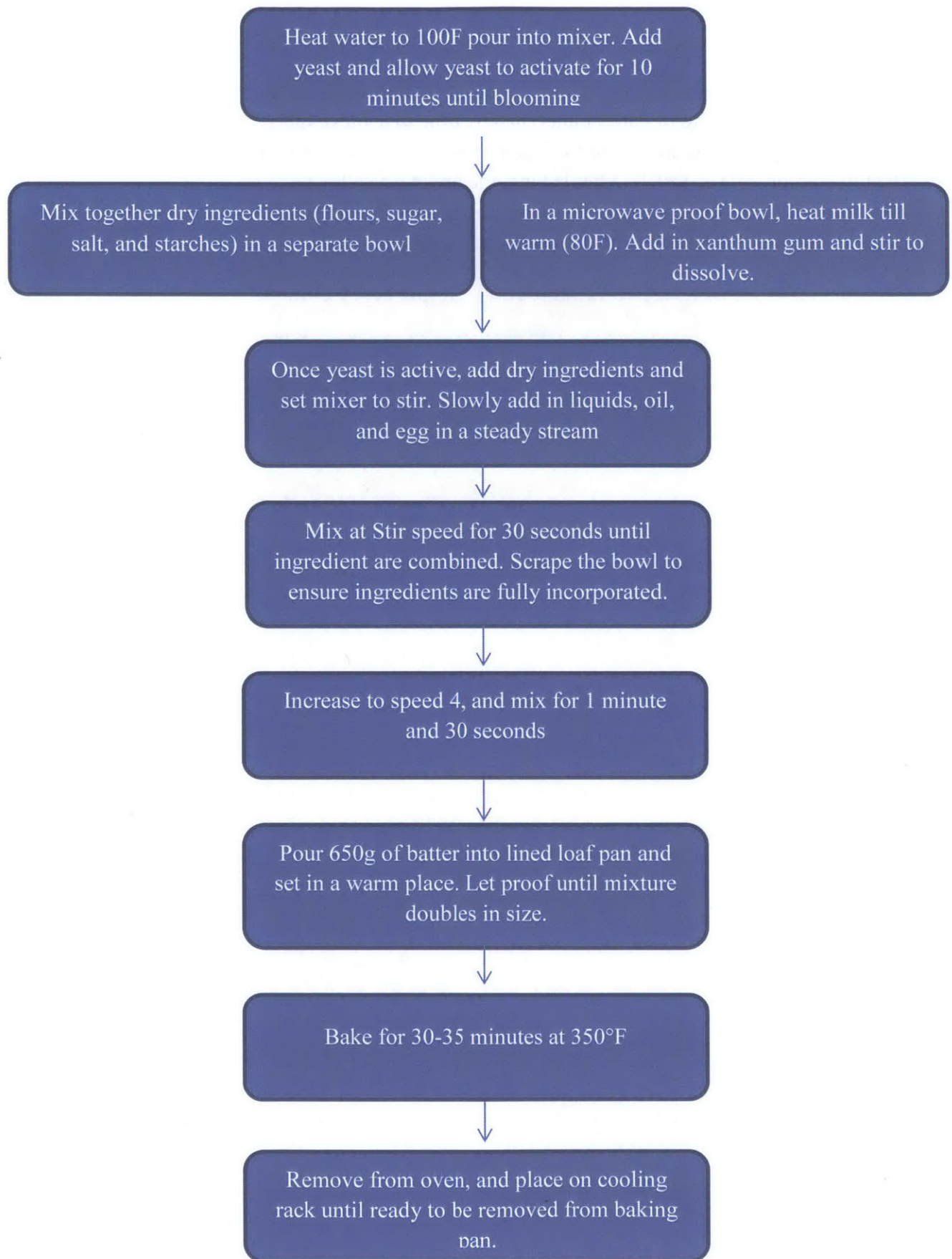


Figure 3: Gluten-free baking methodology

Basic Bread Formulations

Sample 11- Gluten Free Bread Formulation

Ingredient	Total Percentage	Weight as Prepared (g)
Brown Rice Flour	20.42%	136.5
Quinoa Flour	10.05%	67.2
Sorghum Flour	6.61%	44.17
Sugar	2.09%	14
Salt	0.61%	4.06
Potato Starch	6.61%	44.17
Xanthum Gum	0.31%	2.1
Water	27.75%	185.5
Egg	7.85%	52.5
Canola	3.46%	23.1
Yeast	0.63%	4.2
Milk	13.61%	91
Total	100.00%	668.5

Table 2: Gluten-free bread formulation including brown rice, quinoa, and sorghum flour

The commercial based bread mix was purchased from Bob's Red Mill and was prepared according to the directions on the package. The exact percentage of the ingredients was not discovered, but the ingredients of the package in descending proportions is as follows: garbanzo bean flour, potato starch, corn starch, white sorghum flour, cane sugar, fava bean flour, xanthan gum, yeast, potato flour, sea salt, guar gum, soy lecithin, milk, egg, oil, and cider vinegar (GF Homemade Wonderful Bread).

The gluten-based sandwich bread formulation was taken from King Arthur Flour recommendation in the following proportions.

Gluten-based Bread Formulation

Ingredient	Total Percentage	Weight as Prepared (g)
Flour	52.63%	400
Water	18.55%	141
Milk	15.79%	120
Oil	6.32%	48
Sugar	3.29%	25
Yeast	2.76%	21
Salt	0.66%	5
Total	100.00%	760

Table 3: Gluten-based sandwich bread formulation from King Arthur Flour

Bread Evaluation Techniques

Scanning Electron Microscopy (SEM)

The structure of the samples was analyzed using a JEOM JSM 6610LV scanning electron microscope. Baked samples were attached onto an aluminum specimen stub using double-sided carbon tape and examined under high vacuum in a scanning electron microscope. Secondary electron images were acquired at an accelerating voltage of 15kV.

Evaluation of Moisture Content

Once bread was fully cooled, at least two hours after baking, loaves were double wrapped in plastic wrap, labeled, and stored in a freezer until further tests could be conducted. For moisture analysis tests, loaves were removed from freezer and allowed to thaw at room temperature for at least three hours prior to testing.

1 cm thick slices of bread were cut from the middle half of each loaf. Then, each slice was further divided at a height of 3 cm to obtain a 3cm x 9cm x 1cm rectangle of bread. After bread was cut, weight in grams of each rectangle was measured and recorded. Samples were then baked at 350°F for 15 minutes before removing, cooling, and re-measuring. Percent weight loss was calculated and used to analyze moisture content by assuming that mass loss was equal to moisture lost during baking process.

Furthermore, by baking the bread for a second time, not only was the moisture content evaluated, the bread's ability to brown and toast was discovered. As gluten-free bread has been highly criticized for its dense and gummy structure and light color, the ability for the bread to toast was an important parameter that could be used to compare the formulation to its gluten-based counterpart.

Rheological properties of the dough

The dough samples were prepared via method shown in Figure 3, and a small portion was removed after proofing for rheological testing. Rheologica data was collected at a constant temperature of 25°C, using an Anton Paar Modular Compact Rheometer MCR 302. All samples were conducted using measuring plate PP25 which had a diameter of 25mm. The following test protocol was adhered to:

1) Frequency Sweep

The complex viscosity, storage modulus (G') and loss modulus (G'') were measured at a constant strain of 1% with varying angular frequency from 100 to 0.1 rad/s with a ramp logarithmic profile.

2) Flow Curve

Viscosity of the sample was measured when shear rate was varied logarithmically from 0.001 – 100 1/s.

3) Sample was held for 10 minutes at 25°C.

4) Strain Sweep

At a constant angular frequency of 10 rad/s, the range of viscoelasticity was measured based on the storage modulus (G') and the loss modulus (G'') at a varying strain from 0.1 to 100%.

These dynamic mechanical test methods collect a more accurate representation than prior test methods such as a farinograph, extensograph, or Intsrn as these test methods only determine single point measurements and cannot provide detailed visco-elastic properties of the sample (Crockett 2011). Thus, using a frequency sweep and strain sweep test allowed for the many variables such as elastic and loss modulus, loss factor, and viscosity to be detected. Experimental data was described by the following equations.

$$G' = \frac{\sigma}{\epsilon} \cos \delta$$

$$G'' = \frac{\sigma}{\epsilon} \sin \delta$$

$$\tan \delta = \frac{G''}{G'}$$

Where G' – storage modulus [Pa], G'' – loss modulus [Pa], σ – stress, ϵ - strain, and δ - phase shift. G' displays the dough's ability to store energy whereas G'' measures the dough's ability to dissipate energy in the form of heat. In the case of bread, the elastic properties affect crust formation and gas retention while the liquid/viscous properties are largely affected by water and gas retention as well as gas expansion (Crockett 2011).

