

Chemically leavened gluten free sorghum bread

by

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Abstract

Sorghum is unique in terms of its resistance to drought and heat and is grown and consumed around the globe. Moreover, sorghum does not contain gluten and has potential in the gluten-free market. A blend of non-wheat flour, starch and hydrocolloid typically provide the structure of gluten-free products. Most research on sorghum bread uses a yeast leavened process, HPMC gum, rice flour and corn, potato, or tapioca starch. Little is known about the functionality or interactions of different starches and hydrocolloids in sorghum batter. The objectives of this study were to examine starch-hydrocolloid interaction in chemically leavened gluten free sorghum bread; to evaluate the effects of different ingredients on gluten free bread quality made with sorghum flour: starch (tapioca starch, rice flour and potato starch): hydrocolloid (HPMC, locust bean gum and xanthan) and to develop a chemically leavened gluten free sorghum bread method. Bread was baked as pup loaves. Volume index was measured using the AACCI Method 10-91.01 template, crumb grain was evaluated using the C-Cell Imaging System and texture was determined with the TA.XT Plus Texture Analyzer. The base formula was commercial sorghum flour, water, starch, hydrocolloid, sugar, salt, shortening and double acting baking powder. Sorghum flour: starch (tapioca starch, rice flour and potato starch) ratios of 70:30, 80:20 and 90:10 were tested. Loaves containing all levels of rice flour had the same volume index (~165) as 100% sorghum flour (168) while all levels of tapioca starch and potato starch produced significantly smaller loaves (~150). The ratio of 90% sorghum flour and 10% starch (tapioca starch, rice flour and potato starch) was selected. The type and level of hydrocolloid significantly impacted loaf volume, grain and texture. Starch-hydrocolloid combinations which produced the best loaves were tapioca starch + 3% HPMC, rice flour + 3% xanthan and potato starch + 4% xanthan. Following initial optimization experiment, egg ingredients, fat, baking

powder and water were added and evaluated individually to develop an optimized formulation. In general, addition of egg ingredients, shortening and oil did not improve the overall quality of sorghum based bread and were not added to the formula. However, emulsified shortening was effective. The best level of emulsified shortening was determined to be 3% for the breads with sorghum flour: tapioca starch or sorghum flour: potato starch and 5% for bread made with sorghum flour: rice flour. The best baking powder (SALP and MCP) levels were 5, 8 and 5% for sorghum flour: tapioca starch bread, sorghum flour: rice flour bread and sorghum flour: potato starch bread, respectively. Optimum levels of water for sorghum flour: tapioca starch bread, sorghum flour: rice flour bread, and sorghum flour: potato starch bread were 120, 110 and 120%, respectively. This research showed that different starch sources have different interactions with other ingredients in chemically leavened sorghum based gluten free bread.

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Dedication

This dissertation is dedicated to my mother, Nergiz Ari, who offered me unconditional love and encouragement. She has been a perfect example as a friend, mother, successful dentist and women so that I may follow in her food steps. I am following in her footsteps. I always will be thankful for her infinite support.

Chapter 1. Introduction

Most sorghum based gluten free breads in the literature are prepared using yeast as the leavening agent and do not have good quality. In general, yeast leavened sorghum bread contains at least 20% added starch. Dilution of the sorghum flour in the sorghum bread formula with this added starch reduces the dietary fiber, protein and micronutrient content in the bread and may also lead to a higher glycemic response. The breads often have a short shelf life and excessively firm texture. There are several reasons why chemically leavened sorghum bread may be more desirable than yeast breads. Yeast is more expensive compared to baking powder and should be kept at cool temperatures while baking powder can be stored at room temperature. Specialized equipment, such as a fermentation cabinet, is also needed for yeast bread processing. Therefore, making yeast leavened bread requires more energy, time and space than making a chemically leavened bread. In yeast leavened systems, the carbon dioxide is produced over time during fermentation. For wheat bread this time can be between two and three hours or even longer. However, the fermentation time of the published gluten free yeast methods are relatively short (30-45 minutes) so only a small quantity of gas is produced. Gluten free batters do not have a developed gluten network to retain the gas produced. Thus, the fermentation time used in the majority of the published methods is not long enough to produce high quality bread. These factors result in bread with low volume, tough crumb, hard structure and short shelf life. Using chemical leavening may improve loaf volume by increasing gas generation and controlling the timing of gas release. Bread with an expanded structure is often softer and has a longer shelf life. The first objective of this project was to develop a chemically leavened gluten free sorghum bread method.

The experimental design started with an initial basic formula containing flour, water, sugar, baking powder and salt. Other ingredients including starch, hydrocolloids, egg ingredients and fat

were then added individually to evaluate their effect. If the ingredient improved the volume and the crumb characteristics of gluten free sorghum bread, it was kept in the formula. If it did not improve the bread, it was omitted. In this way, the final formula was developed. The goal was increased volume and a fine crumb grain containing a large number of cells with thin cell walls and small cell diameter.

Three satisfactory formulas were developed, each using a different starch. Interestingly the starches performed differently in combination with different hydrocolloids. Thus, the other objectives of the project were to further evaluate starch-hydrocolloid interaction in the chemically leavened gluten free sorghum bread formula and the effects of the different ingredients on the volume index and crumb characteristics.

Objectives

The objectives of this study were:

- 1) to examine starch-hydrocolloid interaction in chemically leavened gluten free sorghum bread.
- 2) to evaluate the effects of different ingredients in chemically leavened gluten free sorghum bread.
- 3) to develop an optimized chemically leavened gluten free sorghum bread method.

Chapter 2. Literature Review

Wheat bread is a staple food for many countries. Wheat flour doughs are viscoelastic materials which exhibit an intermediate rheological behavior between a viscous liquid and an elastic solid. The viscoelastic protein network has an important role in dough processing and the textural characteristics of the finished bread (Torbica et al 2010). Because of the unique characteristics of wheat bread, it is highly preferred by consumers. The most functional component of wheat flour is the gluten protein. Gluten is made up of gliadin and glutenin. These two groups of proteins provide elasticity and cohesiveness to the dough and develop into a network in dough which is able to retain gas (Delcour and Hoseney 2010). The gluten matrix is important for water absorption capacity, cohesiveness, viscosity, extensibility, elasticity, resistance to stretch, mixing tolerance and gas holding capacity (Lazaridou et al 2007; Wieser 2007). Furthermore, the solid matrix of the bread crumb is also formed of a perpetual phase of gelatinized starch which is surrounded by the continuous gluten network (Hüttner and Arendth 2010).

Celiac disease (CD) is also known as gluten sensitive enteropathy (Wieser et al 2012). The existence of CD was underreported for a long time but with the help of sensitive serological tests, it is now known that CD is one of the most encountered food intolerances around the world (Catassi and Fasano 2009). CD is a permanent food intolerance caused by ingestion of gluten from wheat, rye and barley which harms the enterocytes and causes malabsorption of significant nutrients in the intestine (Catassi and Fasano 2009). It is reported to affect 0.7% of the total U.S. and European populations and is also a prevalent disease in North Africa, the Middle East and India (Catassi and Fasano 2009). Chronic diarrhea, stunting, anemia and increased mortality are some symptoms of CD, which sometimes is a painful illness (Catassi and Fasano 2009).

Certain genes contribute to the genetic inclination of CD, but the main factor is the environment. The major genetic factors are HLA-DQ genes which are DQ2 and DQ8 in the HLA complex on 6p21. Almost 95% of celiac patients have DQ2 (DQB1*02 and DA1*05) and the remaining 5% have DQ8 (DQB1*302 and DQA1*03) heterodimer. Homozygous people with DQB1*02 and DA1*05 in *cis* on both chromosomes are at a higher risk of having complex forms of CD (Verbeek et al 2009). The best and only treatment for CD is a lifelong totally gluten free diet (Pelkowski and Viera 2014).

GLUTEN FREE BREAD

It is challenging to produce good quality gluten free breads because there are no ingredients available to simply replace gluten. The lack of gluten in a bread formulation commonly leads to a liquid batter instead of a developed dough. Gluten free breads are known to have low quality, poor crumb and crust characteristics, rapid staling and poor mouth feel (Gallagher et al 2004). However, the increasing demand for gluten free diets requires cereal technologists and bakers to develop gluten free products which taste good and have desirable textural properties.

There are controversial issues about the definition of “the hydrated mixture of gluten free ingredients” among researchers. Some researchers refer to it as a “batter” while others call it a “dough.” According to Cauvin and Young (2007a), the formulation and techniques to keep gas bubbles stabilized during processing and baking in starch based gluten free “dough” are very close to those of cake batter; therefore, the viscosity of gluten free “dough” is more similar to cake batter than traditional bread dough.

The major ingredients in gluten free bread products are gluten free flours, starches, hydrocolloids (gums), animal proteins and vegetable proteins. Gluten free flours are obtained

from cereals (i.e. maize, rice, millet and sorghum), legumes (i.e. soya, chickpea and pea), pseudo cereals (i.e. amaranth, quinoa and buckwheat) and nut flours (chesnut and tiger nut flours) (Vivas 2013; Puig 2014).

Barley, rye, triticale and their products are also eliminated from a gluten free diet because they contain amino acid sequences in their proteins which are identical to those that cause the allergic reaction from wheat. Oats are considered a safe cereal for people who suffer from celiac disease. However, the risk of cross contamination of oats with wheat and barley is an issue (Bianchi 2013). For this reason, many people with CD avoid consuming oat products even though research has shown that daily consumption of oats does not harm celiac patients.

Cereal Flours

Maize flour is a gluten free flour. Different formulations of maize flour bread were developed through research by Olatunji et al (1992a) and Sanni et al (1998). In these projects, maize flour was blended with raw cassava (tapioca) starch, maize starch or soy flour. The effect of HPMC (Hydroxypropyl Methyl Cellulose), xanthan and their combination on gluten free bread with maize flour and other flours (rice, buckwheat and teff) was investigated. HPMC was found to increase the volume and decrease the crumb softness while xanthan decreased the volume of maize bread (Hager and Arendt 2013).

Gluten free rice bread has a high volume and a bland flavor (Schober 2009). The formulation of rice bread often utilizes white rice flour, high water levels (75–110% on a flour basis) and HPMC which was shown to be the best hydrocolloid for rice bread (Schober 2009). Addition of isolated starch is not necessary in rice bread formulations. No negative effect was observed by the addition of moderate levels of 5% rice bran; however, higher levels of rice bran caused low volumes. Renzetti et al (2009) found that enzymatic treatment of the rice bran had a

positive effect on bread quality by significantly increasing specific volume and reducing both the crumb hardness and chewiness.

Teff flour is used in gluten free bread. The effects of HPMC, xanthan, HPMC combined with xanthan and transglutaminase on gluten free teff flour breads were evaluated (Renzetti et al 2008; Hager and Arendt 2013). Renzetti et al (2008) investigated the effect of the application of glucose oxidase and protease commercial preparations on the bread making performance of four different gluten free flours (buckwheat, corn, sorghum and teff) and found no significant effects on buckwheat and teff breads. Hager and Arendt (2013) found that HPMC improved the volume and increased softness while xanthan decreased the volume and softness of teff bread.

Legume Flours

Legumes are good sources of protein in the human diet. They are rich in lysine, leucine, aspartic acid, glutamic acid and arginine. Moreover, they offer a well-balanced essential amino acid profile if consumed with cereals and other foods that contain high amounts of sulphur-containing amino acids and tryptophan (Vivas 2013). In addition to their nutritional profile, legume proteins have functional properties that are important for food formulation and processing. The functional characteristics of legume proteins, such as chickpea flour (*Cicer arietinum* L.), pea protein isolate (*Pisum sativum* L.) and carob germ flour (*Ceratonia siliqua* L.) have been used in the preparation and development of bakery products. According to Smith et al (2012), carob germ flour and HPMC created a true dough which is unusual in gluten free breads. The quality of bread made with carob germ flour and HPMC was similar to wheat bread.

According to Minarro et al (2012), legume flours have positive effects on the physicochemical characteristics and contribute an adequate sensory profile to gluten free breads. Batters containing carob germ flour have good rheological properties; nevertheless, the resulting

breads have poor characteristics such as low specific volume and hard crumb. Breads made with chickpea flour and pea isolate had good baking, rheological and sensory characteristics.

Soy makes several contributions to gluten free bread. In addition to increasing the consistency of gluten free batters and improving the specific volume of the gluten free breads, soy also helps to reduce staling and firmness of the crumb due to the high water absorption capacity of its protein. However, soy is an allergen and can cause digestive problems. This concern leads researchers to find alternative protein sources which are not allergens (Puig 2014).

Pseudo Cereal Flours

According to the botanical view, pseudo cereals are dicotyledons while cereals are monocotyledons. Since their seeds are rich in starch, they can be made into flour (Vivas 2013). The most commonly used pseudo cereals in gluten free baking applications are amaranth, quinoa and buckwheat. Because of their high starch contents, they are excellent sources of energy. They have great nutrient profiles, good quality protein, dietary fiber and lipids which are rich in unsaturated fats. Research showed that the replacement of potato starch with pseudo cereal flour provided higher amounts of important nutrients such as protein, fiber, calcium, iron and vitamin E (Jubete et al 2009). No information was reported on baking quality.

Nut Flours

Chestnut and tiger nut flours are other types of alternative flours used in gluten free breads (Puig 2014). Demirkesen et al (2010) evaluated gluten free bread with chestnut and rice starch blends at different levels (0/100, 10/90, 20/80, 30/70, 40/60, 50/50 and 100/0 %). They also investigated how hydrocolloid blends (xanthan–locust bean gum, xanthan–guar gum blend) and the emulsifier DATEM affected the rheological properties of the batter and quality characteristics of the breads made with chestnut/rice flour levels of 10/90, 20/80, 30/70 and

40/60%. In terms of hardness, specific volume, color and sensory values, the best quality was obtained with 30% chestnut flour, 70% rice flour, xanthan–guar blend and DATEM. Irrespective of gum blend and emulsifier addition, increasing the level of chestnut flour caused lower quality (low volume, harder texture and darker color) in the final bread (Demirkesen et al 2010).

Paciulli et al (2016) investigated the physicochemical (proximate composition, color, texture and crumb grain characteristics) and nutritional (antioxidant capacity and in vitro digestion) properties of gluten free breads made with two commercial gluten free mixture enriched with chestnut flour during three days of storage. The enrichment of chestnut flour caused darker color, lower volume, larger holes in the crumb holes, faster staling, increased crumb gumminess and decreased crumb elasticity. Enriched breads had a higher fiber content and antioxidant activity. There was no change in starch digestibility between enriched and non-enriched flours.

Demirkesen et al (2013) evaluated the quality of gluten free bread made with different tiger nut flour/rice flour ratios (0/100, 5/95, 10/90, 15/85, 20/80 and 25/75%) baked in an infrared-microwave combination oven and a conventional oven. In terms of hardness and specific volumes, breads made with tiger nut flour/rice flour ratio of 10/90 baked in a conventional oven and breads made with tiger nut/rice flour ratio of 20/80 baked in the infrared–microwave combination oven were the most acceptable.

Starches and Hydrocolloids as Flour Supplements

Corn, tapioca, potato and rice starches are different types of starches commonly used in gluten free formulations (Sanchez et al 2002; Kobylanski et al 2004; Moore et al 2004). Nevertheless, their individual effects on the microstructure and textural characteristics of bread is not well known since they are used with other ingredients for optimizing formulations.

Miyazaki et al (2006) investigated the impact of modified starches on the textural properties of bread. The researchers found that modified starches helped control the texture of the final products and created unique breads. Onyongo et al (2011) found that bread made from sorghum flour and native cassava (tapioca) starch had better crumb characteristics (springier, softer, less chewy, more adhesive) than bread made with pre-gelatinized tapioca starch. Krupa-Kozak et al (2010) investigated native and hydrothermally modified bean starch as an ingredient for gluten free bread. They reported that modified bean starch increased the protein content and crumb elasticity of the bread and made the crumb more homogeneous. Moreover, modified starch improved the chemical composition and quality of fresh bread. However, it decreased crumb toughness and specific volume and increased staling of the bread.

Mancebo et al (2015) compared rice, maize and wheat starches in their research and found that wheat starch produced bread with better acceptability and higher volume than maize starch containing bread. Bread containing a blend of rice flour (59%) and wheat starch (41%) had the highest sensorial acceptability. To understand the effect of different starch types on the quality properties of gluten free breads, more studies are required.

Although starch based breads are the simplest gluten free bread, their formulations require a hydrocolloid to avoid settling of starch granules and a loss of gas bubbles during fermentation (Schober 2009). Common hydrocolloids used in gluten free bread formulations are HPMC, xanthan, guar, locust bean, carrageenan, pectin, CMC (carboxymethyl cellulose), konjac, agarose, β -glucan, alginate, gelatin, k-carrageenan, high β -glucan oat bran, agar, arabic, tragacanth and propylene glycol alginate (Vivas 2013; Puig 2014).

GLUTEN FREE SORGHUM BREADS

Several studies have been conducted with sorghum bread. Different levels of various starches have been used in sorghum based bread (Hart et al 1970; Olatunji et al 1992a; Olatunji et al 1992b; Hugo et al 1997; Schober et al 2005; Schober et al 2007). The total amount of flour/starch blend was specified as 100% where starch was generally 20-30% of the total blend and the remaining 70-80% was sorghum flour. Maize, raw cassava, gelatinized cassava and potato were the starch sources most commonly used.

