EFFECT OF REDUCING AGENTS ON BATTER CONSISTENCY AND PHYSICAL CHARACTERISTICS OF BREAD FROM SORGHUM FLOUR

by

EMILY L. FORT

B.S., Kansas State University, 2013

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Major Professor
Dr. Rebecca Regan

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Abstract

Sorghum is a vital cereal crop grown in many regions around the world. Tolerance to harsh climates and low moisture conditions are unique traits making sorghum an economical choice in an era of global water scarcity. In recent years, sorghum has gained greater recognition as a gluten-free grain and is a safe alternative for individuals suffering from gluten sensitivities or celiac disease. Still, the lack of gluten proteins does not allow sorghum to form a viscoelastic dough. In this study reducing agents were added to improve functional properties of sorghum kafirins for bread baking. Study objectives were to determine the effect of reducing agents on protein body structure of sorghum kafirins, investigate the influence on the sorghum batter consistency, and evaluate the effects on the physical characteristics of sorghum bread. Protein analysis, accomplished using RP-HPLC, showed reducing agents, L-cysteine and sodium metabisulfite, reduced protein structure; increasing RP-HPLC total peak area up to 747% and 681%, respectively. Batter consistency was obtained using a RVA. Treatments of L-cysteine (2.5% fwb) expressed increased RVA peak viscosity and decreased final viscosity. Samples treated with sodium metabisulfite (500 ppm fwb) had increased peak viscosity, holding strength and final viscosity. Yeast activity of batter treated with ≥3000 ppm (fwb) sodium metabisulfite caused volume loss of 95% yet at 500 ppm (fwb) sodium metabisulfite did not have an effect. Batter with 2.5% (fwb) L-cysteine experienced reduced yeast activity after 20 min. Sorghum bread characteristics were altered. Loaf volume and crumb grain characteristics of bread produced using sodium metabisulfite (500 ppm) were equal to that of the control, while initial texture and staling were improved. The addition of L-cysteine (2.5% fwb) to breads lowered loaf volume but produced softer initial crumb texture and improved *in-vitro* protein digestibility by 18.8%.

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CHAPTER 1

Introduction

Grain sorghum is grown in many regions around the world. The crop's ability to withstand harsh climates and low moisture conditions aids in its versatility. In recent years, sorghum has gained recognition as a gluten-free grain, making it a popular alternative for individuals suffering from celiac disease or other gluten sensitivities. White sorghum varieties are preferred for food use. Nevertheless, because sorghum grain does not possess gluten proteins it is unable to form a viscoelastic dough. Without an elastic and extensible network of proteins, dough does not have the ability to retain gases produced during fermentation, which is required to properly leaven and to ultimately evolve into a quality loaf of bread.

1.1 Statement of Problem

Many studies have evaluated sorghum in composite bread applications (Carson et al., 2000,Goodall et al., 2012,Mkandawire, 2013), investigated the carbohydrate composition and functionality (Subramanian et al., 1994, Beta et al., 2001, Sang et al., 2008, Taylor et al., 2010), as well as protein characterization, functionality and digestibility (Hamaker et al., 1987, Duodu et al., 2003, Ioerger et al., 2007, Oom et al., 2008, Miller, 2013). Yet little work has been done to alter the native protein body structure of sorghum kafirin proteins. Previous research has indicated reducing agents may be employed to alter protein body structure for functional purposes (Hamaker et al., 1987, Elkhalifa et al., 1999, Yano, 2010, Guo et at al., 2012). In a study conducted by Yano (2010), the reducing agent, glutathione was evaluated for its effects on rice bread batter. The study concluded the reduction of rice proteins lead to a significant improvement of gas retention in batter. A subsequent study performed by Guo et al. (2012) investigated the effects of reducing agents on

stored rice. They discovered the reducing agent, ascorbic acid, increased peak viscosity while also improving water absorption capacity, soluble solid content and granule swelling capacity of aged rice. Similar results were found by Elkhalifa et al. (1999) when cysteine, sodium metabisulfite and ascorbic acid were added to cooked sorghum gruel. Treatment with reducing agents improved *in-vitro* starch digestibility of sorghum gruel, up to 58% when sodium metabisulfite was used. Additionally, a study carried out by Hamaker et al. (1987) found protein digestibility of cooked sorghum was improved in the presence of reducing agents, sodium bisulfite and L-cysteine. These studies indicate there is potential for reducing agents to be used in gluten-free breads, for the purpose of improving functional properties of batter and finished products.