Porosity

Porosity of samples was determined using image analysis. A quarter way through the loaf, a cross sectional picture was taken. The picture was converted to a binary form, and image analysis was performed using ImageJ to calculate average pore size and percent area of pores. Images were cropped in order to ensure that only bread was analyzed, and no background was taken into account.

Results

Volume percentages for all gluten-free bread formulations are detailed in Table 4 below. Sample 9 is excluded as it represents the commercial gluten-free bread mix.

	Ingredients	Weight Percentage		Ingredients	Weight Percentage
Sample 1	Brown Rice Flour	20.28	Sample 8	Brown Rice Flour	20.42
	Sorghum Flour	20.28		Quinoa Flour	9.95
	Potato Starch	0.62		Sorghum Flour	10.47
Sample 2	Brown Rice Flour	20.75	Potato Starch	0.52	
	Quinoa Flour	20.75	Xanthan Gum	0.21	
	Potato Starch	0.62	Flour	52.63	
Sample 3	Quinoa Flour	20.75	Sample 10 Gluten- Based Bread	Water	18.55
	Sorghum Flour	20.75		Milk	15.79
	Potato Starch	0.62		Oil	6.32
Brown Rice Flour	13.76	Sugar		3.29	
Sample 4	Quinoa Flour	13.76	Sample 11	Brown Rice Flour	20.42
	Sorghum Flour	13.76		Quinoa Flour	10.05
	Potato Starch	0.62		Sorghum Flour	6.61
Sample 5	Brown Rice Flour	20.42	Potato Starch	6.61	
	Quinoa Flour	20.42	Xanthan Gum	0.31	
	Potato Starch	0.77	Brown Rice Flour	20.42	
	Corn Starch	0.77	Quinoa Flour	10.05	
Sample 6	Brown Rice Flour	20.42	Sample 12	Sorghum Flour	6.61
	Quinoa Flour	20.42		Potato Starch	6.61
	Potato Starch	0.52		Xanthan Gum	0.63
	Corn Starch	0.52	Brown Rice Flour	20.42	
Sample 7	Brown Rice Flour	20.42	Sample 13	Quinoa Flour	10.05
	Quinoa Flour	20.42		Sorghum Flour	6.61
	Potato Starch	0.53		Potato Starch	6.61
	Xanthan Gum	0.73		Xanthan Gum	0.00

Table 4: Formulations by volume percentages for flour, starches, and gums

Bread Imaging

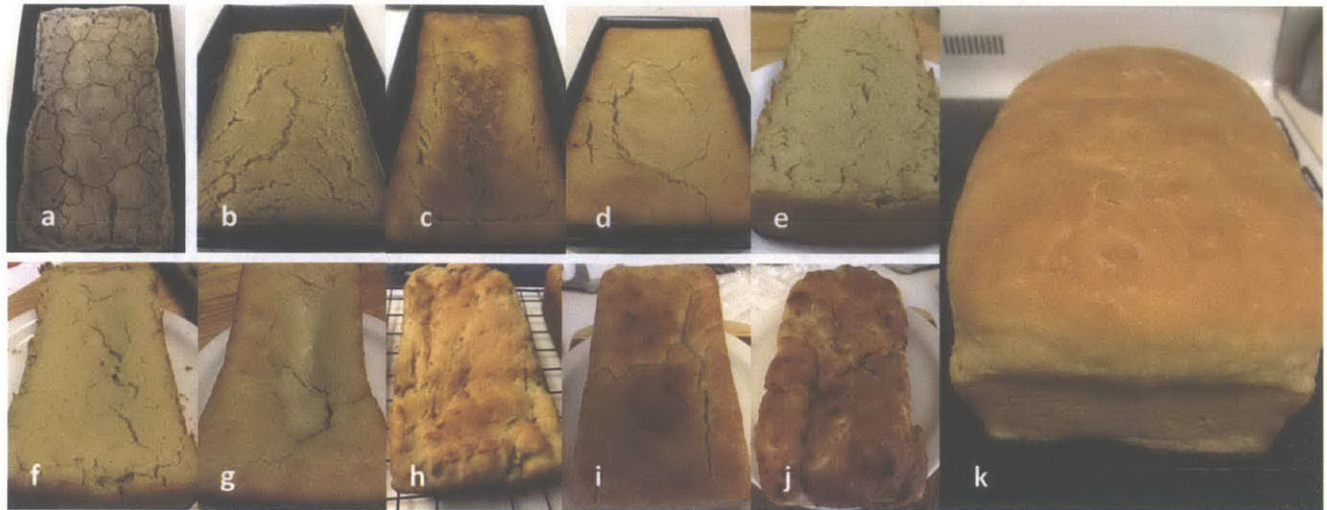


Figure 4: Gluten-free bread formulations (post-baking)

(a) SF only (b) BRF, SF (c) BRF, QF (d) QF, SF (e) BRF, QF, SF (f) BRF, QF, corn starch (g) BRF, QF, corn starch (dec.) (h) BRF, QF, xanthan (i) BRF, QF, SF xanthan (dec.) (j) Commercial GF bread mix (k) Regular Bread

The sorghum only formulation was modified off of Boswell's laboratory gluten-free yeast formulation however was modified to fit the objectives of this paper. Therefore, non-fat dry milk powder was substituted with milk in an appropriate liquid proportions and expandex, a modified tapioca starch, was removed. Next, varying proportions of brown rice flour and quinoa flour were added until a desirable bread quality, loaf volume, and loaf color were achieved (see Table 4 for more details). In Figure 4, as samples progress from a-i, loaf color darkens to a more golden brown and loaves are less susceptible to fallen center seen in sample g.

In Figure 4, the center of some of the breads fell dramatically when exposed to the hot oven temperature or when removed from the hot oven temperature. As this happened in various formulations with all different flours, starches, and gums, the falling center was not due to any specific type of flour. The fallen center was due to a low ratio of solid to liquid in the formulation. Thus, by adding xanthan gum in samples h-j, which increased the solid properties of the dough, the fallen center was not present post-baking.

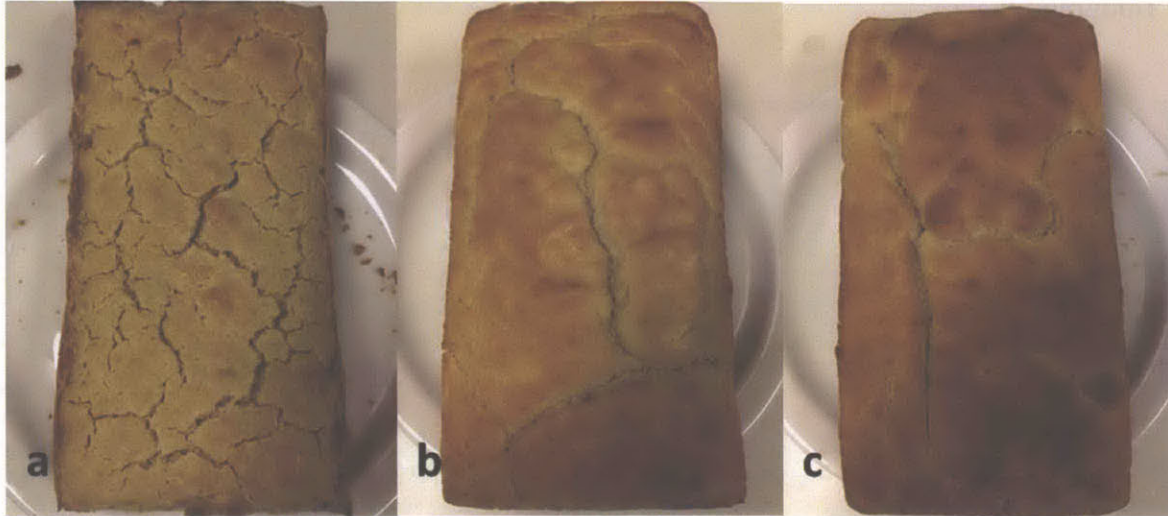


Figure 5: : Xanthan gum samples represented by samples 11-13 in Table 4 (a) 0% xanthan (b) 0.3% xanthan (c) 0.6% xanthan

Figure 5 displays the affects that varying xanthan gum concentration had on the loaves. With no xanthan gum, sample A was not able to properly retain the gas released during proofing and did not rise as much as expected. In addition, the bread cracked which could be due to the dough being insufficiently mixed. As xanthan gum is an emulsifying agent, without xanthan gum, the particles in the batter are more susceptible to separation during the proofing process and may not have been thoroughly distributed.