Many formulations of sorghum bread in the literature commonly contain sorghum flour, starch, sugar, salt, yeast and water. However, the levels of the common ingredients vary widely. Most of the published research on gluten free sorghum bread (Hart et al 1970; Olatunji et al 1992a; Hugo et al 1997; Schober et al 2005; Schober et al 2007) used 2% (fwb) yeast while Olatunji et al (1992b) used 1% yeast. No studies were found that used chemical leavening. The water absorption ranged between 80-120% (fwb) in these studies. In one study, Olatunji et al (1992a), used 70% sorghum flour and 30% raw cassava starch with fungal amylase while a second study used 70% sorghum flour, 10% raw cassava starch and 20% gelatinized cassava starch with monoglycerol palmitate as an additional ingredient (Olatunji 1992b). Schober et al (2005) used a formulation with 70% sorghum flour and 30% potato starch and found that sorghum bread formulated with potato starch was less prone to collapse but the results were not constant. Substituting HPMC for the xanthan in the formulation with potato starch helped to produce more consistent results. However, the specific loaf volume of the bread was still low ($1.8 \text{ cm}^3/\text{g}$). Later work by Schober et al (2007) reported that modifying their original formula to include replacing xanthan with HPMC caused specific loaf volume to increase from $1.8 \text{ cm}^3/\text{g}$ to $2.7 \text{ cm}^3/\text{g}$. That study also evaluated the effect of sourdough fermentation by adding HPMC,

bacterial alpha-amylase, starter culture and calcium carbonate compared to breads with sorghum flour: corn starch; sorghum flour: potato starch; the breads with sorghum flour: potato starch with HPMC; breads chemically acidified with lactic acid, bacterial alpha-amylase, calcium carbonate and HPMC. They concluded that sourdough fermentation helped to improve the crumb structure and resolve the problems of flat tops and large holes in the crumb. The researchers concluded that “a strong starch gel, without interference of aggregated protein, is desirable for this type of bread”.

Common Ingredients in Sorghum Gluten Free Bread

Sorghum Flour

Sorghum is the fifth most prevalent grain in the world (Anglani 1998). Sorghum is an essential food, which provides people in semi-arid regions of Africa and Asia with over 70% of their daily caloric intake (Hulse et al 1980). The traditional products made with sorghum are thick and thin porridges (tuwo, ugali, ogi), bread (injera, kisra, roti), couscous and beer (Murtz and Kumar 1995). Sorghum has a broad genetic variability. Over 30,000 selections exist in nature, making it cumbersome to classify them. Nutritionally, sorghum is a source of carbohydrate, fiber, protein, vitamins and minerals (Hubbard et al 1950).

Protein content and amino acid composition of sorghum depend on genotype, environment and fertilizer application (Buckner 1997). Lysine, histidine and the sulfur-containing amino acids are the most restrictive amino acids in sorghum while proline, alanine, leucine and glutamate have the highest concentrations. Hamaker et al (1995) showed that 68-73% of whole sorghum flour protein is kafirin (sorghum prolamin). According to Shull et al (1991) kafirins have three subdivisions (α , β and γ). Sorghum is defined as a poor source of protein since the concentration of lysine in all three subdivisions is low (Buckner 1997).

Various studies illustrated the role the specific protein fractions play in determining digestibility (Buckner 1997). After cooking, the kafirin fraction became less extractable and less digestible (Hamaker et al 1986). Scanning electron microscopy (SEM) studies showed that uncooked kafirin protein bodies had wide pitting on their surface when exposed to pepsin digestion, whereas cooked kafirin protein bodies remained smooth after the same treatment (Rom et al 1992). Research also indicates that sorghum proteins, particularly the kafirins, participated in thermally induced disulfide-sulfhydryl interchanges that have a negative impact on digestibility (Buckner 1997). Although kafirin is the most prominent, small amounts of glutelins, albumins and globulins are also found in kafirin protein bodies (Taylor et al 1984).

The main storage form of carbohydrates in sorghum is starch which comprises 73.8% of the grain (Murtz and Kumar 1995; Belhadi et al 2012). The barrier formed by the protein matrix in which the starch granules are embedded inhibits access of amylases to the starch (Rooney and Pflugfelder 1986). This limited accessibility gives sorghum starch a low digestibility which is lowered even more after cooking (Rooney and Pflugfelder 1986; Zhang and Hamaker 1998).

Tannins are high molecular weight polyphenols which can complex with carbohydrates and proteins (Hagerman and Butler 1980; Dykes et al 2005). Condensed tannins influence the sensory and nutritional quality of the sorghum (Anglani 1997; Kulamarva et al 2009). Studies show that tannins have anti-carcinogenic and anti-inflammatory impact (Awika et al 2009; Huang et al 2010; Burdette et al 2010; Hargrove et al 2011). Sorghum also contains phenolic acids including caffeic, syringic, protocatechuic, p-coumaric and sinapic which exhibit good antioxidant activity *in vitro* (Hahn 1984, Barros et al 2012). Antioxidants can reduce the risk of certain illnesses such as cancer, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease and cataracts (Kulamarva et al 2009). However, tannins also reduce the nutritional value

of sorghum grain because they can link to dietary proteins, carbohydrates, digestive enzymes, minerals such as iron and B vitamins, such as thiamin and vitamin B6 (Wang and Kies 1991; Duodu et al 2003; Schons et al 2011).

Starch Sources

Gluten free products often contain a mixture of a non-wheat flours, starches and hydrocolloids as the “flour” component. Typical starches used in gluten free breads are corn, tapioca, potato and rice.

Potato Starch

Potato starch is highly utilized in the food industry. It has some beneficial characteristics which include higher purity compared to cereal starches, a neutral taste, ability to make clear pastes and high viscosity gels as well as having a low gelatinization temperature (Grommers and van der Krog 2009). However, potato starch is also sensitive to shear and heat. The overall shape of potato starch is oval and granular with a diameter between 5-100 µm. The pasting temperature of potato starch was determined by Kofler hot stage microscopy as 58-63-68 °C (onset-midpoint-end) (Grommers and van der Krog 2009). Potato starch has high phosphorous content (0.06-0.1%) because of the presence of phosphate ester groups (Whistler and BeMiller 1997).

Tapioca (Cassava) Starch

Tapioca starch has low lipid (<0.1%) and low protein (0.1%) contents. This makes native tapioca a unique starch which is used in many food and industrial applications and which is an excellent starting material for modification into specialty products (Breuninger et al 2009). Nevertheless, tapioca starch is not useful for some food products because it tends to break down under heat and shear and has poor freeze-thaw stability (Taggart 2004). Tapioca starch granules

range in size from 5-40 µm. Gelatinization temperature was determined by Kofler hot stage microscopy as 59 – 64 – 69°C (onset-midpoint-end) (Breuninger et al 2009).

Rice Flour

Starch is the main component of rice. Milled rice contains about 90% starch based on dry weight. Depending on cultivar, the gelatinization temperatures can be different. The difference of gelatinization temperature may be 10 °C. Another important component is protein in rice flour. The protein level is commonly less than 10% in milled rice and brown rice. White rice protein has approximately 3 to 5 % albumin, 8 to 10 % globulin, more than 80% glutelin and almost 5% prolamins. The fat and fiber content are very low in rice flour (Alhusaini, 1985)

Hydrocolloids

Hydrocolloids are often used to obtain better texture and appearance in gluten free products because the characteristics of some hydrocolloids and hydrocolloid mixtures can imitate some of the properties of gluten (BeMiller 2009). Their functionality is related to the source, process, chemical structure, modification, addition level and their interactions with other ingredients (Zaninni et al 2012).

Xanthan Gum

Xanthan gum is a linear, branched and anionic polysaccharide (Houben et al 2012). Xanthan can create a stable high viscosity gel or a weak and cold-set gel (Houben et al 2012). The uniqueness of xanthan gum is that it maintains a stable viscosity over a broad range of temperature, whereas most gums thin as their aqueous forms are heated (Hoefler 2004). Xanthan has been used as a gluten substitute in the development of gluten free bread for amending dough elasticity (Peressini et al 2011).

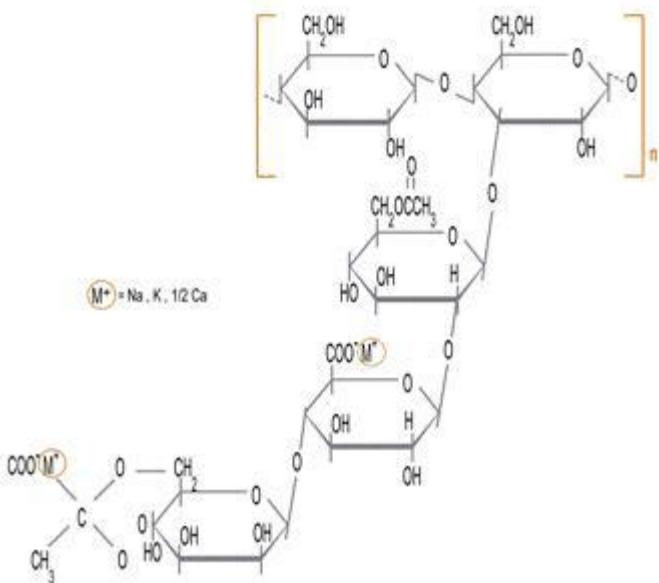


Figure 1. Xanthan chemical structure.

Hydroxypropyl-Methyl-Cellulose (HPMC)

Hydroxypropyl-methyl-cellulose (HPMC) is a modified cellulose derivative (Zaninni et al 2012). It has been shown to improve specific volume, enhance gas retention, increase water absorption, enhance sensory properties, improve crumb texture, increase crumb softness and raise the moisture content of gluten free bread (Dziezak 1991; Kohajdova and Karovicova 2009; Huttner and Arendt 2010). HPMC creates a reversible and heat set gel network which results in a rise in dough viscosity and continuity of boundaries of expanding gas cells (Houben et al 2012).

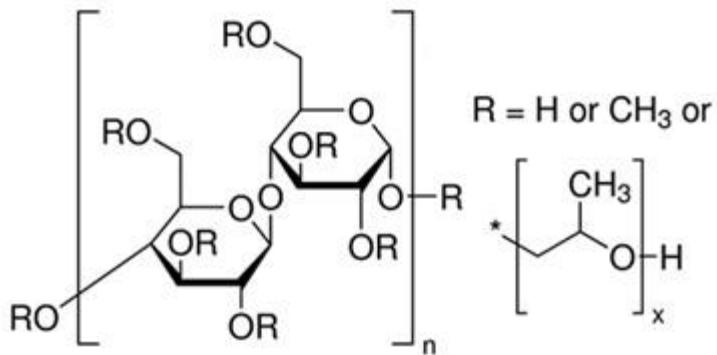


Figure 2. HPMC chemical structure

Locust Bean Gum

Locust bean gum is not highly affected by pH, salt or heat processing and forms very viscous solutions at relatively low amounts, making it popular in food and industrial applications (Golcalves et al 2004). Locust bean gum is used in the food industry as a thickener, viscosity modifier, free water binder and suspending agent or stabilizer in cheeses, frozen confections, bakery products and pie fillings (Kohajdova and Karovicova 2009). In order to solubilize completely in water, locust bean gum has to be heated to approximately 82 °C (180 °F) (Pyler and Gorton 2009a). It has been shown to improve the height of gluten free bread loaves and delay bread staling (Zannini et al 2012).

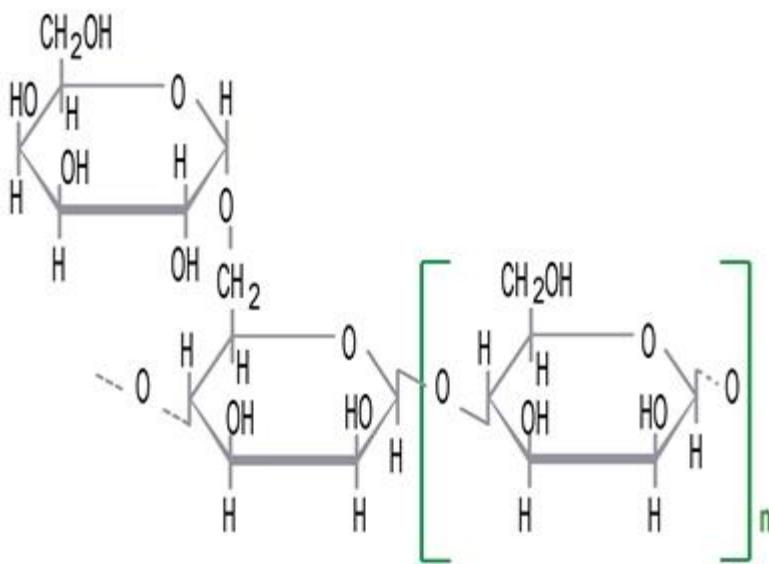


Figure 3. Locust Bean Gum chemical structure.

Egg

Egg proteins are able to form strong cohesive viscoelastic films, which are essential for stable foaming and gas retention (Jonagh et al 1968; Ibanoglu and Ercelebi 2007). As a gluten replacer in gluten free baking, egg proteins behave as foaming agents and crumb stabilizers and enhance bread volume and crumb grain characteristics (Moore et al 2006). The best results for the crumb texture of gluten-free bread was obtained by the addition of whole egg powder compared with other protein sources (Houben et al 2012).

Water

Water affects dough consistency, dough rheology and dough temperatures (Ngemakwe et al 2015; Pyler and Gorton 2009b). Water solubilizes formula ingredient so they rehydrate and interact with each other (Vivas 2013). The quantity of water added to the bread formula depends on the water absorption properties of the formula ingredients (Ngemakwe et al 2015). Water content and its distribution play an important role in textural characteristics, such as crumb softness and shelf life (Vivas 2013).

Yeast

Baker's yeast (*Saccharomyces cerevisiae*) is the main leavening agent in bread-type products. Yeast produces carbon dioxide gas (CO₂) as a by-product of the fermentation of the sugar in the formula. A viscoelastic network formed by the gluten in a fully developed dough is able to hold the carbon dioxide gas bubbles (Peighambardoust et al 2010). Fermentation gases are released into gas cells thereby expanding the dough during fermentation and proofing stages. Sheeting and molding increase the number and size distribution of gas cells by subdividing the existing gas cells (Sroan et al 2009). The gluten-starch matrix which encompasses the growing air bubbles in bread dough is a primary reinforcement and is highly important to keep equal growth of expanding gas cells. Stability of the gluten-starch matrix controls the uniformity of cell size in proofing and baking stages. This provides good crumb structure. Because of the rheology of the gluten-starch matrix and its extensibility, gas cells are able to expand and not collapse (Sroan et al 2009). As temperature increases during baking, the gas bubbles in the dough expand, giving a rapid increase in volume known as oven spring (Cauvin and Young 2007b). Yeast not only produces gas, but the fermentation products also contribute to bread flavor and affect dough rheology (Rezaei et al 2014).

Baking Powder

Baking soda (sodium bicarbonate) and one or more leavening acids are combined to form baking powder (Vivas 2013). The reaction of the sodium bicarbonate with the leavening acid generates carbon dioxide gas. Depending on their reaction rates, baking powders are categorized as fast acting, slow acting or double acting. Fast acting leavening acids release carbon dioxide gas in the first few minutes of mixing when they come together with liquids. Slow acting leavening acids do not release gas during mixing or at low temperatures but they release carbon

dioxide gas in the oven (Pyler and Gorton 2009c). Double acting baking powder contains two leavening acids, a fast acting acid which reacts during mixing to expand gas bubbles incorporated into the batter during mixing and a slow acting acid which reacts in the oven to increase the volume of the product. Monocalcium phosphate monohydrate (MCP) is a very fast leavening acid. It generates 60-70% carbon dioxide of sodium bicarbonate during the last 2 minutes in the mixing. This leads to a cake batter with high viscosity and higher volume which also increases pan fill. Sodium Aluminium Phosphate (SALP) is widely used as a second leavener in double acting baking powder. SALP generates carbon dioxide during the baking. There is no reaction between SALP and other ingredients at lower temperature (Pyler and Gorton 2009d).

Fat

Fat gives many functional and sensory characteristics to food products (Giese 1996). It can be in the form of liquid oil or solid shortening. Shortening is a term which is derived from the fact that fat in a bakery product “shortens” (tenderizes) the texture of the final product. Shortening contributes tenderness, moist mouthfeel, lubricity, flavor and structure to the product. Moreover, shortening reduces the staling and increases the shelf life of many baked products (Stauffer 1996).

Other Ingredient

Emulsifiers aid in air incorporation, stabilize dough stability, improve texture, increase volume and extend shelf life of bakery foods (Glossary of Baking Terms 2001). Salt (sodium chloride) is of major importance in gluten free baking. The main reason is for flavor enhancement. The amount of salt added to gluten free bread formulas is about the 1.5% of gluten

free flour weight for taste (Ngemakwe et al 2015). Sucrose is the most commonly used sugar in baking. Sucrose provides flavor to gluten-free bread.

GLUTEN FREE BREAD PRODUCTION PROCESS

Milling

Milling plays an important role in the gluten free bread process because the particle size of the alternative flour can have an effect on dough characteristics and final bread quality, particularly volume. Frederick (2009) milled white food grade sorghum into flour with three different extraction rates (60, 80 and 100%) and then pin milled at various speeds (no pin-milling, low speed and high speed). Additionally, two commercial sorghum flours were pin milled using the same conditions. The characteristics of the resulting flours were evaluated by analyzing flour composition, total starch content, particle size distribution, damaged starch and water absorption. For the baking test, specific volume, crumb characteristics and crumb firmness were analyzed. Extraction rate and speed of pin milling of sorghum significantly affected fiber content, total starch content, flour particle size and starch damage. Although there was not a consistent relationship between extraction level and starch damage, increasing speed of pin-milling increased the starch damage. In no and low speed pin milling process, highest extraction level was found in the 80% extracted flours while the lowest starch damage belonged to the commercially milled sample. The best baking result was obtained with 60% extraction flour in terms of specific volume, crumb property and crumb toughness. It was found that baking properties of the milled flours were highly affected by flour characteristics, specifically particle size, starch damage and fiber content. However, the breads baked with the pin milled commercial flours had lower specific volume, poorer crumb characteristics and denser textures than the laboratory milled sorghum flour. In another study, breads made with 60% extraction sorghum flour with small particle size gave higher

volume, softer crumb and better crumb structure than breads made with 80 or 100% extraction with large particle sizes (Tappey et al 2014). De La Hela et al (2013) showed that breads made with coarse particle size rice flour with high water absorption (90 and 110% fwb) gave higher volume and better texture than breads made with fine particle size rice flour. Recent research also found that breads made with coarse particle size corn flour had higher volume and softer crumb than breads made with fine particle size corn flour (De La Hela et al 2014).

Mixing

Mixing is an important part of the gluten free bread production process. Water content and its distribution are highly significant for textural properties such as crumb softness, crust crispness and shelf life (Wagner et al 2007). Mixing helps to disperse and hydrate formula ingredients. Air bubbles are trapped in the batter or dough matrix during mixing. Mixing also prepares a dough to be in a suitable form for next steps in the process (Cauvin and Young 2007c). Research done by Gomez et al (2013) evaluated the impact of mixing on two gluten free bread formulas which contained 80 or 110% (fwb) water. The researchers used a flat paddle and dough hook to mix batter with lower water (80%), while they used a flat paddle and wire whip to mix the batter with higher water (110%). No difference was observed in the batters mixed with different mixing arms (flat paddle or dough hook) in breads made with 80% water, but mixing time was found to make a difference in batters with 80% water. As the mixing time increased, specific volume improved. In breads with 110% water, the researchers found that both mixer arm and mixing speed affected the volume and texture of the bread. Using a wire whip with lower mixing speeds and longer mixing time resulted in higher specific volumes and softer loaves compared to those made with the flat paddle.

Enzyme Treatment

Enzymes are commonly used in the baking industry and have found success in gluten free systems. Glucose oxidase (GO) is used as a structure agent and has been shown to increase dough strain, strain hardening and bread volume and to improve the crumb grain of wheat bread. Gujral and Rosell (2004a) used GO in gluten free rice breads and found that it increased the specific volume and reduced the crumb toughness.

Adding transglutaminase (TG) to a rice flour based gluten free bread increased both the elastic and viscous behavior of the dough which allowed it to hold carbon dioxide produced during proofing. This also improved the quality in terms of specific volume and softer crumb texture (Gujral and Rosell 2004b).

Several studies describe the effects of proteases in gluten free products. Renzetti and Arendt (2009) found that protease increased gluten free bread quality in terms of specific volume and crumb texture of brown rice flour bread. Rezetti et al (2010) evaluated the effect of protease (0.001% and 0.01% fwb) on bread making quality of gluten free oat flour. Both addition levels increased specific volume but reduced crumb toughness and chewiness. The researchers concluded that increased batter softness, deformability and elasticity improved the quality of the gluten free oat breads. Hamada et al (2013) found that protease treatment improved the gas holding ability of gluten free rice batters.

Sourdough Fermentation

Sourdough is a process in which flour, water, lactic acid bacteria (LAB) and yeast are allowed to ferment for an extended period of time. The sourdough process has been used in the production of wheat and rye bread to increase volume, texture, flavor and the nutritional value. Sourdough aids in increasing the shelf life by delaying the staling process and by conserving

bread from mold and bacterial contamination. Bread produced from quinoa flour fermented with antifungal *L. amylovorus* DSM19280 had increased nutritional value, better bread quality and extended shelf life (Axel et al 2015). The use of sourdough in a buckwheat batter hindered CO₂ production by the yeast which decreased volume and increased crumb hardness in the final bread (Moroni et al 2010). Schober et al (2007) concluded that sourdough fermentation improved the crumb structure of gluten free sorghum bread by minimizing the problems of flat loaf top and large, open cells and holes in the crumb.

Gluten Free Bread Baking

The final step in bread making is baking. Heat transfer by radiation, convection or conduction and steam injection can be used to cause the dough to transform into bread which is lighter, readily digestible and flavorful (Puig 2014).

According to Demirkesen et al (2011), an infrared–microwave combination oven reduced the baking time of rice-chestnut gluten free breads. The color, specific volume and firmness of the breads baked in a conventional oven were statistically the same as the characteristics of breads baked in an infrared–microwave combination oven. Demirkesen et al (2013) conducted a study to evaluate the quality of gluten free bread made with a tiger nut/rice flour blend baked in convectional and infrared–microwave combination ovens.

LEAVENING SYSTEMS IN GLUTEN FREE BREAD

The traditional bread process has five steps, which are mixing, bulk fermentation, dividing/molding, proofing and baking. When the main ingredients of bread dough (i.e. wheat flour, water, sugar, salt and yeast) are blended, the salt and sugar dissolve and the yeast is hydrated. As mixing commences, a three-dimensional viscoelastic gluten network is formed that gives cohesiveness to the dough and holds air cells which are incorporated during mixing.

During fermentation and proofing, the gas cells increase in size as the carbon dioxide gas produced during yeast fermentation fills them. The viscoelastic network hinders the diffusion of the expanded air cells out of the dough, thus the size of the dough is increased. During baking, the air cells expand further, starch granules gelatinize and moisture is withdrawn from the gluten network. The gas cell walls go through strain hardening and rupture, causing the cells to connect; thus, the discontinuous gas phase of the bread dough turns into the continuous gas phase of the baked bread. In cooling, the elastic gluten network is maintained, providing the structure of the loaf and giving the chewy texture found in good bread. In a gluten free batter system, air bubbles are integrated into the batter in the mixing stage and held by the viscosity of the batter. Most gluten free bread systems mimic the traditional batter systems.

According to Casper and Atwell (2014), “the air bubbles are suspended in a viscous matrix consisting of a substance (gum, for example) that is often unstable and susceptible to coalescence.” Therefore, the batter system must be stabilized by high viscosity and surfactants (emulsifiers) (Casper and Atwell 2014). The matrix is more similar a cake batter than a traditional bread dough due to the lack of a developed gluten network. Compared to the traditional bread process, mixing and proofing times are shorter in gluten free bread procedures because the matrix is weak, unstable and porous (Cauvin and Young 2007a).

ISSUES WITH GLUTEN FREE BREAD

The common negative characteristics of gluten free breads are low volume, pale crust color, crumbly texture, poor crumb structure, short shelf life, low dietary fiber and low levels of micronutrients (Schober 2009; Houben et al 2012; Puig 2014). Wheat flour is often enriched with niacin (vitamin B), thiamin (vitamin B1), riboflavin (vitamin B2), folic acid, iron and

sometimes calcium. Since celiac patients cannot eat wheat based products, deficiency in these vitamins and minerals can be cause health issues for them (Corazza et al 1995).

Chapter 3. Materials & Methods

MATERIALS

Sorghum flour containing 10.95% flour weight basis (fwb) moisture, 0.926% (fwb) ash, 7.73% (fwb) protein and 7.87% (fwb) damaged starch, was purchased from Nu Life Market (Scott City, KS). Double acting baking powder, containing sodium aluminum phosphate (SALP) and monocalcium phosphate (MCP) was provided by Corbion Caravan (Kansas City, MO). Whole egg powder, and dried egg white were purchased from Honeyville Farms (Rancho Cucamonga, CA). Potato starch, rice flour (95 % starch), tapioca starch and xanthan gum were purchased from Bob's Red Mill Company (Milwaukie, OR). GMS 540 emulsified shortening containing 35% hydrated distilled glycerol monostearate was provided by Corbion Caravan Company (Lenexa, KS). Locust bean gum was purchased from TIC (White Marsh, MD). Shortening (Crisco; Orrville, OH), salt, sugar and canola oil were obtained from local supermarkets. All ingredients were gluten free.

FORMULA DEVELOPMENT

In order to evaluate starch-hydrocolloid interactions in a chemically leavened sorghum bread, a formula and baking procedure first had to be developed. The gluten free sorghum bread formula and procedure of Schober et al 2005 was used as a model for the development of a chemically leavened gluten free sorghum bread. This formula contained (fwb; where the sum of sorghum flour and starch was taken as the flour) 70% sorghum flour, 30% corn starch, 105% water, 1.75% salt, 1% sugar and 2 % instant active dry yeast. The formula was modified as follows. Sugar content was increased to 6% to improve the flavor. Salt content was reduced to 1.5% due to a current trend in the baking industry to reduce sodium intake. The yeast was removed and replaced with double acting baking powder. Based on preliminary work, the levels

of baking powder and water were initially set at 3% and 100% (fwb). These levels were optimized in later experiments after other formula adjustments had been made. Thus, the initial base formula for chemically leavened gluten free sorghum bread contained (fwb) 100% flour, 100% water, 6% sugar, 3% baking powder and 1.5% salt. Ingredient levels are given on a flour weight basis (% fwb) meaning each ingredient is listed as a percentage of the flour weight. Mixing time was determined as on speed 1 for 0.5 min. and on speed 2 for 2 min but there are some air tunnels in the breads; therefore, the second mixing time was reduced from 2 min to 1.5 min.

Throughout this project, the “flour” was either 100% sorghum flour or a blend of sorghum flour and starch. The effect of starch (rice flour ,tapioca starch or potato starch), hydrocolloids (xanthan, HPMC or locust bean gum), egg ingredients (dry whole egg or dry egg white), fat (oil, shortening or emulsified shortening), baking powder level and water absorption were evaluated and used to create a satisfactory chemically leavened gluten free sorghum bread formulation.

Level of Starch Sources

First, type and level of starch were determined. Rice flour, tapioca and potato starches are typical starches used in gluten free breads and were selected for evaluation. Sorghum flour/starch percentages of 90:10, 80:20 and 70:30 were examined. Control bread had 100% sorghum flour. The formula used contained (fwb) 100% flour, 100% water, 6% sugar, 3% baking powder and 1.5% salt.

Type and Level of Hydrocolloid

After selecting the optimum ratios of sorghum flour and starch for the flour blend, different hydrocolloids (xanthan, HPMC and locust bean gum) were added at 1, 2 and 3% (fwb) and the optimum type and level of hydrocolloid for each type of starch in the flour blend was determined. Xanthan showed the best improvement in breads made with sorghum flour: rice flour and sorghum flour: potato starch. Therefore, the level of xanthan was also examined at 4, 5 and 6% for breads made with sorghum flour: rice flour (90:10) and with sorghum flour: potato starch (90:10). Control bread had no hydrocolloid. The formula used was 100% flour, 100% water, 6% sugar, 3% baking powder and 1.5% salt. The flour was either 100% sorghum flour or 90% sorghum flour plus 10% starch (rice flour , potato or tapioca starch) blend. From 90 to 160% water (fwb) were used with 1, 2 and 3% xanthan, HPMC and locust bean gum in preliminary work. The breads appeared to be under baked looked like uncooked with 130, 140, 150 and 160% water (30 min baking time). Although higher levels of hydrocolloids were supposed to hold more water was not a large difference between the breads with 90, 100, 110 and 120% water. Based on visual evaluation, initial water level (100%) was the optimum water level for this experiment. Optimum starch and hydrocolloid combination was chosen at 100% water.

Type and Level of Egg Ingredients

After selecting the optimum level and type of hydrocolloid for each 90:10 sorghum flour/starch blend, egg ingredients were added to the formula at 1, 3 and 5% (fwb). The egg ingredients used in the formulation were dry egg white and dry whole egg. Control bread had no egg ingredient. The formula used was 90% sorghum flour, 10% starch (rice, tapioca or potato), 100% water, 6% sugar, 3% baking powder, optimum hydrocolloid and 1.5% salt. The optimum

hydrocolloids were 3% HPMC with tapioca starch, 4% xanthan with potato starch and 3% xanthan with rice flour.

Type of Fat

Different types of fat were added to the formula at 3% (fwb). The fats used were canola oil, shortening and emulsified shortening which contained 35% hydrated distilled glycerol monostearate. Control bread had no fat. The formula used was 90% sorghum flour, 10% starch (rice, tapioca or potato), 100% water, 6% sugar, 3% baking powder, optimum hydrocolloid and 1.5% salt. The optimum hydrocolloids were 3% HPMC with tapioca starch, 4% xanthan with potato starch and 3% xanthan with rice flour.



Figure 4. Breads with different levels or different mixing method of shorthening (Left to right control (no shorthening), 3% (fwb) shorthening (batch method), 3% (fwb) shorthening, 6% (fwb) shorthening, 9% (fwb) shorthening and 3% (fwb) oil.

Level of Emulsified Shortening

Higher levels of emulsified shortening were used in the formulation because it has the potential to increase the shelf life, improve taste and texture. Emulsified shortening was further optimized by evaluation at 3, 5 and 8% (fwb). Control bread had no emulsified shortening. The formula used was 90% sorghum flour, 10% starch (rice flour, tapioca or potato starch), 100% water, 6% sugar, 3% baking powder, optimum hydrocolloid and 1.5% salt. The optimum

hydrocolloids were 3% HPMC with tapioca starch, 4% xanthan with potato starch and 3% xanthan with rice flour.

Level of Baking Powder

Baking powder levels of 3, 5, 8 and 16% (fwb) were examined. Control bread had no baking powder. The modified formula was 100% flour, 100% water, 6% sugar, optimum hydrocolloid, 1.5% salt and optimum level of GMS 540 emulsified shortening. The formula used was 90% sorghum flour, 10% starch (rice flour, tapioca starch or potato starch), 100% water, 6% sugar, 3% baking powder, optimum hydrocolloid, 1.5% salt and optimum GMS 540 emulsified shortening. The optimum hydrocolloids were 3% HPMC with tapioca starch, 4% xanthan with potato starch and 3% xanthan with rice flour. The optimum levels of GMS 540 emulsified shortening were 3% with tapioca and potato starch and 5% with rice flour.

Level of Water

The last step in optimizing the formula was to determine the optimum water absorption. Water level was adjusted to 110, 120, 130 and 140% (fwb). Control bread had 100% water. The formula used was 90% sorghum flour, 10% starch (rice flour, tapioca or potato starch), 100% water, 6% sugar, optimum baking powder, optimum hydrocolloid, 1.5% salt and optimum GMS 540 emulsified shortening. The optimum hydrocolloids were 3% HPMC with tapioca starch, 4% xanthan with potato starch and 3% xanthan with rice flour. The optimum levels of GMS 540 emulsified shortening were 3% with tapioca and potato starch and 5% with rice flour. The optimum levels of baking powder were 5% with tapioca and potato starch and 8% with rice flour. Water levels of 130 and 140% requires longer baking time.

SORGHUM BREADMAKING PROCEDURE

Dry ingredients were scaled into a container and mixed by hand with a spatula to make a uniform blend. First, the water was added to the stainless steel Hobart N-50 mixing bowl (Hobart Mfg., Troy, OH). In the experiment, different mixing techniques were tried. First, a large batch of fat and sugar was prepared. To provide better distribution, creaming stage was used during batter preparation in fat experiment with the solid shortening. First, the sugar and shortening were mixed at low speed for 1 minute, medium speed for 1 minute and high for 30 seconds. The mixture was scraped with a rubber spatula between each mixing step.

In another experiment, the solid shortening was melted and added directly to mixture of the water and dry ingredients (Figure 4.). Oil was added to the water. Then, the dry ingredients were added to mixture of water and oil. Since there is no big difference between the breads visually, the creaming stage was eliminated from the mixing procedure.

The final procedure was: mix fat and dry ingredients together by hand then add to mixing bowl containing water, mix with a flat beater paddle on speed 1 for 0.5 min. The batter was scraped down with a rubber spatula and mixed on speed 2 for 1.5 min. The batter (350 g) was weighed into greased metal pup loaf baking pans (14.3 x 7.9 cm top inside; 12.9 x 6.4 cm bottom outside; 5.7 cm inside depth) and baked in rotary baking oven (National Manufacturing Co., Lincoln, NE) at 204 °C for 30 min. Two beakers of water were placed in the over prior to and during baking to saturate the oven with steam to delay setting of the crust. Loaves were depanned immediately after removal from the oven and cooled for 2 hours on wire racks. After the breads cooled, they were bagged individually in polyethylene bags and stored at room temperature (24° C) overnight.

EVALUATION OF SORGHUM BREAD CHARACTERISTICS

A single 2.54 cm wide slice was cut along the long dimension from the center of each loaf using a slice regulator. Volume index was determined using a cake template as described in AACC International Approved Method 10-91.01(Figure 1). The bread slice was imaged using the C-Cell Imaging System (Calibre Control International Ltd., Appleton, Warrington, United Kingdom) to measure the crumb characteristics of number of cells, cell wall thickness and cell diameter. Each slice was analyzed for crumb firmness and elasticity using the TA. XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA/Stable Micro Systems, Godalming, Surrey, UK) with a modified version of AACCI Method 74-10.02. The slice was positioned with the cut edge facing up under a 2.54 cm diameter cylindrical probe and compressed 40% of the slice thickness at a speed of 1 mm/sec. The compression was held for 30 sec. Measurements were taken in the center of the slice. Firmness is the force in grams required for a 25% compression of the slice. Elasticity is the force recorded after holding the compression for 30 sec divided by the peak force at 40% compression and multiplied by 100 to convert to percentage. Higher values indicate that the crumb was more elastic and sprung back after the compression while lower values indicate the crumb was gummy and compacted during the compression.

One-way analysis of variance (ANOVA) was conducted using Minitab 17 Statistical Software (Minitab Inc., Pennsylvania, USA) with Fisher's least significant difference (LSD) testing at the p<0.05 level of significance. All tests were done in triplicate.

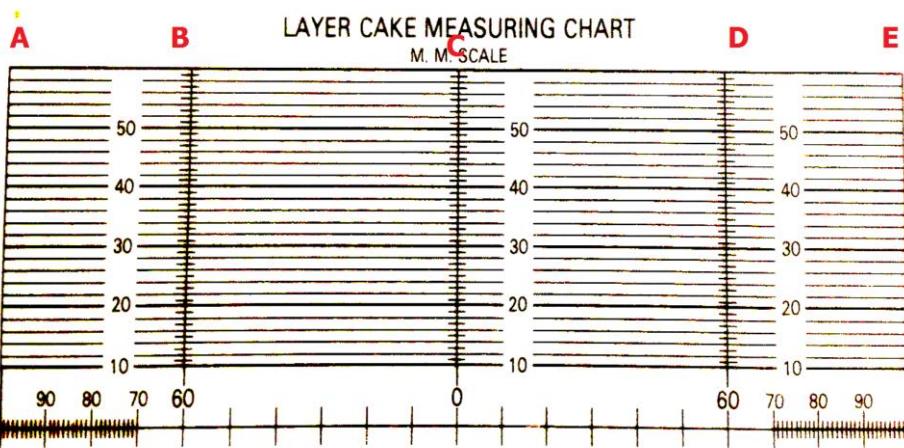


Figure 5. Layer cake measuring chart.