Figure 6: Cross-sectional view of commercial and gluten-based bread. Gluten-free bread mix made according to Bob's Red Mill (left), Sandwich Bread Recipe according to King Arthur Flour (right)

Figure 6 highlights the two controls used as a baseline for this experiment. The commercial gluten-free bread contained a much higher ratio of eggs than any other formulation

and this resulted in a bread loaf similar to brioche (egg-based gluten bread). The commercial grade bread proofed quickly and had a higher loaf height than experimental formulations. However, post-baking, the bread volume decreased rapidly once out of the oven and a unique mushroom –like structure was made once the bread was removed from the pan (Figure 6-left).

The gluten-based sandwich bread seen on the right hand side of Figure 6 had the highest loaf volume of all of the formulations. The gluten network allowed for rapid gas retention and gas expansion thus resulting in a tall loaf that maintained in shape once removed from the oven. The resulting texture was also more airy and cohesive in comparison to the more dense and egg-like texture of the commercial gluten-free bread mix.

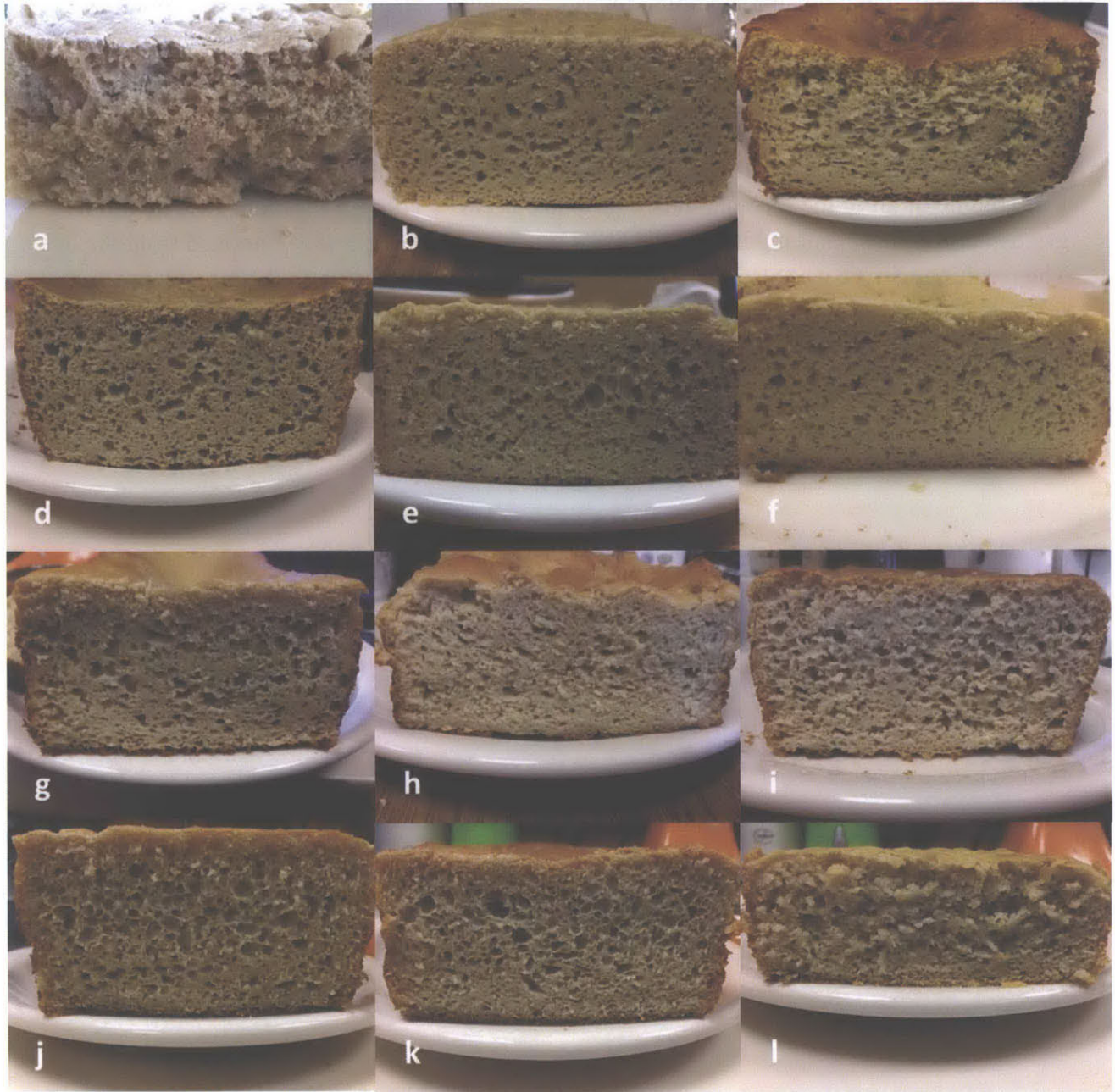


Figure 7: Cross-sectional view of gluten-free bread samples.

(a) SF only (b) BRF, SF (c) BRF, QF (d) QF, SF (e) BRF, QF, SF (f) BRF, QF, corn starch (g) BRF, QF, corn starch (dec.) (h) BRF, QF, xanthan (i) BRF, QF, SF xanthan (dec.) (j) BRF, QF, SF, xanthan 0.3% (k) BRF, QF, SF, xanthan 0.6% (l) BRF, QF, SF, xanthan 0%

The sorghum flour only bread (a) produced very dry and crumbly bread. However, when combined in equal ratio with brown rice flour or quinoa flour, the bread produced a much more desirable texture, crumb quality, and taste. Due to sorghum and quinoa's distinct flavor a higher ratio of brown rice flour was used as a baseline and then blended with equal percent of both

sorghum flour and quinoa flour to even the protein content and density of the bread. This combination of flours produced a much more desirable taste and crumb texture.

Sorghum flour alone also prevented a browning of the bread. In Figure 7, the bread made with sorghum flour (a) as well as formulations using greater than 20% sorghum flour (b and d) tended to be lighter color beige color in comparison to the other breads. However, in formulations where SF was between ~10-15% (samples e and i), the bread exhibited a slight browning effect.

Addition of xanthan gum also played a significant role on springiness and gumminess of bread. When xanthan gum concentrations were greater than 0.6%, the resulting bread had an unnatural springiness and texture was gummy. When the sample was torn, the sample stretched rather than crumbled further perpetuating the gummy property. However, without xanthan gum, the bread did not rise properly resulting in a short, dense loaf shown in Figure 8 below.



Figure 8: Post-baked loaves for loaves at varying xanthan concentrations (Left to right: 0% xanthan (sample 13), 0.3% xanthan (sample 11), 0.6% xanthan (sample 12))

The post baked loaves show significant differences in loaf volume given the same mass of dough in the pan. The 0.3% xanthan sample had the highest loaf volume followed closely by the 0.6% xanthan gum sample. The 0% xanthan gum sample had difficulty in the initial rising and did not rise to the top of the loaf pan even after double the allotted proofing time given

Scanning Electron Microscope Imaging

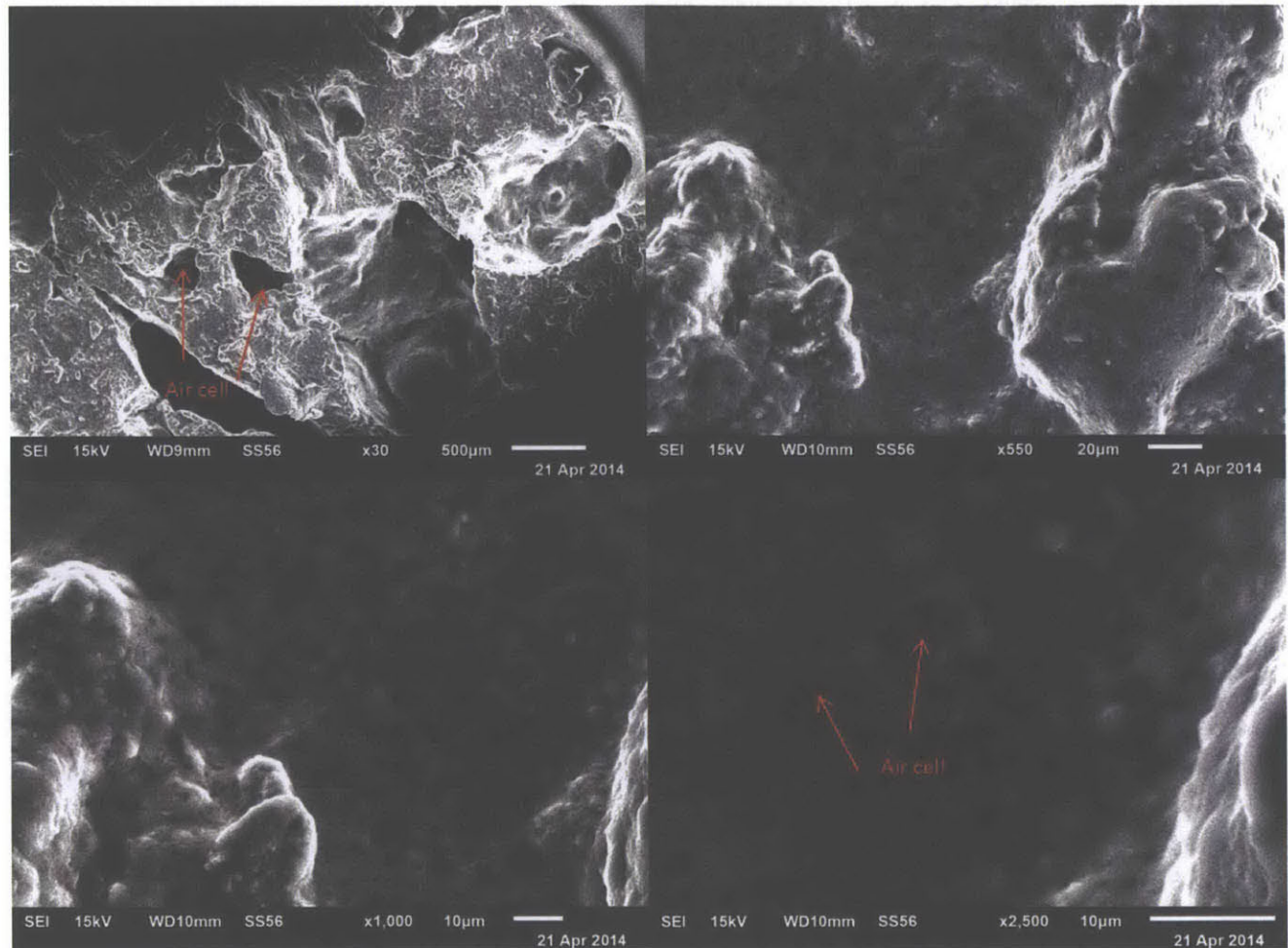


Figure 9: SEM images for Brown Rice Flour and Quinoa Flour (Sample 2) baked bread at varying magnifications (top left X30; top right X550; bottom left X1,000; bottom right X2,500)

The SEM images in Figure 9 depict the air pockets that form within the dough during the baking process. The image shown in the bottom right image at x2500 magnification show pores from 2-8µm in diameter integrated all throughout the bread.

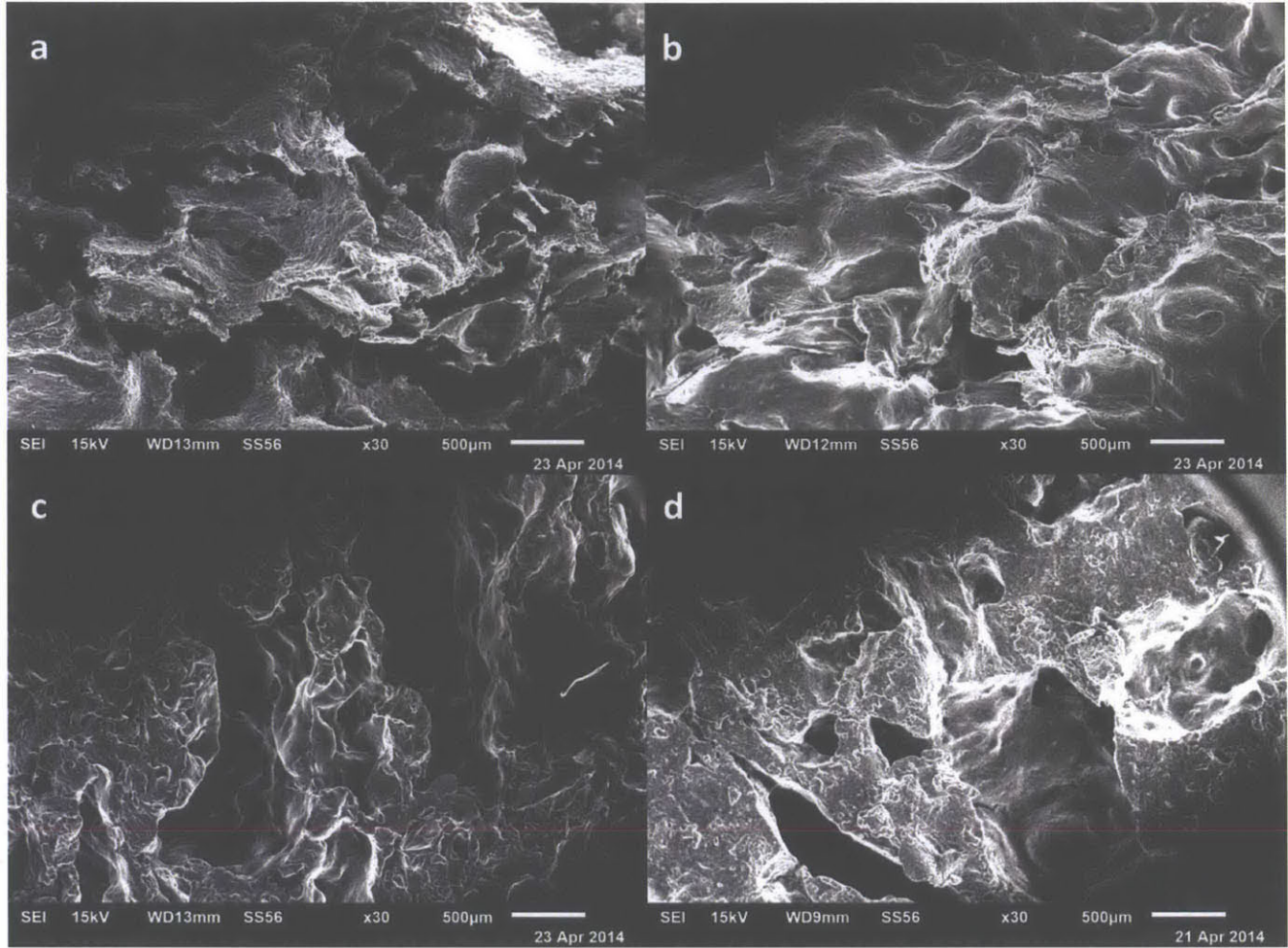


Figure 10: SEM pictures of different bread loaves (a) gluten-based bread (b) commercial gluten-free bread mix (c) BRF, QF, and xanthan gum (d) BRF and QF

From the SEM images shown in Figure 10, the density differences in samples can be identified. Sample A and Sample C contain larger distinct air pockets while Samples B and D are dense and more compact. Furthermore, since Sample B is comprised mostly of garbanzo bean flour, it can be observed that the resulting bread is more fibrous and contains longer strands in comparison to samples C and D in which particles tend to glob together. Comparing the two control breads specimen, Sample A and B, it can be noted that sample A contains more air between the gluten network. This is to be expected as gluten provides the structure for the dough to trap air during expansion and rise to generate light and airy bread. This is also reflected in the Figure 6 cross-section of gluten-based bread where pores are high in density all throughout the top and bottom sections of the loaf.

Rheological Measurements

Figure 11 shows the results of the frequency sweep test on the analyzed dough samples. In all of the graphs, the storage modulus G' was greater than the loss modulus G'' indicating that all dough had more elastic properties than viscous properties. In addition, the storage modulus and the loss modulus of the gluten-free dough samples were smaller than the moduli of the gluten-based dough.