Chapter 4. Results & Discussion

Throughout this project, the “flour” in the formula was either 100% sorghum flour or a blend of sorghum flour and starch. The initial base formula for chemically leavened gluten free sorghum bread is given in Table 4.1.

Table 4.1. Initial chemically leavened gluten free formula

Ingredient	Level (% fwb)
Flour ^a	100
Sugar	6
Salt	1.5
Baking Powder	3
Water	100

^aFlour weight basis (fwb) where ‘‘flour’’ was a blend of sorghum flour and starch (potato, rice or tapioca) or 100 % sorghum flour.

STARCH

First, type and level of starch were examined. Rice flour, tapioca and potato starches were added at 10, 20 and 30% of the flour weight. Loaves made with 100% sorghum flour were baked as controls. Loaf volume index data is shown in Table 4.2. The volume index of loaves made with 100% sorghum flour was 168. Replacing 20 or 30% of the sorghum flour with rice flour did not cause a significant change in volume compared to the 100% sorghum flour loaves. While there was not a significant difference in loaf volume index between loaves containing all levels of rice flour, loaves containing 90% sorghum flour and 10% rice flour were significantly smaller than the 100% sorghum flour loaves. Sorghum flour and tapioca starch blends of 90:10, 80:20 and 70:30 did cause a significant reduction in loaf volume compared to the volume index of breads made with 100% sorghum flour and 70:30 and 80:20 sorghum flour and rice flour

blends. The smallest loaf volume index was obtained with 70% sorghum flour and 30% potato starch. Blends of 80:20 and 90:10 sorghum flour and potato starch produced loaves with similar volume as loaves made with all three levels of tapioca starch and 90:10 sorghum flour and rice flour.

It has been reported that dilution of the sorghum flour with higher levels of starch reduced the dietary fiber, protein and micronutrients in the bread and may also lead to a higher glycemic response. Additionally, the breads often have a short shelf life and excessively firm texture (Schober 2009). Therefore, a lower level of starch is more desirable for the gluten free bread formulation. Since there was not a significant difference between the volume index of bread made with each type of starch or the addition level, 90% sorghum flour and 10% starch was chosen as the best levels for the chemically leavened formula.

Table 4.2. Effect of sorghum: starch ratio and type of starch on chemically leavened sorghum bread

Sorghum (% fwb)	Starch (% fwb)	Starch Source	Volume Index ^a
100	0	n/a	168 a
70	30	Rice Flour	166 ab
80	20	Rice Flour	165 abc
90	10	Rice Flour	158 bcd
70	30	Tapioca Starch	150 de
80	20	Tapioca Starch	152 de
90	10	Tapioca Starch	150 de
70	30	Potato Starch	120 f
80	20	Potato Starch	149 e
90	10	Potato Starch	157 cde

^a Means in the same column with different letters are significantly different at p<0.05.

HYDROCOLLOIDS

Evaluation of Hydrocolloids in 100% Sorghum Flour Bread

The next step was to determine the effect of adding hydrocolloids. The base formula listed in Table 4.3 was used. The effect of xanthan, HPMC and locust bean gum were evaluated in a formula made with 100% sorghum flour. The initial levels of xanthan, HPMC and locust bean gum were 1, 2 and 3%.

Table 4.3. Base formula used to evaluate type and level of hydrocolloids.

Ingredient	Level (% fwb)
Sorghum Flour	100, 90
Starch ^a	0, 10
Hydrocolloid	Variable ^b
Sugar	6
Salt	1.5
Baking Powder	3
Water	100

^a Starch (rice, potato or tapioca starch).

^b Xanthan, HPMC and locust bean was added at 1, 2 or 3% fwb in formula with 100% sorghum flour and 90% sorghum flour and 10% tapioca starch; xanthan was added at 1, 2, 3, 4, 5 or 6% (fwb) in formulas with 90% sorghum flour: 10% rice blends and 90% sorghum flour: 10% potato starch while HPMC and locust bean gum were added at 1, 2 or 3% (fwb).

Xanthan

The volume index of the control loaves was 159 (Table 4.4). Adding 1, 2 or 3% xanthan gum to bread made with 100% sorghum flour significantly increased the volume index compared to the control. There was not a significant difference in volume index between loaves made with 1, 2 or 3% xanthan gum. All levels of xanthan gum (1, 2 and 3%) significantly increased the number of cells compared to the control. Cell wall thickness in the breads without any hydrocolloid was statistically the same as in the loaves with 1, 2 or 3 % xanthan. Cell diameters in loaves without any hydrocolloid was the same as the loaves with 1 and 2% xanthan but were larger than in the loaves made with 3% xanthan. The volume index and number of cells were increased by addition of xanthan but the thickness of cell walls was not affected. Only 3% xanthan reduced the diameter of the cells in the bread, thus creating a finer crumb grain. Overall

xanthan improved the bread with 3% selected as the best level for breads made with 100% sorghum flour.

HPMC

Loaves containing all levels of HPMC (1, 2 and 3%) had significantly higher volume than the control loaves (Table 4.4). Addition of 1% HPMC did not change the number of cells in loaves made with 100% sorghum flour; however, loaves containing 2 and 3% HPMC had significantly more cells than loaves made with 100% sorghum flour. Addition of HPMC at all levels did not significantly affect the cell wall thickness and cell diameter of loaves containing 100% sorghum flour. In conclusion, volume index was improved by addition of HPMC to the bread. At addition levels of 2 and 3%, HPMC increased the number of cells of the bread; however, the cell wall thickness or cell diameter of the bread was not improved by addition of 1, 2 and 3% HPMC. The best HPMC levels were 2 or 3% for breads made with 100% sorghum flour.

Locust Bean Gum

Loaves made with 2 and 3% locust bean gum had significantly higher volumes compared to the loaves with no hydrocolloids (Table 4.4). Loaves with no hydrocolloids and 1% locust bean gum had statistically the same volume. Loaves containing 1, 2 and 3 % locust bean gum had significantly higher number of cells than the loaves without hydrocolloids. All levels of locust bean gum did not significantly affect the cell wall thickness and cell diameter of loaves containing 100% sorghum flour. To sum up, addition of 2 or 3% locust bean gum improved the volume index of the bread but had no effect on crumb grain characteristics. The best locust bean levels were 2 or 3% for breads made with 100% sorghum flour.

Selection of Best Hydrocolloid for 100% Sorghum Flour Bread

In general, the addition of 1, 2 or 3% xanthan gum to the bread formula containing 100% sorghum flour significantly increased loaf volume index and the number of air cells but had no effect on cell wall thickness and cell diameter. HPMC increased loaf volume index but did not impact crumb grain. Locust bean gum increased both loaf volume index and number of cells but had no impact on cell wall thickness or cell diameter. Thus, while the addition of xanthan, HPMC or locust bean gum at levels up to 3% (fwb) did significantly increase loaf volume index, all measured aspects of crumb grain were not improved. Therefore, bread made with 100% sorghum flour was eliminated from further research.

Table 4.4. Volume and crumb grain characteristics of chemically leavened bread made with 100% sorghum flour and hydrocolloids^a

Hydrocolloid	Level (% fwb)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	159 c	3483 e	0.462 abc	3.77 a
Xanthan	1	176 ab	4154 bcd	0.463 abc	3.10 ab
Xanthan	2	181 ab	4571 ab	0.470 abc	3.18 ab
Xanthan	3	179 ab	4975 a	0.447 c	2.24 b
HPMC	1	173 b	3702 de	0.483 a	3.71 a
HPMC	2	183 a	4220 bc	0.467 abc	3.10 ab
HPMC	3	177 ab	4015 cd	0.480 ab	3.93 a
Locust Bean	1	159 c	4124 bcd	0.459 abc	3.26 ab
Locust Bean	2	176 ab	4178 bcd	0.456 abc	2.90 ab
Locust Bean	3	172 b	4128 bcd	0.456 abc	3.07 ab

^a Means in the same column with different letters are significantly different at p<0.05.

^b The control loaves contained no hydrocolloids.

Evaluation of Hydrocolloids in Sorghum Flour/Tapioca Starch Bread

The following experiment was done to evaluate the effect of hydrocolloids on the volume and crumb characteristics of breads made with 90% sorghum flour and 10% tapioca starch blends. The hydrocolloids evaluated were xanthan, HPMC and locust bean. The control loaves contained no hydrocolloids.

Xanthan

Adding 1, 2 or 3% xanthan significantly increased the volume index and number of cells of bread made with 90% sorghum flour and 10% tapioca starch compared to the control (Table 4.5). Compared to the cell wall thickness of the control, loaves made with 1 or 2% xanthan had

thinner cell walls while loaves with 3% xanthan were the same statistically as loaves with 1 or 2% xanthan and the control. Addition of 3% xanthan reduced the cell diameter of the breads compared to the control while addition of 1 or 2% had no effect on the cell diameter of the breads. The best levels of xanthan were 1 and 2% in terms of increasing the volume and number of cells of the breads.

HPMC

Addition of 1% HPMC to the breads made with sorghum flour and tapioca starch blends of 90:10 did not affect the volume index while the loaves made with 2 and 3% HPMC had a significantly higher volume index than the control (Table 4.5). Breads made with each level of HPMC had significantly more cells than the control. The highest number of cells was obtained with addition of 3% HPMC. The cell wall thickness of the breads containing all levels of HPMC were similar but significantly thinner than the control breads. Breads made with all levels of HPMC had cells with significantly smaller diameter than the control. The best level of HPMC was 3%.

Locust Bean Gum

Breads made with sorghum flour and tapioca starch blends of 90:10 with 3% locust bean gum had the same volume index as the control while those with 1 or 2% locust bean gum had significantly higher volume index than the control (Table 4.5). The number of cells were higher in breads made with all levels of locust bean gum compared to the control. Breads made with all levels of locust bean gum had the same cell wall thickness and cell diameter as the control. Bread with 1 or 2 % locust bean gum were the best level in terms of higher volume index compared to the other levels.

Selection of Best Hydrocolloid for Tapioca Starch

Overall, addition of 3% HPMC to bread made with the sorghum flour and tapioca starch (90:10) blends gave the best results. At that level, HPMC increased the number of cells and reduced the cell wall thickness and cell diameter. Although the HPMC-containing doughs were sticky and difficult to stir during mixing, the bread had a good structure and produced intact slices. Bread made with xanthan and locust bean gum were difficult to cut as they tended to crumble during slicing. Moreover, the surface of the breads made with HPMC had a better appearance with less cracking than bread made with xanthan and locust bean gum.

Table 4.5. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: tapioca starch blends of 90:10 and hydrocolloids^a

Hydrocolloid	Levels (% fwb)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	148 d	3501 d	0.457 ab	3.25 a
Xanthan	1	176 a	4261 bc	0.437 c	2.72 abcd
Xanthan	2	174 a	4137 c	0.438 c	2.79 abcd
Xanthan	3	160 bc	4279 bc	0.440 bc	2.47 de
HPMC	1	156 cd	4148 c	0.436 c	2.09 e
HPMC	2	177 a	4564 b	0.436 c	2.54 cde
HPMC	3	181 a	4944 a	0.430 c	2.56 bcde
Locust Bean	1	175 a	3931 c	0.467 a	3.17 ab
Locust Bean	2	171 ab	4112 c	0.460 a	3.01 abcd
Locust Bean	3	157 cd	4049 c	0.457 ab	3.15 abc

^a Means in the same column with different letters are significantly different at p<0.05.

^b The control loaves contained no hydrocolloids.

Evaluation of Hydrocolloids in Sorghum Flour/ Rice Flour Bread

The effect of hydrocolloids on the volume and crumb characteristics of breads made with 90% sorghum flour and 10% rice flour blends was determined. The hydrocolloids evaluated were xanthan, HPMC and locust bean. Xanthan showed the best improvement in breads made with blend of sorghum flour and rice flour(90:10). Thus, xanthan was also evaluated at 4, 5 and 6% for breads made with sorghum flour and rice flour(90:10). Desirable crumb characteristics are increased cell numbers with thin cell walls and small diameter. The control loaves contained no hydrocolloids.

Xanthan

The addition of xanthan at the initial levels of 1, 2 and 3% showed increasing improvement in the number of cells and cell wall thickness (Table 4.6). Therefore, the addition level was increased to 4, 5 and 6%. There was not a significant difference in loaf volume index between the loaves containing all levels of xanthan and the control. Breads with sorghum flour and rice flour blends with 1% xanthan had the same number of cells, cell wall thickness and cell diameter as the control. Addition of 2% xanthan significantly increased cell number but did not affect cell wall thickness or cell diameter compared to the control. However, breads with 3, 4, 5 or 6% xanthan had significantly more cells with significantly thinner cell walls and significantly smaller diameter than the control. Statistically, breads made with 3, 4 or 5% xanthan had the same number of cells but the breads made with 6% xanthan had the highest number of cells among the breads with all levels of xanthan. The cell wall thickness of the breads with 1 and 2 % xanthan was statistically the same as the control but they were thicker than in breads with 3, 4, 5 or 6% xanthan. The cell diameter of the breads with 1% xanthan were the same as the control while cell diameter in bread with 2, 3, 4, 5 and 6% xanthan were smaller than the control.

Although the breads made with 6% xanthan had better crumb characteristics (higher number of cells, thinner cell wall thickness and smaller cell diameter), they also had a gummy appearing crumb (data now shown) and were deemed unacceptable. The breads containing 3 or 4% xanthan had good crumb characteristics. No difference was observed between adding 3 or 4% xanthan. Since cost is an important criterion in choosing between two levels of an ingredient 3% xanthan was chosen as the best level for bread made with 90% sorghum flour and 10% rice flour breads.

HPMC

The volume index of the breads made with 1, 2 and 3% HPMC was statistically the same as the volume index of the control (Table 4.6). Breads with 1% HPMC had the same number of cells while those with 2 or 3% HPMC had significantly more cells than the control. The breads with 1% HPMC had thicker cell walls while those with 2 or 3% xanthan had the same cell wall thickness as the control. Addition of HPMC did not significantly affect cell diameter compared to the control. Loaves with 1% HPMC had significantly larger cell diameters than the higher levels of HPMC. During mixing, HPMC formed a sticky dough which was hard to stir. The surface of the sorghum flour and rice flour blends with 1, 2 and 3% HPMC had less cracking. The best level of HPMC for 90% sorghum flour: 10% rice flour was 3% HPMC based on the fact that it increased the number of cells.

Locust Bean Gum

The volume index of the breads made with 90% sorghum flour and 10% rice flour blend was not affected by the addition of 1, 2 and 3% locust bean gum (Table 4.6). All levels of locust bean gum in sorghum flour and rice flour blends had statistically the same cell wall thickness and cell diameter as the control. The best level of locust bean gum was 2 or 3 % for sorghum flour

and rice flour bread because it created more cells in the bread than the breads with 1 % locust bean gum.

Selection of Best Hydrocolloid for Rice Flour

The best hydrocolloid for use in chemically leavened sorghum bread was 3% xanthan. Addition of 3% xanthan increased the number of cells and reduced cell wall thickness and cell diameters in the bread.

Table 4.6. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: rice flourblends of 90:10 and hydrocolloids^a

Hydrocolloid	Level (fwb %)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	161 abc	3252 f	0.469 bcd	3.84 ab
Xanthan	1	170 ab	3527 ef	0.477 bc	3.78 abc
Xanthan	2	169 ab	4423 c	0.451 d	2.69 cd
Xanthan	3	178 a	5704 b	0.419 e	1.86 de
Xanthan	4	164 abc	5598 b	0.419 e	1.9 de
Xanthan	5	157 bc	5633 b	0.421 e	1.9 de
Xanthan	6	155 bc	6800 a	0.394 f	1.55 e
HPMC	1	148 c	3376 ef	0.498 a	4.65 a
HPMC	2	162 abc	3744 de	0.480 abc	3.53 bc
HPMC	3	164 abc	4096 cd	0.461 cd	3.04 bc
Locust Bean	1	146 c	3440 ef	0.470 bcd	3.82 ab
Locust Bean	2	154 bc	4006 bcd	0.483 ab	3.59 abc
Locust Bean	3	164 abc	3810 cde	0.469 bcd	3.47 bc

^aMeans in the same column with different letters are significantly different at p<0.05.

^bThe control loaves contained no hydrocolloids.

Evaluation of Hydrocolloids in Sorghum Flour/Potato Starch Bread

The effect of hydrocolloids on the volume and crumb characteristics of breads made with 90% sorghum flour and 10% potato starch blends was also determined. Xanthan showed the best improvement in breads made with sorghum flour and potato starch (90:10). Thus, xanthan was also evaluated at 4, 5 and 6% for breads made with sorghum flour and potato starch (90:10). The control loaves contained no hydrocolloids.

Xanthan

The addition of xanthan at the initial levels of 1, 2 and 3% showed increasing improvement in the volume index, number of cells and cell diameter (Table 4.7). Therefore, the addition level was increased to 4, 5 and 6%. All levels of xanthan significantly increased volume index and number of cells compared to the control. Except for breads made with 6% xanthan, the wall thickness of the breads made with 1, 2, 3, 4 and 5% xanthan had statistically the same as the control. The cell wall thickness of the breads made with 6% xanthan were lower than the control. Addition of 2% and higher xanthan significantly decreased cell diameter. The highest volume index was reached with 4% xanthan. Loaves with 6% xanthan had the largest number of cells, thinnest cells and smallest cell diameter. The best level of xanthan was selected as 4% based on the volume index.

HPMC

Breads made with 1% HPMC had the same volume index as the control; however, higher levels of HPMC significantly increased volume index of the breads (Table 4.7). Addition of all levels of HPMC had the same number of cells as the control but those cells were thicker than the control bread. The cell diameter of the breads made with 3% HPMC were the same as the control while the cell diameter of the breads made with 1 or 2% HPMC were larger than the control. Overall addition of HPMC increased loaf volume index but had a negative impact on crumb grain characteristics.