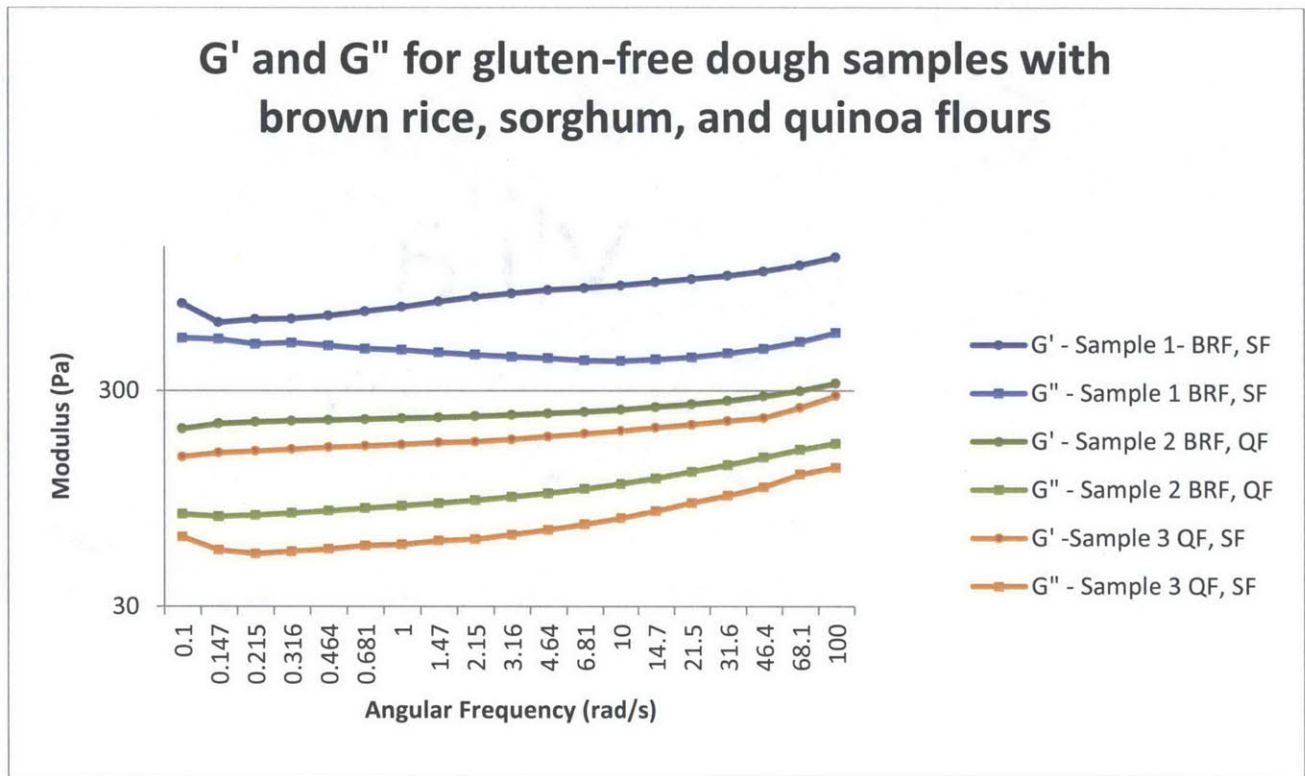


Figure 11: Storage and Loss Modulus graph for Sample 1 (BRF, SF), Sample 2 (BRF, QF), and Sample 3 (QF, SF)

From the data shown in Figure 11, the effect of flour composition on G' and G'' can be observed. Samples with brown rice flour tended to have a higher storage and loss modulus than samples lacking brown rice flour. Conversely, the formulations with quinoa flour have an overall lower G' and G'' behavior.

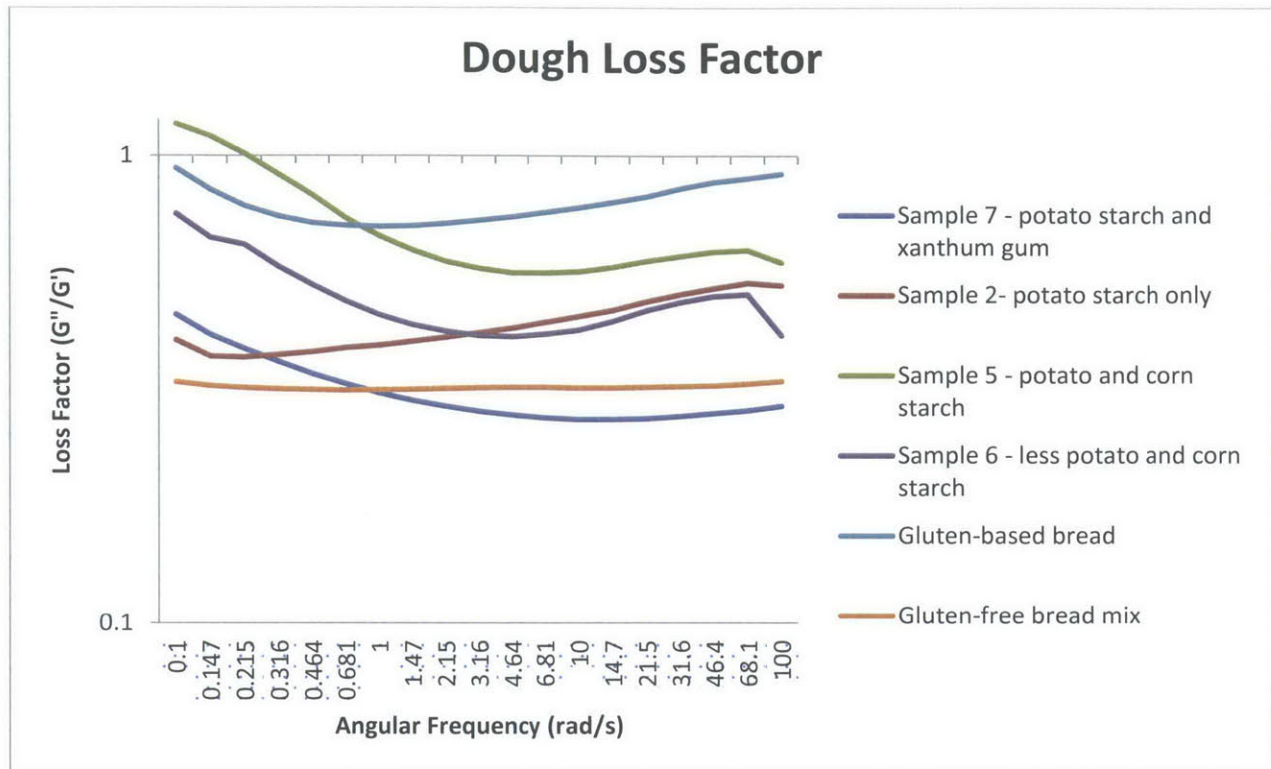


Figure 12: Dough loss factor for all brown rice and quinoa flour samples compared to regular bread and gluten-free bread mix

From Figure 12, it can be observed that for most samples, the dough samples exhibited a solid-like behavior with a loss factor less than 1. The commercially available gluten-free mix had the lowest loss factors across all frequencies. In addition, the gluten-free formulations had a wide range of loss factors ranging from 0.372 to 1.1. From recent research (Korus 2009), gluten-free dough is expected to have a higher ratio of viscous to elastic properties due to the lack of gluten network. These concepts are supported by the results in Figure 12 above as some of the gluten-free formulations are close to 1 and Sample 5 has a relaxation time where the loss factor is greater than 1.

Figure 12 also displays the commercially available gluten-free dough sample and the xanthan gum gluten-free formulation having a flat curve. This indicates that these two dough samples are independent of angular frequency and their storage and loss moduli are parallel at both high and low angular frequencies. On the other hand, the other gluten-free formulations, except for sample 2, behave as expected on a curve basis. At low frequencies, the dough had more time to relax and a higher loss factor was observed at lower frequencies. As the angular frequency increased, the dough had less time to relax and behaved more like a solid and thus had a lower loss factor. An interesting phenomenon in the gluten-based bread was detected as it displayed a loss factor close to 1, but never relaxed fully. The gluten-based bread also had a

greater loss factor than the commercial gluten-free dough displaying that it has more viscous properties than previously expected (loss factor below gluten-free formulations).

Addition of starches to formulations can also be analyzed in the above graph. An increase of corn starch resulted in an increase in the loss factor exhibiting that corn starch increased the viscous properties of the material rather than the elastic properties. Xanthan gum had the opposite effect and the addition of xanthan gum decreased the loss factor and increased the elastic properties of the dough.

In separate formulations in which xanthan gum concentrations were varied, the same trend was confirmed. Below, in Figure 13 as xanthan gum concentration increases, the loss factor decreases. This data confirms the hydrocolloid properties that xanthan gum strengthens the dough's viscoelastic properties and the dough acts more like a solid than a liquid.