Locust Bean Gum

Breads made with all levels of locust bean gum had statistically higher volume index than the control (Table 4.7). Breads with 1% locust bean gum had lower number of cells than the control while breads with 2 or 3% locust bean gum had the same number of cells as the control.

While breads made with 2 or 3% locust bean gum had statistically the same size of cells as the control, breads made with 1% locust bean gum had bigger cell diameters. The cell diameter of the breads with 1% locust bean gum bigger than the control while the cell diameter of breads with 2 or 3% were statistically the same as the control. Different levels (1, 2 or 3%) increased the volume index of the breads but the levels of locust bean gum did not improve the crumb characteristics (number of cells, cell wall thickness and cell diameter).

Selection of Best Hydrocolloid for Potato Starch

The bread made with sorghum flour and potato starch blends (90:10) and 4% xanthan gave the best results. Xanthan increased the number of cells, reduced the cell wall thickness and cell diameter.

Table 4.7. Volume and crumb grain characteristics of bread made with sorghum flour: potato starch blends of 90:10 and hydrocolloids ^a

Hydrocolloid	Level (fwb %)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	145 f	3887 e	0.427 c	2.87 cd
Xanthan	1	158 de	4514 d	0.440 c	2.6 de
Xanthan	2	178 b	5191 c	0.431 c	2.1 e
Xanthan	3	177 b	5601 bc	0.433 c	2.0 e
Xanthan	4	193 a	6122 b	0.429 c	1.9 e
Xanthan	5	178 b	5900 b	0.429 c	2.14 e
Xanthan	6	175 b	7337 a	0.362 d	1.19 f
HPMC	1	151 ef	3713 ef	0.472 ab	3.81 ab
HPMC	2	173 bc	3803 ef	0.482 a	3.89 ab
HPMC	3	174 bc	3940 de	0.469 ab	3.39 bc
Locust Bean	1	165 cd	3256 f	0.481 a	4.09 a
Locust Bean	2	165 cd	3992 de	0.451 bc	2.92 cd
Locust Bean	3	165 cd	3784 ef	0.452 bc	2.92 cd

^a Means in the same column with different letters are significantly different at a p<0.05

^b The control loaves contained no hydrocolloids.

EGG INGREDIENTS

After determining the best level and hydrocolloid for different types of starch and sorghum flour combinations, the next step was evaluating the effects of egg ingredients (egg white and whole egg) on the volume index and crumb characteristics (number of cells, cell wall

thickness and cell diameter) of the breads made with optimum level of hydrocolloid and sorghum flour and starch (rice flour, tapioca and potato starch) combinations.

Evaluation of Egg Ingredients in Sorghum Flour/Tapioca Starch Bread

The base formula for breads with sorghum flour and tapioca starch blend of 90:10 is given in Table 4.8. The control loaves contained no egg ingredients.

Table 4.8. Base formula used to evaluate type and level of egg ingredients in chemically leavened bread made with 90% sorghum flour and 10% tapioca starch.

Ingredient	Level (fwb %)
Sorghum Flour	90
Tapioca Starch	10
Sugar	6
HPMC	3
Baking Powder	3
Salt	1.5
Egg Ingredient	Variable ^a
Water	100

^a Egg white powder or whole egg powder were added at 1, 3 or 5% (fwb). \

Breads with sorghum flour and tapioca starch blend with 1 or 5% egg white had lower volume index than the control (Table 4.9). However, breads with 3% egg white had statistically the same volume index as the control. Adding 1, 2 and 3% egg white did not affect the number of cells, cell wall thickness and cell diameter. Therefore, addition of egg white had no effect on bread made with sorghum flour and tapioca starch blend.

The volume index of the breads made with 5% whole egg were higher than the control.

Nevertheless, 1 and 3% whole egg did not improve the volume index of the breads (Table 4.9).

Breads with 1% whole egg had significantly lower number of cells than the control while the breads with 3 or 5% whole egg had statistically the same number of cells as the control. The cell wall thickness of the breads was increased by adding 1 and 5% whole egg. Adding 3% whole egg to the breads did not change the cell wall thickness. Moreover, the cell diameter of the breads with 1 or 3% whole egg was statistically the same as the control but 5% whole egg increased the cell diameter of breads compared to the control. Addition of whole egg did not have an improving effect on bread made with sorghum flour and tapioca starch.

Selection of Best Egg Ingredient for Tapioca Starch

Overall, neither whole egg nor egg white increased volume index or number of cells, decreased the cell wall thickness or cell diameter in sorghum flour and tapioca starch bread. Therefore, egg white and whole egg were eliminated from the formulation of sorghum flour and tapioca starch bread.

Table 4.9. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and egg ingredients ^a

Treatment	Level (fwb%)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	181 b	4944 ab	0.430 cd	2.09 bc
Egg White	1	161 d	4998 ab	0.436 bcd	2.27 abc
Egg White	3	175 bc	4337 bc	0.453 abc	2.74 abc
Egg White	5	171 c	5199 a	0.416 d	1.97 c
Whole Egg	1	171 c	4087 c	0.469 a	2.85 ab
Whole Egg	3	174 bc	4349 bc	0.458 abc	2.68 abc
Whole Egg	5	194 a	4745 abc	0.461 ab	2.97 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no egg ingredients.

Evaluation of Egg Ingredients in Sorghum Flour/Rice Flour Bread

The base formula for breads with sorghum flour and rice flour blend of 90:10 is given in Table 4.10. The control loaves contained no egg ingredients.

Table 4.10. Base formula used to evaluate type and level of egg ingredients in chemically leavened bread made with 90% sorghum flour and 10% rice flour.

Ingredients	Levels (fwb %)
Sorghum Flour	90
Rice flour	10
Sugar	6
Xanthan	3
Baking Powder	3
Salt	1.5
Egg Ingredient	Variable ^a
Water	100

^a Egg white powder or whole egg powder were added at 1, 3 or 5% (fwb).

The levels of egg white (1, 3 or 5%) did not significantly affect the volume index of the breads (Table 4.11). The breads with 1 or 5% egg white did not significantly change the number of cells. However, adding 3% egg white reduced the number of cells in the bread. All levels of egg white increased the cell wall thickness and cell diameter of the bread. Overall, addition of egg white had a negative impact on the bread.

All levels of whole egg did not affect the volume index and the number of cells of the bread (Table 4.11). While adding 1% whole egg increased the cell wall thickness and cell diameter of the bread, the other levels of whole egg (3 or 5%) had no effect on the cell wall thickness and cell diameter of the bread compared to the control. Overall, whole egg did not improve the bread.

Selection of Best Egg Ingredient for Rice Flour

Although addition of various levels of whole egg or white egg improved one or two quality criteria of the chemically leavened sorghum flour and rice flour bread, none of them improve the volume index and crumb characteristics of the breads at the same addition level. Moreover, egg ingredients are allergenic. Therefore, the egg ingredients were not added the formulation of the chemically leavened sorghum flour and rice flour bread.

Table 4.11. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: rice flour (90-10%) and egg ingredients ^a

Treatment	Level (fwb %)	Volume Index (mm)	Numbers of Cell	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	178 ab	5704 ab	0.419 b	1.86 d
Egg White	1	165 b	4914 bc	0.454 a	2.47 abc
Egg White	3	179 ab	4683 c	0.458 a	2.72 a
Egg White	5	183 a	5236 abc	0.465 a	2.59 ab
Whole Egg	1	186 a	4990 abc	0.463 a	2.54 ab
Whole Egg	3	187 a	5399 abc	0.438 ab	2.07 bcd
Whole Egg	5	190 a	5827 a	0.422 b	1.86 cd

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no egg ingredients.

Evaluation of Egg Ingredients in Sorghum Flour/Potato Starch Bread

The base formula for breads with sorghum flour and potato starch blend of 90:10 is given in Table 4.12. The control loaves contained no egg ingredients. Although 4% xanthan was determined as the best hydrocolloid for the breads made with sorghum flour and potato starch in the hydrocolloid experiment, it had negative effects on the breads when egg ingredients were

added to the formulation. After breads were cool, they shrank and the edges of the breads curled. Reducing the xanthan level to 2% xanthan in the formulation of sorghum flour and potato starch bread resulted in satisfactory breads.

Table 4.12. Base formula used to evaluate type and level of egg ingredients in chemically leavened bread made with 90% sorghum flour and 10% potato starch.

Ingredient	Levels (fwb%)
Sorghum Flour	90
Potato Starch	10
Sugar	6
Xanthan ^b	2
Baking Powder	3
Salt	1.5
Egg	Variable ^a
Water	100

^a Egg white powder or whole egg powder were added at 1, 3 or 5% (fwb).

^b The best level was 4% except in this experiment.

Breads made with 3 or 5% egg white had higher volume index than the control while breads with 1% egg white had statistically the same volume index as the control (Table 4.12). The number of cells in loaves made with 1, 3 or 5% egg white were the same as the control. Addition of 1 or 5% egg white did not affect the cell wall thickness and cell diameter of sorghum flour and potato starch breads. However, loaves made with 3% egg white had thicker cell walls and bigger cell diameters than the control while breads made with 1 or 5% egg white had statistically the same size cell diameters as the control. Egg white did not have a positive effect on breads with sorghum flour: potato starch blend.

Loaves with 1 or 3 % whole egg had no effect on the volume index or the number of cells of the bread baked with sorghum flour and potato starch blends of 90:10 ; however those with 5% had higher number of cells than control (Table 4.13). Loaves made with 1, 3 or 5% whole egg had the same cell wall thickness as the control. Breads made with 1% whole egg had bigger cell diameters than the control. However, loaves made with 3 or 5% whole egg had statistically the same size cell diameters. Whole egg did not improve the breads baked with sorghum flour and potato starch blends.

Selection of Best Egg Ingredient for Potato Starch

Addition of whole egg or egg white did not improve bread quality in terms of increased volume and number of cells, thinner cell walls and smaller cell diameters. Therefore, egg ingredients were eliminated from the formulation of sorghum flour and potato starch breads.

Table 4.13. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: potato starch (90-10%) and egg ingredients ^a

Treatment	Level (fwb %)	Volume (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	0	178 b	5191 bc	0.431 bcd	2.12 cd
Egg White	1	183 b	5108 bc	0.446 abc	2.36 abcd
Egg White	3	203 a	5292 abc	0.455 a	2.55 a
Egg White	5	203 a	5664 ab	0.446 abc	2.45 abc
Whole egg	1	168 b	4974 c	0.450 ab	2.51 ab
Whole Egg	3	180 b	5534 abc	0.430 cd	2.18 bcd
Whole Egg	5	184 b	5890 a	0.423 d	2.02 d

^a Means in the same column with different letters are significantly different at a p<0.05

^b The control loaves contained no egg ingredients.

Bize (2012) evaluated three different levels of fresh egg. The levels of the fresh were 20%, 25% and 30%. It was found that eggs increased the specific volume of the yeasted sorghum bread and also improved the crumb characteristics. The data of the present study showed that 5% dried whole egg increased the volume of the bread made with the chemically leavened breads made with 90% sorghum flour and 10% tapioca starch but the same level did not improve the crumb characteristics. Moreover, chemically leavened sorghum flour and rice flourbreads with any level of the dried egg ingredient did not have higher volume index or better crumb characteristics than the breads without any dried egg ingredients. Furthermore, sorghum flour and potato starch breads with 3 or 5 % dried egg white had higher volume index but these levels did not improve the crumb characteristics.

FAT

Evaluation of Fat Type in Sorghum Flour/ Tapioca Starch Bread

Shortening, oil and emulsified shortening are known to give bread a soft texture, and to improve the crumb characteristics and the palatability of breads. The following experiments were done to evaluate the effect of 3% shortening, oil or emulsified shortening in breads made with 90% sorghum flour and 10% starch (rice flour, tapioca and potato starch). The first evaluation of the fat source was done for the breads with sorghum flour and tapioca starch blends of 90:10. The control loaves contained no shortening, oil or emulsified shortening. The base formula is listed in Table 4.14.

Table 4.14. Base formula used to evaluate the types of fat in chemically leavened tapioca starch: sorghum flour bread

Ingredient	Level (fwb%)
Sorghum Flour	90
Tapioca Starch	10
Sugar	6
HPMC	3
Baking Powder	3
Salt	1.5
Fat ^a	3
Water	100

^a Shortening, oil or emulsified shortening.

There was not a difference in volume index or the crumb characteristics (the number of cells, cell wall thickness and cell diameter) between breads with emulsified shortening and the control (Table 4.15). Compared to the control, the volume index of the breads with oil or shortening were significantly lower. The breads with oil or shortening had statistically the same number of cells. However, the cell wall thickness and cell diameter of the breads with oil or shortening were higher than the control. In summary, shortening and oil had a negative impact on the bread while addition of emulsified shortening had no effect.

Table 4.15. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and fat^a

Ingredient Type	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	202 a	4944 ab	0.430 b	2.09 c
Emulsified Shortening	193 a	5345 a	0.450 ab	2.46 bc
Shortening	162 b	4320 b	0.455 a	2.94 a
Oil	153 b	4650 ab	0.460 a	2.72 ab

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no fat.

Evaluation of Fat Type in Sorghum Flour/ Rice Flour Bread

The second experiment was done to evaluate the impact of 3% shortening, oil or emulsified shortening on the volume index and crumb characteristics of the breads made with sorghum flour and rice flour blends of 90:10. The control loaves contained no shortening, oil or emulsified shortening. Table 4.16 lists the base formula.

Table 4.16. Base formula used to evaluate the types of fat in chemically leavened rice flour: sorghum flour bread

Ingredients	Level (fwb%)
Sorghum Flour	90
Rice Flour	10
Sugar	6
Xanthan	3
Baking Powder	3
Salt	1.5
Fat ^a	3
Water	100

^a Shortening, oil or emulsified shortening.

The volume index, cell wall thickness and cell diameter of the breads with emulsified shortening were the same as the control (Table 4.17). However, the breads made with emulsified shortening had significantly more cells than the control. Compared to the control, the volume index of the breads with oil or shortening were significantly lower. Addition of shortening or oil did not affect the crumb characteristics (the number of cells, cell wall thickness or cell diameter) of the breads. The best type of fat was emulsified shortening for the breads made with 90% sorghum flour and 10% rice flour.

Table 4.17. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: rice flour(90-10%) and fat^a

Ingredient	Volume Index (mm)	Numbers of Cell	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	197 a	5704 b	0.419 a	1.86 ab
Emulsified Shortening	196 a	6648 a	0.417 a	1.66 b
Shortening	166 b	5338 b	0.426 a	1.98 ab
Oil	170 b	5385 b	0.431 a	2.03 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no fat.

Evaluation of Fat Type in Sorghum Flour/ Potato Starch Bread

The third experiment evaluated the impact of 3% shortening, oil or emulsified shortening on the volume index and crumb characteristics of the breads made with sorghum flour and potato starch blends of 90:10. The control loaves contained no shortening, oil or emulsified shortening. The base formula is given in Table 4.18.

Table 4.18. Base formula used to evaluate the types of fat in potato starch: sorghum flour bread

Ingredients	Level (fwb%)
Sorghum Flour	90
Potato Starch	10
Sugar	6
Xanthan	4
Baking Powder	3
Salt	1.5
Fat ^a	3
Water	100

^a Shortening, oil or emulsified shortening.

There was no difference in volume index and the crumb characteristics (the number of cells, cell wall thickness or cell diameter) of the breads made with emulsified shortening and the control (Table 4.19). The breads with shortening had a lower volume index than the control but the breads with shortening had statistically the same number of cells, cell wall thickness and cell diameter as the control. Moreover, the breads with oil had statistically the same volume index, number of cells, cell wall thickness and cell diameter as the control. None of the fat sources was specified as the best fat for the breads made with 90% sorghum flour and 10% potato starch.

Table 4.19. Volume and crumb grain characteristics of bread made with sorghum flour: potato starch (90-10%) and fat^a

Ingredient	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	193 a	6122 ab	0.429 a	1.98 a
Emulsified Shortening	201 a	7136 a	0.420 a	1.85 a
Shortening	168 b	5819 b	0.423 a	2.19 a
Oil	190 a	5978 b	0.426 a	1.87 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no fat.

Selection of Best Fat for Tapioca Starch, Rice Flour and Potato Starch

While oil reduced the volume index of sorghum flour and tapioca starch bread; oil in sorghum flour and potato starch bread did not change the volume index of the bread. Adding shortening decreased the volume index of all three types of bread. The breads with emulsified shortening had statistically the same volume index as the control. However, emulsified shortening increased the number of cells in sorghum flour and rice flour bread. Although emulsified shortening did not improve the volume index and crumb characteristics of the sorghum flour and tapioca starch breads or sorghum flour and potato starch breads, it was not eliminated from the formulation because emulsified shortening provides taste aspects and possibly texture improvement to the gluten free bread.

EMULSIFIED SHORTENING

After choosing emulsified shortening as the best fat type for bread made with sorghum flour and starch, different levels (3, 5 and 8%) of emulsified shortening were added to the modified formula to determine the optimum addition level for breads made with sorghum flour and starch(rice flour, tapioca and potato starch). Hart et al (1970) researched the effect of shortening addition in yeast based gluten free sorghum bread and found that shortening was beneficial for crumb softness. Therefore, crumb softness and elasticity measurements were also measured in order to determine the best level of emulsified shortening.

Evaluation of Emulsified Shortening in Sorghum Flour/ Tapioca Starch Bread

First, the breads made with sorghum flour and tapioca starch were evaluated. The control loaves contained no emulsified shortening with sorghum flour and tapioca starch blends of 90:10. The levels of emulsified shortening used in the experiment were 3, 5 and 8% (Table 4.20).

Table 4.20. Base formula used to evaluate the different levels of emulsified shortening in chemically leavened sorghum flour: tapioca starch bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Tapioca Starch	10
Sugar	6
HPMC	3
Baking Powder	3
Salt	1.5
Emulsified Shortening	Variable ^a
Water	100

^a Emulsified Shortening was added at 3, 5 or 8%.

Increasing the levels of emulsified shortening to 5 and 8% did not change the volume index or crumb characteristics of the breads made with sorghum flour and tapioca starch compared to the initial 3% addition level (Table 4.21). However, the sorghum flour and tapioca starch bread with all three levels of emulsified shortening had a higher volume index compared to the control.

Table 4.21. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and emulsified shortening ^a

Level (fwb %)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	181 b	4944 a	0.430 a	2.09 a
3	193 a	5345 a	0.450 a	2.46 a
5	193 a	6148 a	0.429 a	2.12 a
8	199 a	6738 a	0.421 a	2.02 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no emulsified shortening.

Significant differences in firmness between the breads made with sorghum flour and tapioca starch with 0, 3 and 5% emulsified shortening were observed (Table 4.22). The firmness of breads made with sorghum flour and tapioca starch with 5 and 8% were not significant. Moreover, emulsified shortening reduced the elasticity of chemically leavened bread compared to the control; nevertheless, no significant difference between the elasticity of the breads made with sorghum flour and tapioca starch bread with 3, 5 and 8% was observed. In conclusion, breads with 3, 5 or 8% emulsified shortening were softer and less elastic than the control. Although the breads with 5 or 8% emulsified shortening were softer than the breads made with 3% emulsified shortening, the 5 or 8 % emulsified shortening did not distribute in the slices well. Lumps of emulsified shortening was seen in the slices of the breads made with 5 or 8% emulsified shortening. The best level of emulsified shortening was 3% for the bread made with sorghum flour and tapioca starch.

Table 4.22. The firmness and elasticity of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and emulsified shortening^a

Level (fwb %)	Firmness (g)	Elasticity (%)
Control ^b	2346 a	54 a
3	1983 b	43 b
5	1556 c	47 b
8	1355 c	47 b

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no emulsified shortening.

Evaluation of Emulsified Shortening in Sorghum Flour/ Rice Flour Bread

The following evaluation was done to observe the effects of emulsified shortening on the breads made with sorghum flour and rice flour blends of 90:10. The control loaves contained no emulsified shortening. The levels of emulsified shortening used in the experiment were 3, 5 and 8% (Table 4.23).

Table 4.23. Base formula used to evaluate the different levels of emulsified shortening in chemically leavened sorghum flour: rice flour bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Rice Flour	10
Sugar	6
Xanthan	3
Baking Powder	3
Salt	1.5
Emulsified Shortening	Variable ^a
Water	100

^a Emulsified Shortening was added at 3 ,5 or 8%.

The breads made with sorghum flour and rice flour with 3 or 5% emulsified shortening had higher volume index compared to the control (Table 4.24). However, the breads made with sorghum flour and rice flour with 8% emulsified shortening had the same volume index as the control. The breads made with 5% emulsified shortening had more cells with thinner cell wall thickness and smaller cell diameter than the control. However, the breads made with sorghum flour and rice flour with 3 or 8% emulsified shortening had statistically the same number of cells, cell wall thickness and cell diameter as the control.

Table 4.24. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: rice flour(90-10%) and emulsified shortening ^a

Shortening Level (fwb %)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	185 b	5704 b	0.419 a	1.86 a
3	196 a	6648 b	0.417 a	1.67 a
5	196 a	8065 a	0.388 b	1.23 b
8	178 b	6978 ab	0.415 ab	1.62 ab

^aMeans in the same column with different letters are significantly different at a p<0.05.

^bThe control loaves contained no emulsified shortening.

No significant difference in firmness of the breads made with sorghum flour and rice flour with 3, 5 or 8% emulsified shortening was observed (Table 4.25). Nevertheless, the elasticity of the breads made with sorghum flour and rice flour with 5 or 8% emulsified shortening were higher than the control. The elasticity of the breads with 3% emulsified shortening was the same as the control. The level of emulsified shortening selected for bread made with sorghum flour and rice flour was 5%.

Table 4.25. The firmness and elasticity of chemically leavened bread made with sorghum flour: rice flour(90-10%) and emulsified shortening ^a

Shortening Level (fwb%)	Firmness (g)	Elasticity (%)
Control ^a	1914 a	64 b
3	1754 a	64 b
5	1969 a	69 a
8	1921 a	67 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no emulsified shortening.

Evaluation of Emulsified Shortening in Sorghum Flour/ Potato Starch Bread

Next, an evaluation was done to observe the effects of emulsified shortening on the breads made with sorghum flour and potato starch. The control loaves contained no emulsified shortening with sorghum flour and potato starch blends of 90:10. The levels of emulsified shortening used in the experiment are 3, 5 and 8% (Table 4.26).

Table 4.26. Base formula used to evaluate the different levels of emulsified shortening in chemically leavened sorghum flour: potato starch bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Potato Starch	10
Sugar	6
Xanthan	4
Baking Powder	3
Salt	1.5
Emulsified Shortening	Variable ^a
Water	100

^aEmulsified Shortening was added at 3, 5 or 8.

Addition of 3, 5 or 8% emulsified shortening did not change the volume index of sorghum flour and potato starch breads (Table 4.27). The addition of 8% emulsified shortening to the breads increased the number of cells, but reduced cell wall thickness and cell diameter. Breads made with 3 or 5% emulsified shortening had statistically the same volume index, number of cells, cell wall thickness and cell diameter as the control.

Table 4.27. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: potato starch (90-10%) and emulsified shortening ^a

Shortening Level (fwb%)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	193 a	6122 b	0.428 a	1.98 a
3	201 a	7136 b	0.420 a	1.85 a
5	200 a	7445 b	0.498 ab	1.48 ab
8	193 a	8748 a	0.377 b	1.23 b

^aMeans in the same column with different letters are significantly different at a p<0.05.

^bThe control loaves contained no emulsified shortening.

The firmness of the breads with sorghum flour and potato starch with 3, 5 or 8% emulsified shortening were the same as the control (Table 4.28). The elasticity of the breads with 3% emulsified shortening were the same as the control. The elasticity of the breads with 5 or 8% emulsified shortening was lower than the control. Although the breads with 8% emulsified shortening had higher volume, thinner cell wall and smaller cell diameter, the crumb of the breads was gummy. Therefore, the level of 3% emulsified shortening was selected for bread made with sorghum flour and potato starch.

Table 4.28. The firmness and elasticity of chemically leavened bread made with sorghum flour: potato starch (90-10%) and emulsified shortening ^a

Shortening Level (fwb%)	Firmness (g)	Elasticity (%)
Control ^b	1760 a	69 a
3	1472 a	68 ab
5	1868 a	66 bc
8	1790 a	65 c

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no emulsified shortening.

Selection of Best Level of Emulsified Shortening for Tapioca Starch, Rice Flour and Potato Starch

Hart et al (1970) found that shortening was not beneficial in retaining gas in sorghum bread. This study found that volume index of the breads made with sorghum flour and tapioca starch and the breads made with sorghum flour and rice flour increased. Nevertheless, crumb characteristics of the breads made with sorghum flour and tapioca starch were not changed by adding levels 3, 5 or 8% emulsified shortening. Emulsified shortening was also found to reduce firmness of the breads made with sorghum flour and tapioca starch. The breads made with sorghum flour and rice flour with 5% emulsified shortening had better crumb characteristics (more number of cells, less cell wall thickness and smaller cell diameter).

BAKING POWDER

The breads made with 90% sorghum flour and 10% starch (rice flour, tapioca and potato starch) were very compacted. When breads were cut, the crumb was dense and tight. Breads were heavy. The cells of slices did not look open. The purpose of increasing the level of baking powder in the breads was also to obtain a bread with higher volume index and better crumb characteristics (more cells, thinner cell walls and smaller cell diameter).

Evaluation of Baking Powder in Sorghum Flour/ Tapioca Starch Bread

The first evaluation of the different levels of baking powder was done for the breads with sorghum flour and tapioca starch blends of 90:10. The control loaves contained no baking powder (Table 4.29). The levels of baking powder used in the breads were 3, 5, 8 or 16%.

Table 4.29. Base formula used to evaluate the different levels of baking powder in chemically leavened sorghum flour: tapioca starch bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Tapioca Starch	10
Sugar	6
HPMC	3
Baking Powder	Variable ^a
Salt	1.5
Emulsified Shortening	3
Water	100

^a Baking powder were added at 0, 3, 5, 8, 16 %.

The breads made with sorghum flour and tapioca starch blends of 90:10 and 3, 5, 8 or 16% baking powder had a higher volume index than the control (Table 4.30). However, the volume index of the breads made with 3% baking powder were lower than the volume index of the breads with 5, 8 or 16% baking powder. The breads with 5, 8 or 16% baking powder had more cells than the control but the breads made with 3% baking powder had fewer cells than the control. The cell walls of the breads made with 3, 5, 8 or 16% baking powder were thicker than the control. The cell diameter of the breads made with sorghum flour and tapioca starch with 3, 5, 8 or 16% baking powder were larger than the control. The breads with 5 and 16 % baking powder had smaller cell diameter than the breads with 3% baking powder.

Table 4.30. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and baking powder ^a

Baking Powder Level (fwb %)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	123 c	4855 b	0.362 c	1.42 c
3	175 b	4458 c	0.454 a	2.81 a
5	223 a	6364 a	0.439 ab	2.29 b
8	224 a	6104 a	0.446 ab	2.55 ab
16	220 a	6118 a	0.434 b	2.37 b

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no baking powder.

The firmness of the breads made with 3, 5, 8 or 16 % baking powder was lower than the control (Table 4.31). The breads made with 5, 8 or 16 % baking powder were softer than the breads made with 3% baking powder. The elasticity of the breads made with sorghum flour and tapioca starch with 3, 5, 8 or 16 % baking powder were higher than the control. The breads made with sorghum flour and tapioca starch with 3% baking powder were less elastic than the breads made with sorghum flour and tapioca starch with 16% baking powder but more elastic than the breads made with 5% baking powder. The best level of baking powder was 5% for sorghum flour and tapioca starch bread.

Table 4.31. The firmness and elasticity of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and baking powder ^a

Baking Powder Level (fwb %)	Firmness (g)	Elasticity (%)
Control ^b	5619 a	35 d
3	1992 b	54 b
5	1131 c	49 c
8	1218 c	52 bc
16	1311 c	59 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no baking powder.

Evaluation of Baking Powder in Sorghum Flour/ Rice Flour Bread

The second evaluation of the different levels of baking powder was in breads with sorghum flour and rice flour blends of 90:10. The control loaves contained no baking powder (Table 4.32). The volume index of the breads increased by adding 3, 5, 8 or 16% baking powder. The highest volume index belonged to the breads with 8% baking powder (Table 4.33).

Table 4.32. Base formula used to evaluate the different levels of baking powder in chemically leavened sorghum flour: rice flour bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Rice Flour	10
Sugar	6
Xanthan	3
Baking Powder	Variable ^a
Salt	1.5
Emulsified Shortening	5
Water	100

^a Baking powder was added at 0, 3, 5, 8, 16%.

The number of cells in the breads with 3% baking powder was lower than the control.

Addition of 5, 8 or 16% baking powder did not change the number of cells in the breads.

However, addition of 3, 5, 8 or 16% baking powder to the breads increased the cell wall thickness and cell diameters.

Table 4.33. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: rice flour(90-10%) and baking powder ^a

Baking Powder Level (fwb %)	Volume Index (mm)	Number of cells	Wall thickness (mm)	Cell diameter (mm)
Control ^b	153 d	6053 ab	0.367 b	1.44 b
3	182 c	5450 c	0.432 a	2.01 a
5	204 b	6334 a	0.438 a	1.99 a
8	216 a	6085 a	0.444 a	2.31 a
16	204 b	5489 bc	0.445 a	2.41 a

^aMeans in the same column with different letters are significantly different at a p<0.05.

^bThe control loaves contained no baking powder.

The firmness of the breads made with sorghum flour and rice flour blends of 90:10 with 3, 5, 8 or 16% baking powder were lower than the control (Table 4.34). Breads made with 5, 8 or 16% baking powder were softer than the breads made with 3% baking powder. The elasticity of the breads made with sorghum flour and rice flour with 8 or 16% baking powder were the same as the control while breads made with 3 or 5% baking powder were more elastic than the control. The best level of baking powder was 8% for sorghum flour and rice flour breads because they had higher volume index than the breads with 3, 5 or 16% baking powder.

Table 4.34. The firmness and elasticity of chemically leavened bread made with sorghum flour: rice flour(90-10%) and baking powder ^a

Level (fwb %)	Firmness (g)	Elasticity (%)
Control ^b	4501 a	63 c
3	1952 b	69 a
5	1221 c	67 ab
8	1051 c	65 bc
16	935 c	64 c

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no baking powder.

Evaluation of Baking Powder in Sorghum Flour/ Potato Starch Bread

The third evaluation of the different levels of baking powder was done for the breads with sorghum flour and potato starch blends of 90:10. The control loaves contained no baking powder (Table 4.35).

Table 4.35. Base formula used to evaluate the different levels of baking powder in chemically leavened sorghum flour: potato starch bread formula ^a

Ingredients	Level (fwb%)
Sorghum Flour	90
Potato Starch	10
Sugar	6
Xanthan	4
Baking Powder	Variable ^a
Salt	1.5
Emulsified Shortening	3
Water	100

^a Baking powder was added at 0, 3, 5, 8, 16 %.

The volume index of the breads with sorghum flour and potato starch increased by adding 3, 5, 8 or 16% baking powder (Table 4.36). The cell numbers in the breads with sorghum flour and potato starch blends of 90:10 with 3% were the same as the control. Addition of 5, 8 or 16% baking powder reduced the number of cells in the breads but these baking powder levels increased the cell wall thickness and cell diameters. The cell walls of the breads with 3, 5, 8 or 16% baking powder were thicker than the control. The cell walls of the breads with 5, 8 or 16% baking powder were thicker than the cell walls of the breads made with 3% baking powder. The cell diameters of the breads made with 5, 8 or 16% baking powder were larger than the control. The cell diameters of the breads made with 3% baking powder was the same as the control.

Table 4.36. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: potato starch (90-10%) and baking powder ^a

Baking Powder Level (fwb%)	Volume Index (mm)	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)
Control ^b	155 b	6534 a	0.36 c	1.36 c
3	193 a	6253 ab	0.42 b	1.87 bc
5	203 a	5852 bc	0.45 a	2.39 a
8	194 a	5753 bc	0.45 a	2.28 ab
16	198 a	5492 c	0.45 a	2.67 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no baking powder.

The breads made with 3, 5, 8 or 16% were softer than the control. The firmness of the breads made with sorghum flour and potato starch with 5, 8 or 16% was lower than the breads made with 3% baking powder (Table 4.37). The elasticity of the breads made with sorghum flour and potato starch with 5, 8 or 16% baking powder were the same as the control. The breads made with sorghum flour and potato starch with 3% baking powder were more elastic than the control and the other levels of baking powder. The best level of baking powder was 5% for potato starch and sorghum flour breads because the firmness of the breads with 5% baking powder was lower than the breads with 3% baking powder.

Table 4.37. The firmness and elasticity of chemically leavened bread made with sorghum flour: potato starch (90-10%) and baking powder ^a

Level (fbw %)	Firmness (g)	Elasticity (%)
Control ^b	4491 a	66 bc
3	1760 b	69 a
5	1175 c	67 b
8	1190 c	66 bc
16	1212 c	64 c

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained no baking powder.

Selection of Best Level of Baking Powder for Tapioca Starch, Rice Flour and Potato Starch

No study has been done yet on the application of chemical leavening agents in gluten free products (Elgeti et al 2015). The type, composition and levels of the baking powder is significant when the specific characteristics and requirements of the product are taken into consideration. Using 3, 5, 8 or 16% baking powder created a better quality of gluten free sorghum bread, in terms of crumb characteristics and texture. Optimum levels of baking powder were 5% for tapioca starch and sorghum flour and potato starch and sorghum flour and 8% for rice flour and sorghum flour.

WATER

In chemically leavened products, proper water absorption is critical to get the best quality (large volume, soft texture and proper shape) product. The next experiments were done to evaluate the effect of water level in breads made with 90% sorghum flour and 10% starch blends (rice flour, tapioca, and potato starch).

Evaluation of Water in Sorghum Flour/ Tapioca Starch Bread

The first evaluation of water level was done for the breads with sorghum flour and tapioca starch blends of 90:10. The control loaves contained sorghum flour and tapioca starch blends of 90:10 with 100% water. The water levels used in the breads were 110, 120, 130, 140 or 150%. The base formula is listed in Table 4.38.

Table 4.38. Base formula used to evaluate the different levels of water in chemically leavened sorghum flour: tapioca starch bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Tapioca Starch	10
Sugar	6
HPMC	3
Baking Powder	5
Salt	1.5
Emulsified Shortening	3
Water	Variable ^a

^a Water was added at 100, 110, 120, 130, 140, 150 %.

The volume index of breads made with 110, 130 and 140% water were higher than the control (Table 4.39). Moreover, the volume index of breads made with 120 and 150% water were the same as the control. The number of cells in breads made with 130, 140 and 150% water were lower than the control. Furthermore, the number of cells of breads made with 90% sorghum flour

and 10% tapioca starch breads with 110 and 120% water were the same as the control. The cell walls of breads made with 110, 120, 130, 140 and 150% water were thicker than the control. The cell diameters of the breads made with 110 and 120% were the same as the control but the cell diameter of the breads made with 130, 140 and 150% were larger than the control.

Table 4.39. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and variable water absorption^a

Water Level (%)	Volume Index (mm)	Number of Cells	Wall thickness (mm)	Cell Diameter (mm)
Control ^b	209 bc	6232 a	0.427 d	1.96 c
110	222 a	5871 ab	0.448 c	2.39 c
120	221 ab	5933 ab	0.449 c	2.46 c
130	224 a	5239 c	0.474 b	2.98 b
140	222 a	5414 bc	0.482 ab	3.11 ab
150	201 c	4512 d	0.499 a	3.57 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained 100% water.