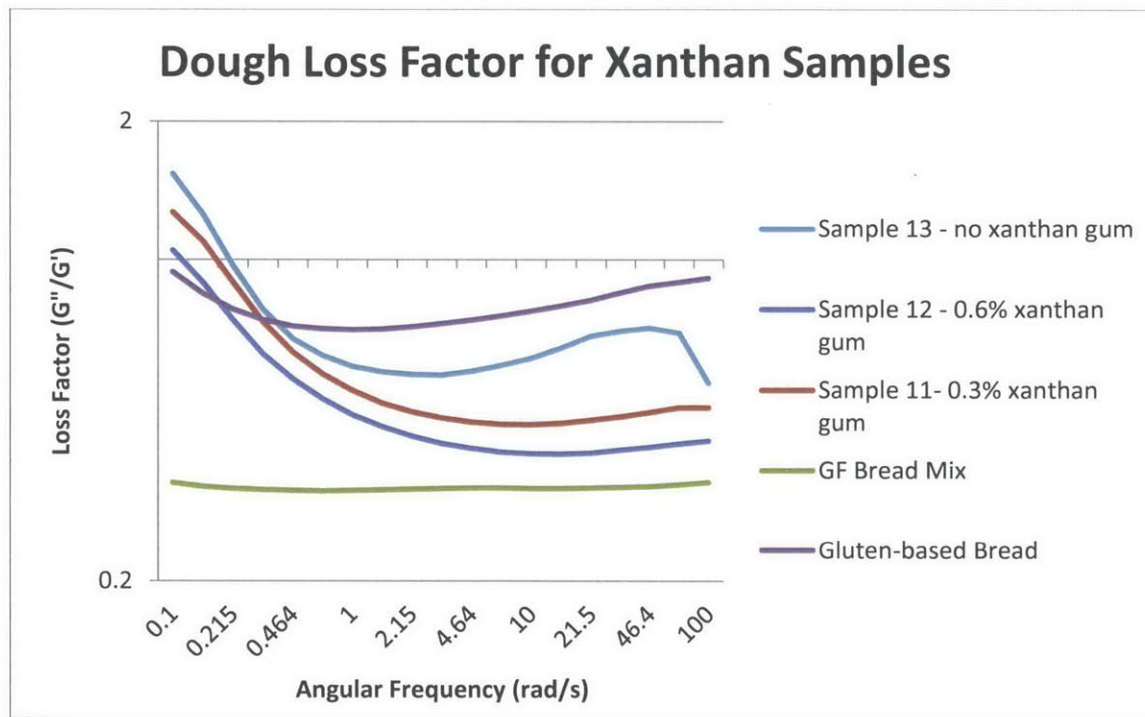


Figure 13: Dough loss factor for gluten-free bread samples with varying xanthan gum concentrations (0%, 0.3%, and 0.6%). Commercial gluten-free bread dough and gluten-based dough graphs are included for reference.

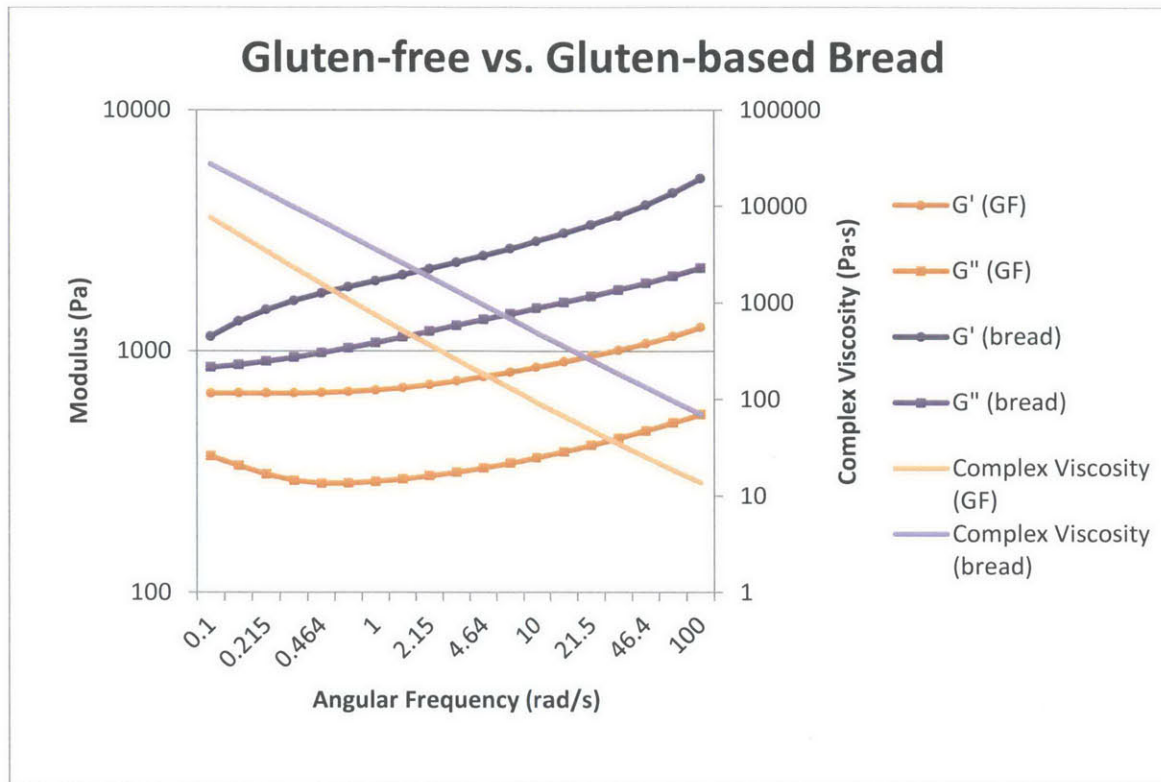


Figure 14: G' , G'' , and complex viscosity graph for gluten-free vs. gluten-based bread. Gluten-free data is based off of Sample 8 (BRF, SF, QF, xanthan gum, and potato starch)

In Figure 14, G' , G'' , and the complex viscosity of bread dough is greater than in gluten-free dough. Due to its batter-like consistency and lack of protein network structure, the gluten-free dough is expected to display more viscous properties which is confirmed by the lower loss modulus in the gluten-free sample. In addition, the loss factor ($\tan\delta$) is less than one for both samples indicating that neither of the dough samples reached their relaxation time even at low angular frequencies.

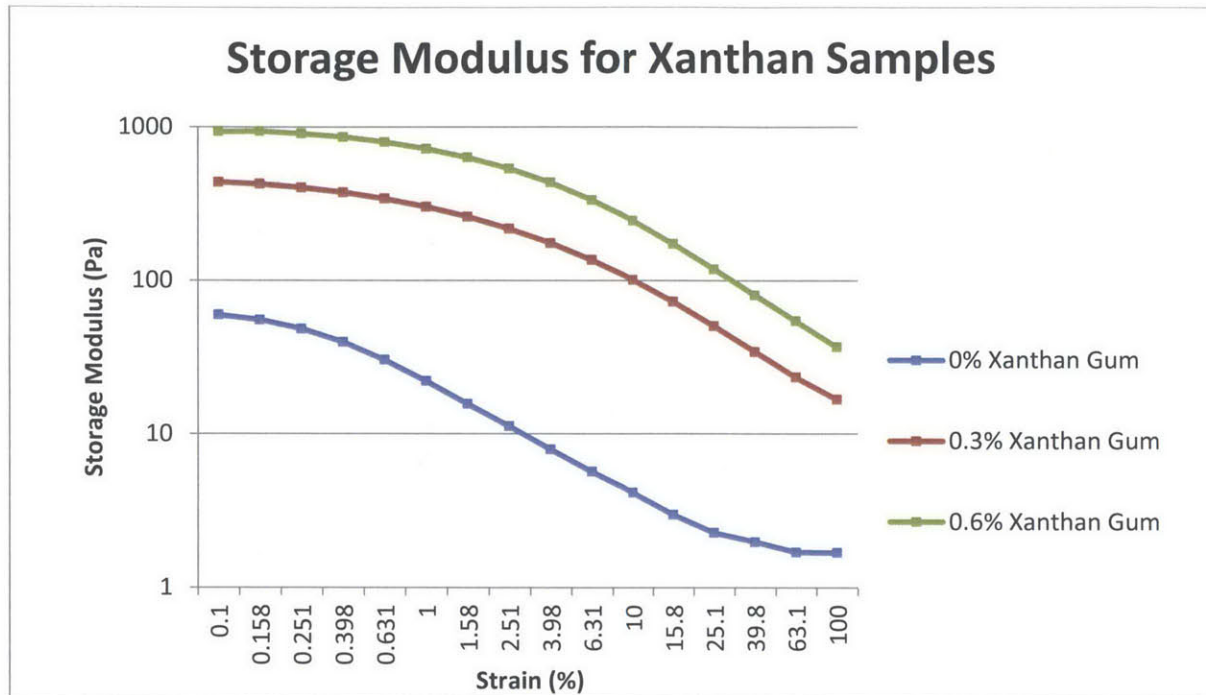


Figure 15: G' for samples (11-13) at varying concentrations of 0%, 0.3%, and 0.6% xanthan gum

Figure 15 displays the storage modulus for xanthan samples ranging from 0% concentration to 0.6% concentration. The graph displays that the storage modulus increases with higher concentrations of xanthan gum. Furthermore, the linear viscoelastic region is also longer for higher concentrations of xanthan as the graph stays linear at higher values of strain. These results further support that xanthan gum has a strengthening effect on the dough's elastic properties which increases almost exponentially with xanthan gum concentration.

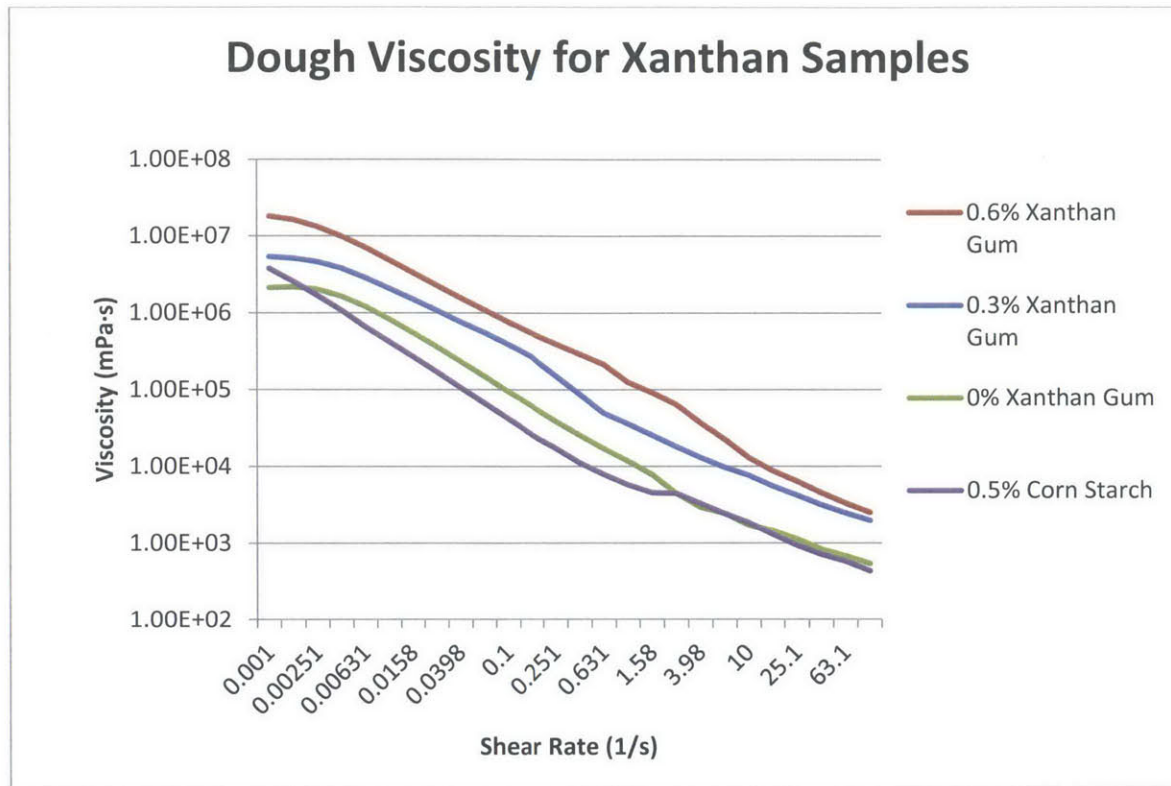


Figure 16: Dough Viscosity for xanthan samples and 0.5% corn starch sample

From Figure 16 it can be inferred that increased levels of xanthan gum resulted in a positive shift in the viscosity curve. From past studies (Lazaridou 2007), xanthan gum is expected to increase the dough viscoelasticity as xanthan gum improves the ability for the dough to absorb water (Korus 2009). Thus, the curve is intuitive to show that as the shear rate increases, the viscosity of the dough would decrease. Furthermore, in comparison to xanthan gum, corn starch has a similar effect on effect on the dough's viscosity by also showing an overall decrease as shear rate increases.

Moisture Content

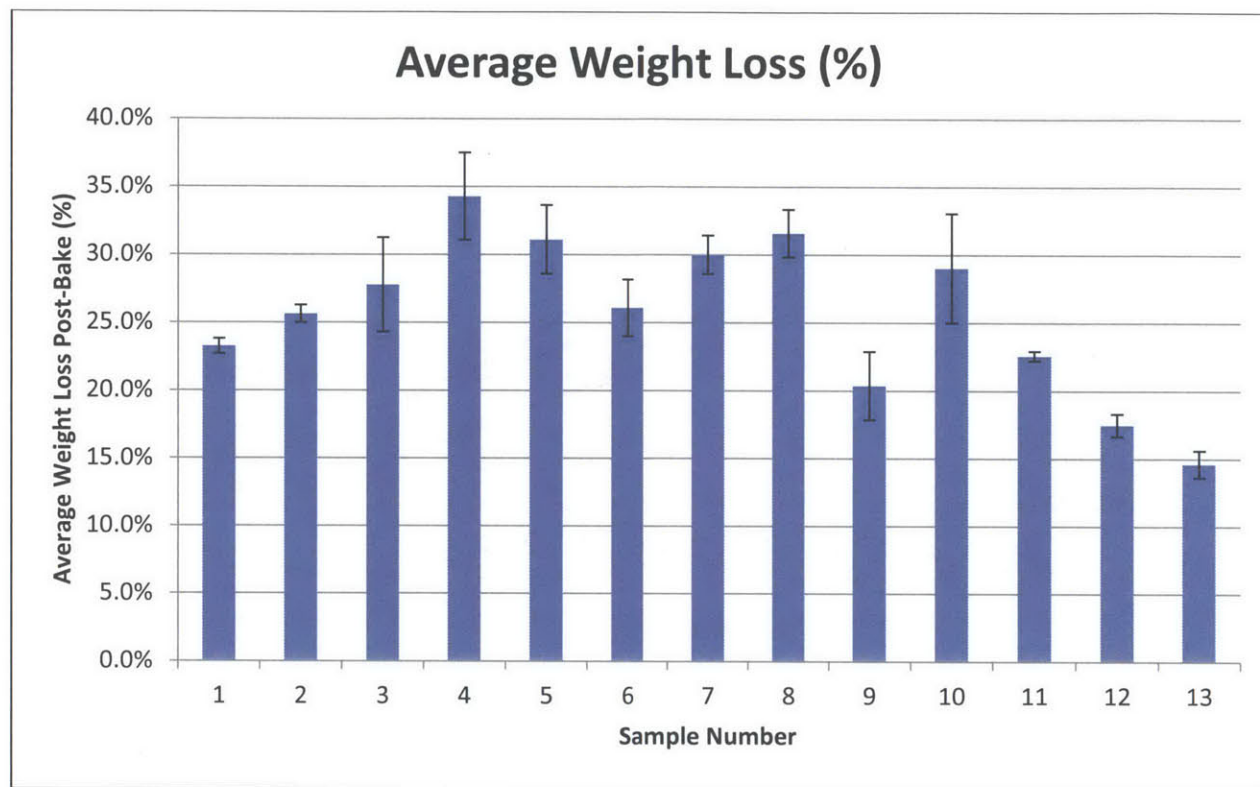


Figure 17: Volumetric weight loss for gluten-free bread (samples 1-9) and gluten-based bread (sample 10), and xanthan samples (samples 11-13)

The percent weight loss of gluten-free bread was on average 25.4% after 15 minutes of baking at 350°F in comparison to the control gluten-based bread sample which lost 29.0% in the same amount of time.

Possible discrepancy during this experiment may have occurred from a couple of separate factors. First, post initial baking of the dough, bread was allowed to cool on a wire rack for up to hours but to as little as 2 hours before bread was wrapped and frozen. Though all bread was thawed for the same amount of time, it is possible that bread moisture was lost after bread was initially baked but before frozen. In addition, the location of the slice could have affected moisture levels. Slices obtained closer to the edge of the pan may have been exposed to more heat in the oven and lost more moisture, where slices in the middle may have been exposed to less heat.