The firmness of breads made with 110, 120, 130, 140 and 150% water was lower than the control (Table 4.40). The elasticity of the breads made with 120, 130, 140 and 150% water were the same as the control. While the elasticity of breads made with 110% water were higher than the control, there was no difference in the volume index, crumb characteristics, firmness and elasticity of the breads made with 110 and 120% water. Since breads with 120% water had higher yield, the best water absorption was specified as 120% for sorghum flour and tapioca bread.

Table 4.40. The firmness and elasticity of chemically leavened bread made with sorghum flour: tapioca starch (90-10%) and variable water absorption ^a

Water Level (fwb%)	Firmness (g)	Elasticity (%)
Control ^b	1545 a	47 b
110	870 b	50 a
120	875 b	49 ab
130	710 b	49 ab
140	617 b	49 ab
150	641 b	49 ab

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained 100% water.

Evaluation of Water in Sorghum Flour/ Rice Flour Bread

The second evaluation of water level was done for the breads with sorghum flour and rice flour blends of 90:10. The control loaves contained sorghum flour and rice flour blends of 90:10 with 100% water. The water levels used in the breads were 110, 120, 130, 140 and 150%. (Table 4.41).

Table 4.41. Base formula used to evaluate the different levels of water in chemically leavened sorghum flour: rice flour bread formula

Ingredients	Level (fwb %)
Sorghum Flour	90
Rice Flour	10
Sugar	6
Xanthan	3
Baking Powder	8
Salt	1.5
Emulsified Shortening	5
Water	Variable ^a

^a Water was added at 100, 110, 120, 130, 140, 150%.

The volume index of the bread with 110, 120, 130, 140 or 150% water were statistically the same as the control (Table 4.42). The number of cells in bread made with 110 or 120% water were the same as the control while the number of cells in bread made with 130, 140 or 150% water were lower than the control. The cell wall thickness of bread made with sorghum flour and rice flour with 110% water absorption was the same as the control while the cell walls in the bread made with 120, 130, 140 or 150% water absorption were thicker than the control. The cell diameters of bread made with sorghum flour and rice flour with 110 or 120% water were the same as the control whereas the cell diameters of bread made with sorghum flour and rice flour with 130, 140 and 150% water were larger than the control.

Table 4.42. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: rice flour (90-10%) and variable water absorption ^a

Water Level (%)	Volume Index (mm)	Number of Cells	Wall thickness (mm)	Cell Diameter (mm)
Control ^b	198 a	6702 a	0.408 c	1.68 c
110	221 a	6502 ab	0.411 c	1.69 c
120	207 a	6240 ab	0.432 b	1.91 bc
130	195 a	5643 bc	0.443 b	2.14 b
140	195 a	5719 bc	0.446 b	2.06 b
150	195 a	5106 c	0.464 a	2.50 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained 100% water.

The firmness of breads made with 110, 120, 130, 140 and 150% water was lower than the control (Table 4.43). The elasticity of the breads with 110, 120, 130 or 140% water was the same as the control while breads made with 150% water were more elastic than the control. The breads made with 110 or 120 % water had statistically the same volume index, number of cells, cell diameter, firmness and elasticity but the cell wall thickness of the breads with 110% water were smaller than the breads with 120% water. Therefore, the best water absorption was specified as 110% for sorghum flour and rice flour bread.

Table 4.43. The firmness and elasticity of bread made with sorghum flour: rice flour (90-10%) and variable water absorption^a

Water Level (fwb %)	Firmness (g)	Elasticity (%)
Control ^b	1390 a	63 b
110	939 b	65 ab
120	794 bc	65 ab
130	1007 b	65 ab
140	872 bc	65 ab
150	644 c	68 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained 100% water.

Evaluation of Water in Sorghum Flour/ Potato Starch Bread

The third evaluation of water level was done for the breads with sorghum flour and potato starch blends of 90:10. The control loaves contained with sorghum flour and potato starch blends with 100% water. The water levels used in the breads were 110, 120, 130, 140 or 150% (Table 4.44).

Table 4.44. Base formula used to evaluate the different levels of baking powder in chemically leavened sorghum flour: potato starch bread formula ^a

Ingredients	Level (fwb %)
Sorghum Flour	90
Potato Starch	10
Sugar	6
Xanthan	4
Baking Powder	5
Salt	1.5
Emulsified Shortening	3
Water	Variable ^a

^a Water was added at 100, 110, 120, 130, 140, 150 %.

Water absorption did not affect volume index in sorghum flour and potato starch breads.

The volume index of the bread made with sorghum flour and potato starch with all water absorptions were statistically the same as the control (Table 4.45). The number of cells in bread made with 110, 120 and 130% water were the same as the control while the number of cells in bread made with 140 and 150% water were lower than in the control. The cell wall thicknesses of bread made with 110 and 120% water were the same as the control. Nevertheless, the cell walls of the bread made with 130, 140 and 150% water were thicker than the control. The cell diameters of bread made with sorghum flour and potato starch with 110, 120 and 130% water was the same as the control. However, the cell diameters of bread made with sorghum flour and potato starch with 140 and 150% water were larger than the control.

Table 4.45. Volume and crumb grain characteristics of chemically leavened bread made with sorghum flour: potato starch (90-10%) and variable water absorption ^a

Level (fwb %)	Volume Index (mm)	Number of Cells	Wall thickness (mm)	Cell Diameter (mm)
Control	206 a	8101 a	0.396 c	1.41 b
110	220 a	7569 a	0.398 bc	1.51 b
120	220 a	7295 a	0.420 bc	1.71 b
130	230 a	6850 ab	0.430 b	1.87 b
140	217 a	5756 bc	0.466 a	2.59 a
150	213 a	5206 c	0.477 a	2.79 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained 100% water.

The firmness of breads made with 110, 120, 130, 140 and 150% water was lower than the control (Table 4.46). Breads with 110 or 120% water were firmer than the breads with 130, 140 or 150% water. Moreover, the elasticity of the breads made with 110, 120 or 130% water was the same as the control. However, the elasticity of breads made with 140 and 150% water was higher than the control. There was no difference in the volume index, crumb characteristics, firmness and elasticity of the breads made with 110 and 120% water. Since breads with 120% water had higher yield, the best water absorption was specified as 120% for sorghum flour and potato bread.

Table 4.46. The firmness and elasticity of chemically leavened bread made with sorghum flour: potato starch (90-10%) and variable water absorption ^a

Level (fwb %)	Firmness (g)	Elasticity (%)
Control ^b	1337 a	64 b
110	1098 b	65 b
120	1009 b	65 b
130	733 c	66 ab
140	772 c	68 a
150	708 c	67 a

^a Means in the same column with different letters are significantly different at a p<0.05.

^b The control loaves contained 100% water.

Selection of Best Level of Water for Tapioca Starch, Rice Flour and Potato Starch

For several significant physicals, chemical and biochemical reactions to occur, the liquid phase of dough is important during the bread making process. For instance, the ability of carbon dioxide gas to diffuse into the dough depends on the water content of the dough (Bellido, 2009). In this research, addition of 110, 130 or 140% water improved the volume index of sorghum flour and tapioca starch bread. However, there was no improvement on the crumb characteristics (number of cells, cell wall thickness and cell diameter) of the breads. Adding 110, 120, 130, 140 or 150% water did not improve the volume index and the crumb characteristics (number of cells, cell wall thickness and cell diameter) of sorghum flour and rice flour bread or sorghum flour and potato starch bread. However, adding 110, 120, 130, 140 or 150% water decreased the firmness of the breads made with all three starches (sorghum flour and tapioca starch bread; sorghum flour and rice flour bread and sorghum flour and potato starch bread).

Chapter 5. Conclusion

The first objective of this study was to examine the interaction of starch-hydrocolloid in chemically leavened gluten free sorghum bread. Based on the results, 10% tapioca starch with 3% HPMC, 10% rice flour with 3% xanthan and 10% potato starch with 4% xanthan formed a breads with higher volume and improved crumb characteristics. According to Schober et al (2007), replacing HPMC with xanthan improved the yeasted sorghum bread quality. Nevertheless, HPMC did not have the same effect in chemically leavened gluten free sorghum bread with potato starch.

The second objective was to evaluate the effects of different ingredients in chemically leavened gluten free sorghum. Egg ingredients, emulsifiers and fat were added to the optimized formulation in each step of the research. Addition of higher levels of whole egg increased the volume index but it reduced the quality of crumb characteristics in the breads made with sorghum flour: tapioca starch. The volume index of the sorghum flour and potato starch was increased by adding 5% egg white. As the best level of emulsified shortening was determined to be 3% for the breads with sorghum flour: tapioca starch or sorghum flour: potato starch. Addition of 5% emulsified shortening increased the volume index and number of cells and cell wall thickness, cell diameter and elasticity in the breads made with sorghum flour: rice flour.

The third objective was to develop a chemically leavened gluten free sorghum bread. To develop a good quality chemically leavened gluten free sorghum bread, the level of baking powder and water increased. Each type of bread with sorghum flour: starch (rice flour, tapioca and potato starch) had its own optimum amount of water and baking powder. The best level of baking powder is 5%, 8% and 5% for sorghum flour: tapioca starch breads, sorghum flour: rice flour breads and sorghum flour: tapioca starch breads, respectively. The optimum level of water

is 120%, 110% and 120% for sorghum flour: tapioca starch bread, sorghum flour: rice flour bread, and sorghum flour: potato starch bread, respectively. The present research showed that different hydrocolloids interact differently with different starches. The effect of formula ingredients varies because of the starch- hydrocolloid interaction in the gluten free batter.

Chapter 6. Future Work

Viscosity is an important aspect of how batters behave during baking. The RVA could be used to measure the viscosity profile of the sorghum flour/starch and sorghum flour/starch/hydrocolloid blends and relate it to bread properties.

Shelf life was not measured in this research. In future work, the effect of the starches and hydrocolloids on shelf life could be determined.

Sensory analysis is another topic of interest. Consumer liking of flavor and texture of the sorghum based breads made with the different starch and hydrocolloids could be measured.

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Appendix A.

Hydrocolloids

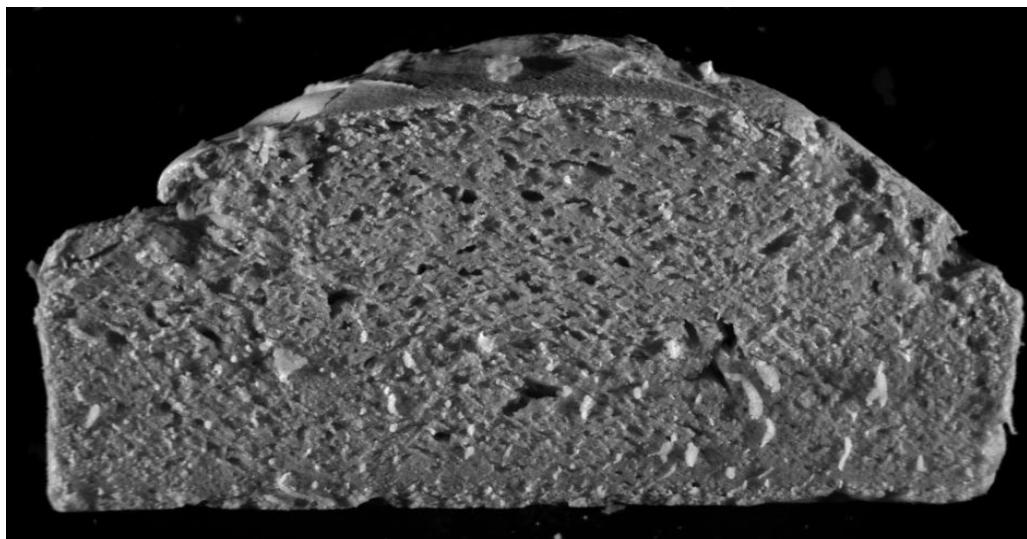


Figure 6. C-Cell image of sorghum bread with rice flour and 3% xanthan.

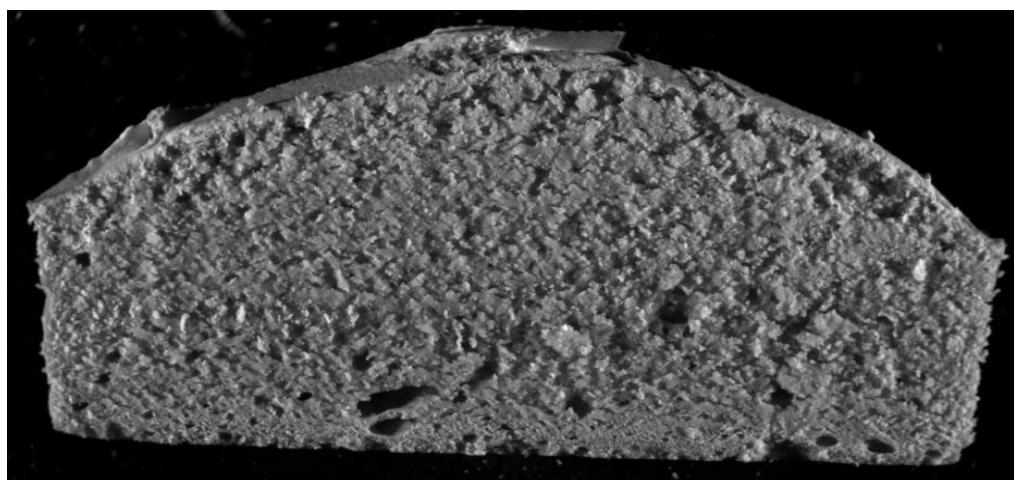


Figure 7. C-Cell image of sorghum bread with tapioca starch and 3% HPMC

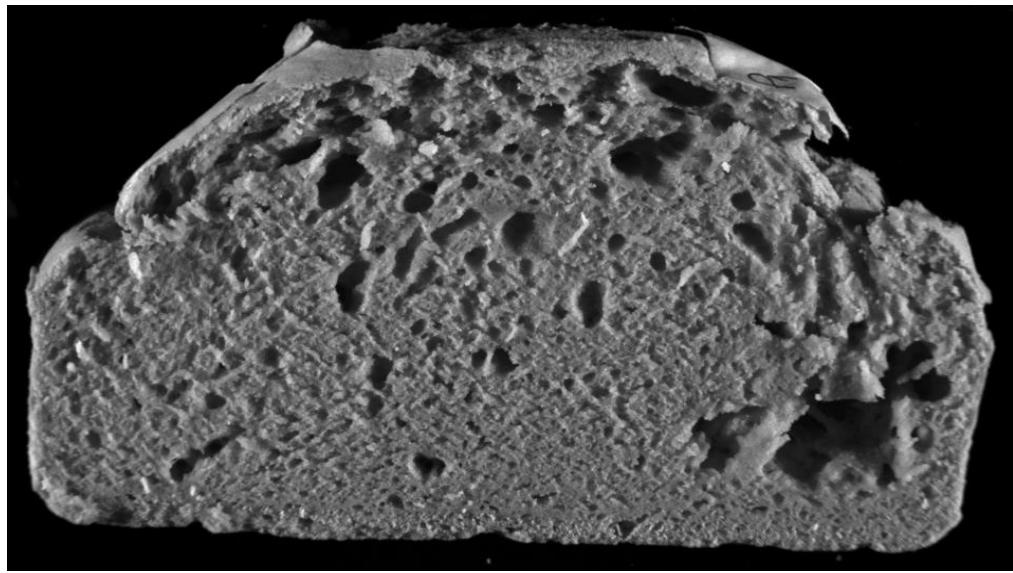


Figure 8. C-Cell image of sorghum bread with potato starch and 4% xanthan.

Fat

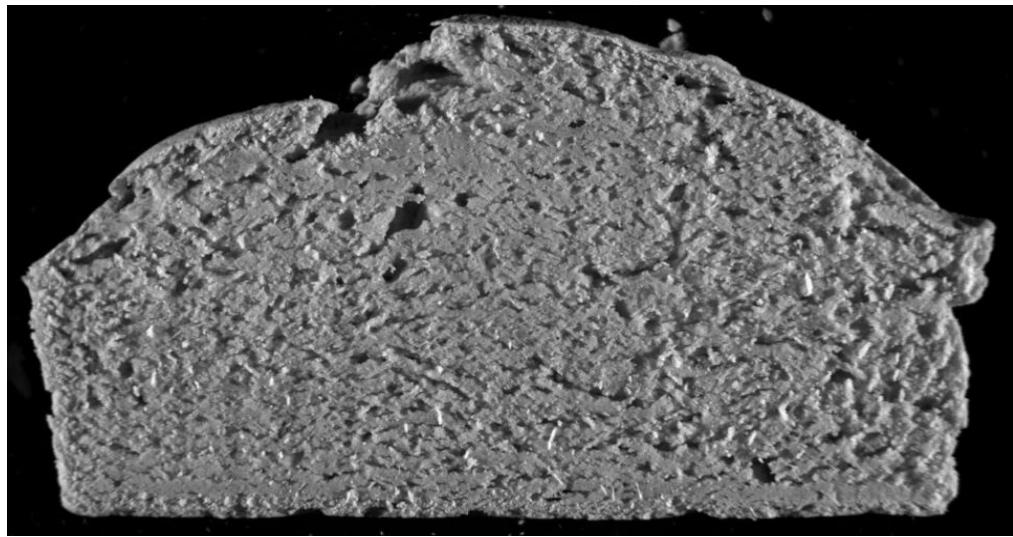


Figure 9. C-Cell image of sorghum bread with rice flour, 3% xanthan, and 3% emulsified shortening.