Porosity

Cross-sectional images of bread were taken and analyzed using the ImageJ analyze particles feature. An analyzed image is shown below in Figure JKFD, where the dark portions of the picture represent the pores in bread.

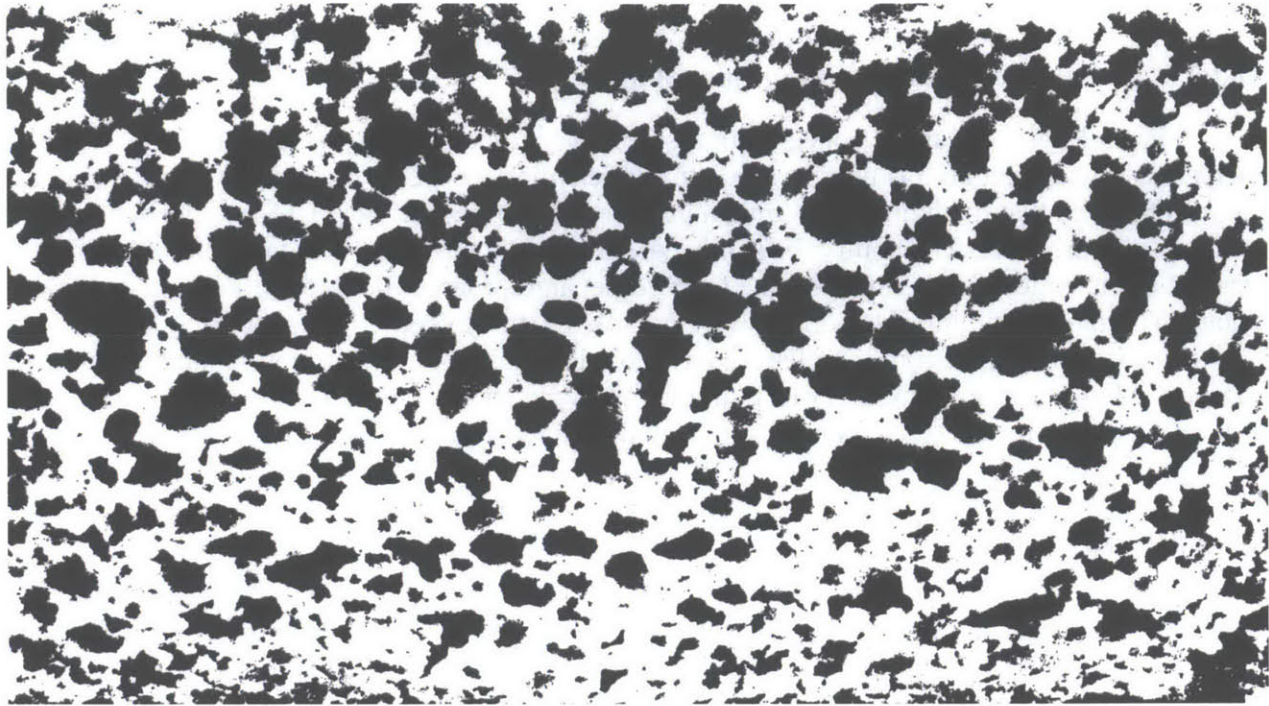


Figure 18: Image of 0.3% Xanthan Gum baked sample after ImageJ Analyze Particle analysis; black (pores), white (bread)

After the data on each pore was collected, the average pore size of the cross-sectional image was analyzed and the results of the xanthan gum samples are shown below. More than one cross-sectional image was used in analysis of average pore size.

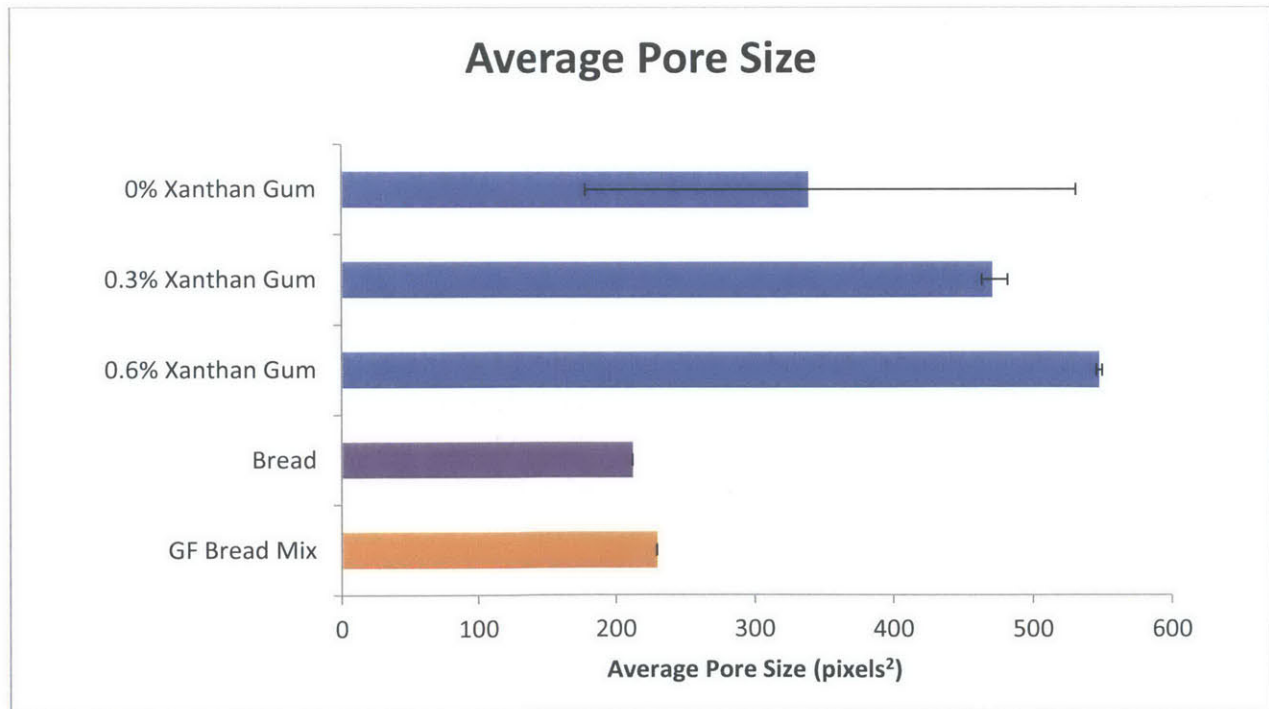


Figure 19: Average pore size of samples 11-13 with varying amounts of gluten (0, 0.3%, and 0.6%). Data was gathered using ImageJ analysis

From Figure 19, as the concentration of xanthan gum increases, the average pore size also increases. As xanthan gum is used to regulate gas retention inside the dough as it is proofing, it can be observed that the higher the xanthan gum concentration, the more CO₂ gas the batter can retain during proofing. The ability for xanthan gum to retain gas allows for larger bubbles to be trapped in the batter, for the loaf volume to increase, and for larger pores to form in the baked dough. Though no volume calculations were completed, this hypothesis is supported by Figure 8 above where no xanthan gum samples are have low loaf height in comparison to xanthan gum samples. Thus, xanthan gum is a crucial ingredient in gluten-free bread as it provides the dough with means to retain the CO₂ gas needed during the rising process.

Conclusion

Xanthan gum provides gluten-free bread with the support and structure necessary for the retention of CO₂ gas during proofing as well as increases gluten-free dough's elastic modulus. The rheological properties of dough were observed to characterize xanthan gum's effect on pre-baked gluten free dough. With increasing xanthan gum concentration, the loss factor decreased indicating a higher ratio of elastic to viscous properties in the dough and a longer linear viscoelastic region. Moreover, decreasing xanthan gum concentration resulted in higher viscosity which further supports xanthan gum's viscoelastic properties. Pore size was also affected by xanthan gum as pore size increased with higher concentrations of xanthan gum in post-baked samples. Without xanthum gum, gluten-free bread formulations did not rise properly and resulted in a dense, unpleasant short loaf. Too much xanthan gum provided proper rise and gas regulation but led to gummy texture from batter thickening too much. Thus, a 0.3% xanthan gum concentration in the gluten-free bread formulation provided the best pre- and post-baked properties.

Acknowledgements

I would like to thank my thesis advisor, Dr. Holten-Andersen for his guidance and support throughout the course of my research. In addition, I would like to thank the other members of Dr. Holten-Andersen's lab group, Scott Grindy and Stephanie Marzen, for their help with the Rheometer. Finally, special thanks to my fellow Materials Science and Engineering undergraduates who also supported me throughout my research.

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