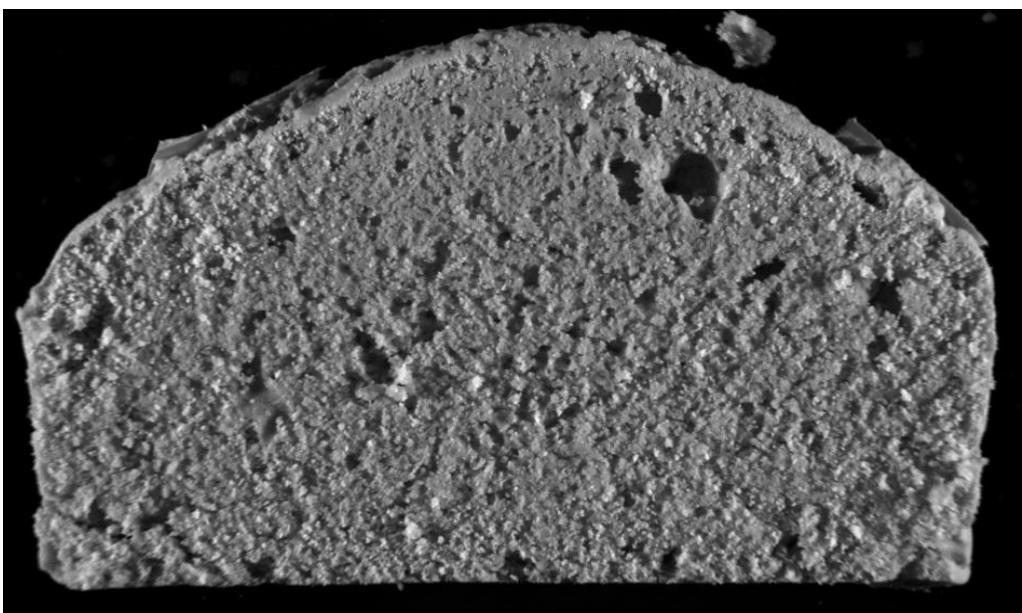


Figure 10. C-Cell image of sorghum bread with 3% tapioca starch, HPMC, and 3% emulsified shortening

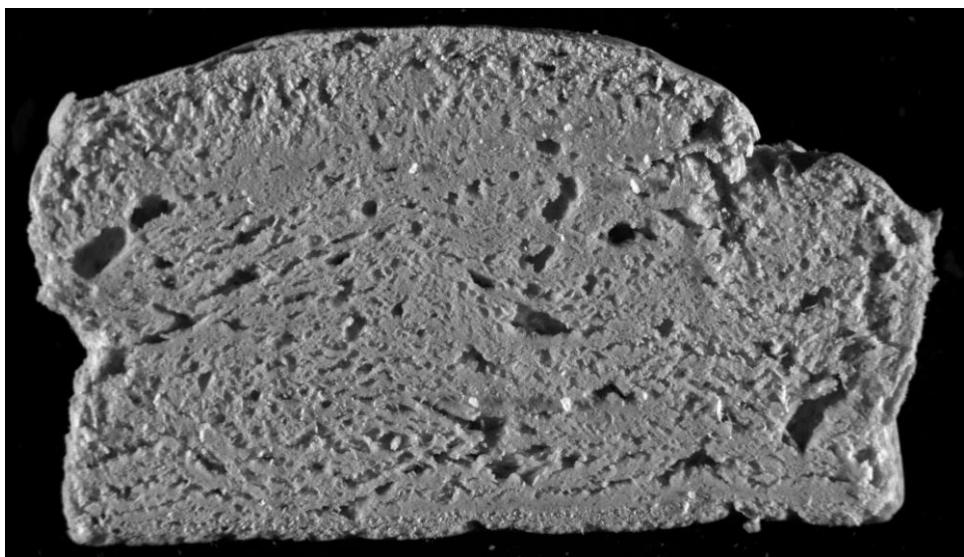


Figure 11. C-Cell image of sorghum bread with potato starch, 4% xanthan, and 3% emulsified shortening

Emulsified Shortening

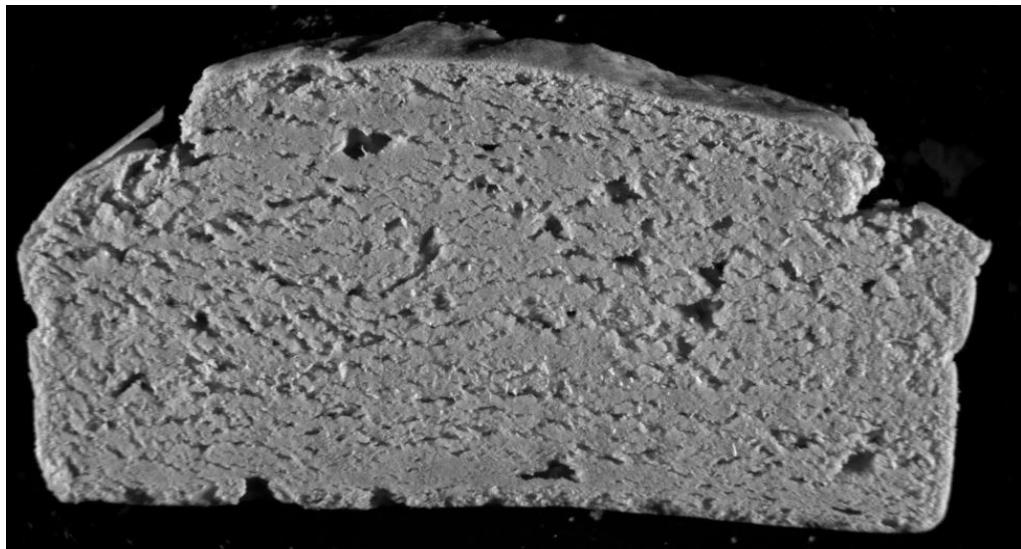


Figure 12. C-Cell image of sorghum bread with rice flour, 3% xanthan and 5% emulsified shortening

Baking Powder

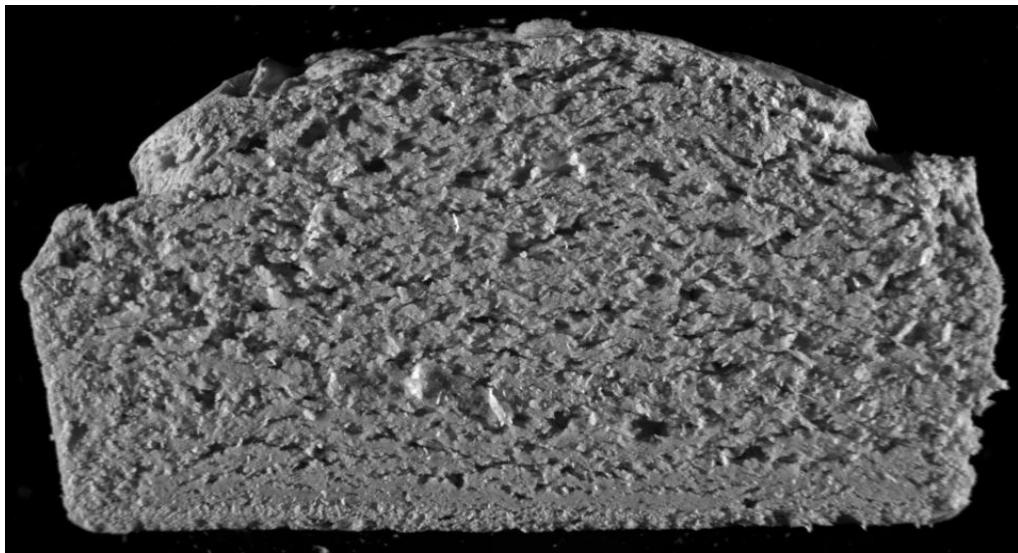


Figure 13. C-Cell image of sorghum bread with rice flour, 3% xanthan, 5% emulsified shortening and 8% baking powder

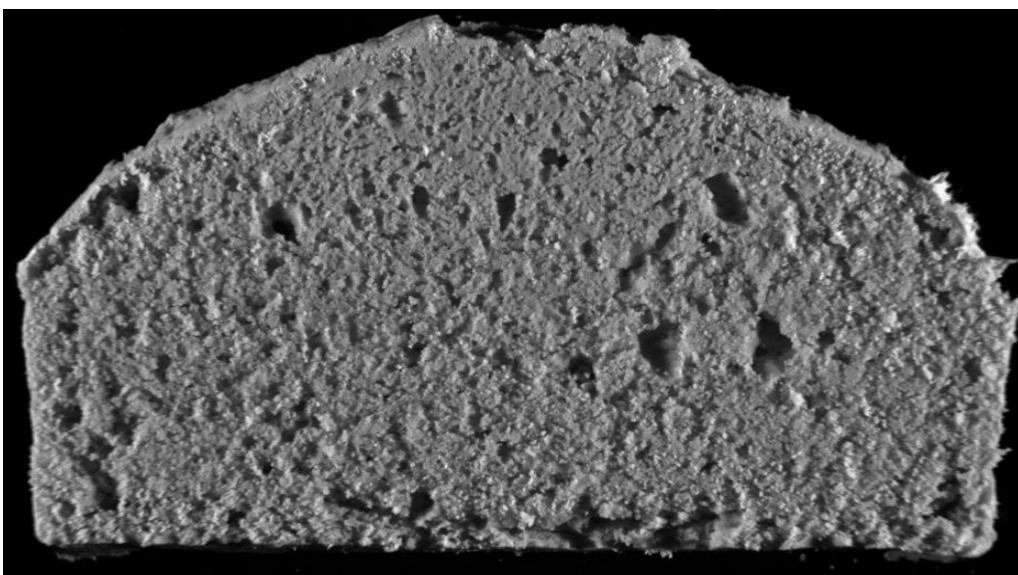


Figure 14. C-Cell image of 3% HPMC, tapioca starch, 3% emulsified shortening and 5% baking powder bread slice.

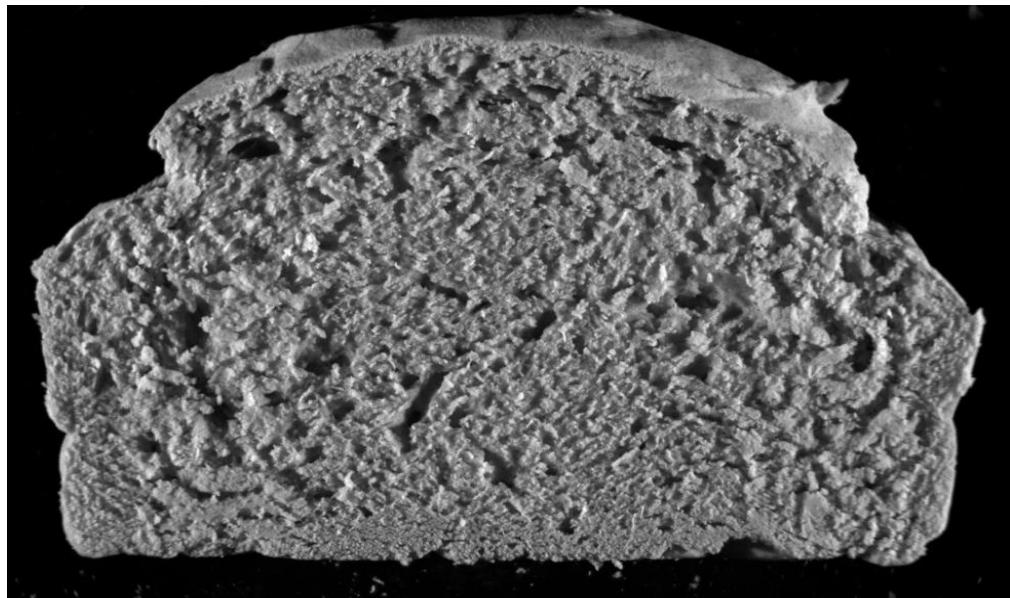


Figure 15. C-Cell image of 4% xanthan, potato starch, 3% emulsified shortening and 5% baking powder bread slice.

Water

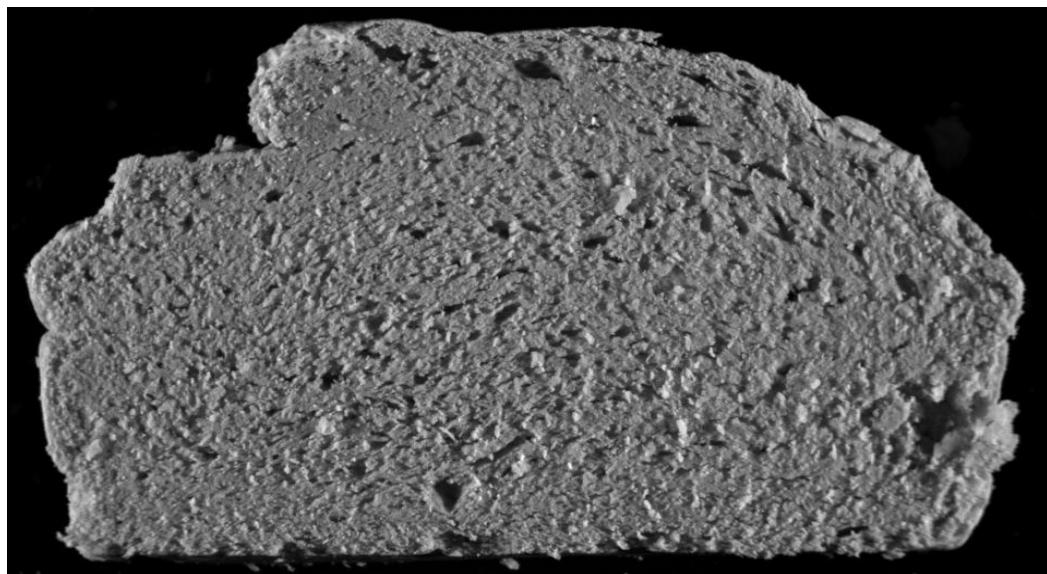


Figure 16. C-Cell image of 3% xanthan, rice flour, 5% emulsified shortening, 8% baking powder and 110% water bread slice

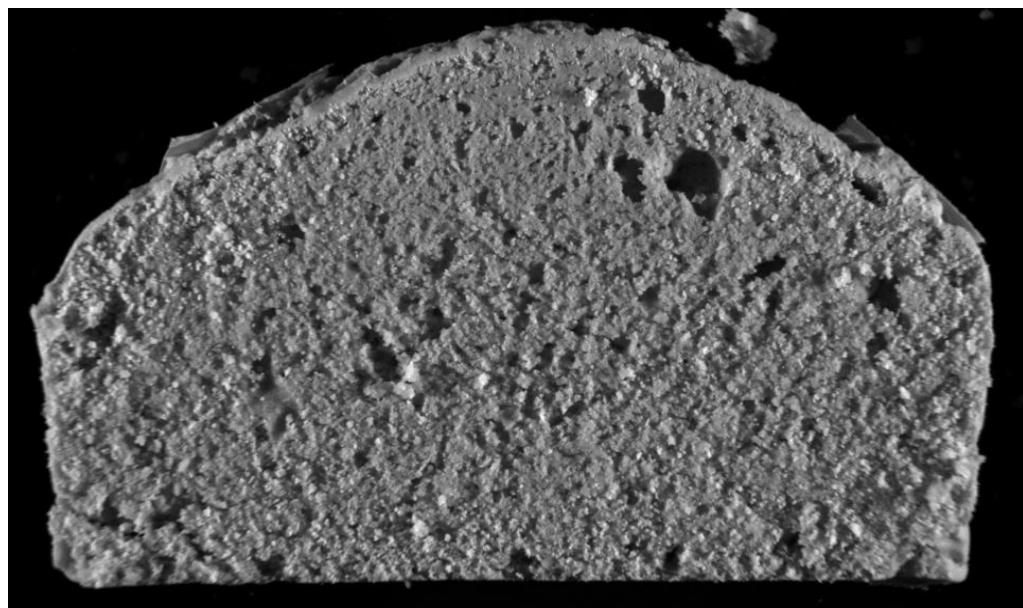


Figure 17. C-Cell image of 3% HPMC, tapioca starch, 3% emulsified shortening, 5% baking powder and 120% water bread slice

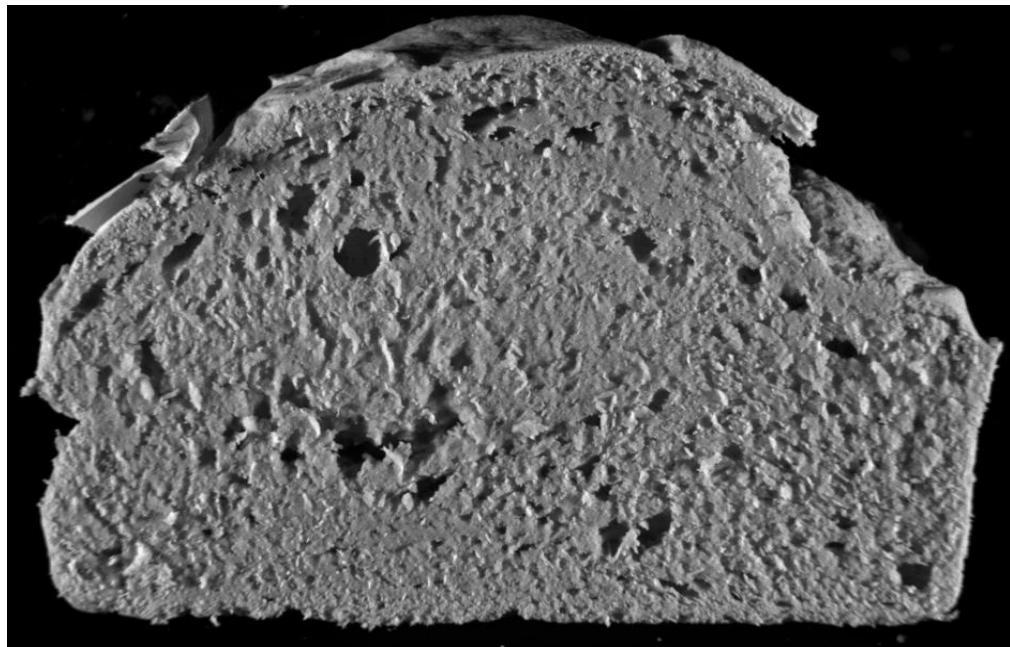


Figure 18. C-Cell image of 4% xanthan, potato starch, 3% emulsified shortening, 5% baking powder and 120% water bread slice.