1.2 Study Objectives

The purpose of this study was to provide further insight into grain sorghum biochemistry, physiochemical composition and protein structure as it relates to bread baking. This was done by evaluating the effects of reducing agents, glutathione, L-cysteine, and sodium metabisulfite, at various concentrations. The objectives of this study were:

1. Determine the effect of reducing agents on the structure of the primary prolamin proteins (kafirins) present in sorghum grain.
2. Investigate the influence of reducing agents on the consistency of sorghum bread batter.
3. Evaluate the effect of reducing agents on the physical characteristics of sorghum bread.

CHAPTER 2

Literature Review

2.1 Celiac Disease

Celiac disease is a chronic, genetic intestinal disease affecting millions of individuals. Awareness of celiac disease has increased in recent years due to improved diagnostic tests. Approximately 1 in 133 Americans is diagnosed with celiac disease (Fasano et al., 2003). This condition is characterized by an autoimmune response to a particular set of gluten storage proteins found in wheat, barley and rye. Gluten is not a singular protein but a heterogeneous mixture of two proteins; gliadin and glutenin. Both proteins provide unique attributes to dough during mixing and baking in addition to the pathogenesis of celiac disease. Gliadins are monomeric proteins, responsible for imparting extensibility to the gluten protein matrix during mixing and facilitating gas retention during leavening. Kagnoff et al. (1982) report a portion of the gliadin proteins exist in the isoform, α-gliadin, which triggers the immune response of celiac disease. Specifically, five peptide sequences derived from a subcategory within the α-gliadin isoform, are highly toxic and have been identified to be the specific instigators of the immune response in celiac patients (Kagnoff et al., 1982,De Vincenzi et al., 1996,Wal et al., 1999,Vader et al., 2002). Glutenins are multimeric proteins, responsible for the elastic characteristic of wheat dough. When glutenin is solubilized as in digestion, it disassociates into low-molecular-weight (LMW) and high-molecular-weight (HMW) subunits. The LMW subunits contain the same peptide sequences found in the toxic gliadin fractions. To a lesser extent than gliadins, the LMW glutenin subunit contribute to the adverse effects of gluten proteins on celiac patients (De Vincenzi et al., 1996).

The triggered immune response to gluten proteins is a combination of genetic predisposition and adaptive response experienced by celiac patients. Spurkland et al. (1997) discovered celiac disease is associated with the HLA-DQ2 and HLA-DQ8 genes. Individuals who possess HLA-DQ2 and/or HLA-DQ8 genotypes have a predisposition for disease development. Interestingly, the strength of association varies between genotypes based on their ability to bind gluten peptides. The association between celiac disease and HLA-DQ8 is weak because it only binds a narrow range of gluten peptides, while HLA-DQ2 binds a wider range; thus, it is strongly associated. A study completed by Tjon et al. (2010) revealed the presence of the HLA-DQ2 gene is fairly common, found in nearly 25% of the European population. Yet, of that fraction of the population, approximately only 4% will develop celiac disease. A visual representation of the affected population, based on genetic markers is shown in Figure 2.1.

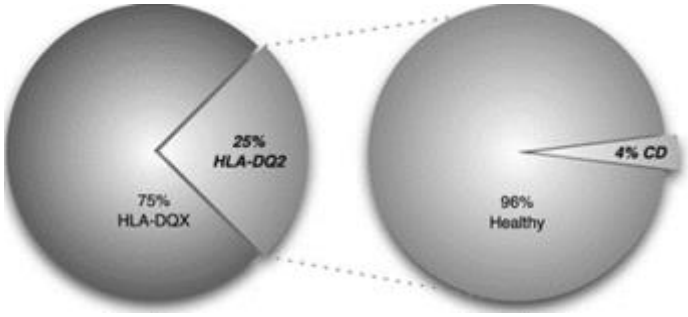


Figure 2.1: Prevalence of celiac disease (CD) based on genetic markers HLA-DQX and HLA-DQ2. Adapted from Tjon et al. (2010).

In contrast to traditional food allergies, celiac disease does not develop as an immediate hypersensitivity to a protein (Green et al., 2015). Instead, celiac disease is a delayed immune response, initiated when gluten enters the intestinal tract. The epidermal tissue transglutaminase (TTG), a protective enzyme lining of the intestine, activates the gluten polymer causing it to present as a pathogenic antigen. In response, the immune system deploys white blood cells with specific

CD4 glycoprotein. The CD4+ T-cells transmit signals to other immune cells equipped to destroy what the body interprets as an infectious agent. While trying to protect the body the cytokines released ultimately result in inflammation of the epithelial layer of the gastrointestinal mucosal lining and atrophy of the intestinal villa. The subsequent clinical expressions of this damage are commonly noted as abdominal pain, weight loss and diarrhea, which can ultimately lead to malabsorption, nutrient deficiency, osteoporosis, anemia and certain cancers (Tjon et al., 2010,Green et al., 2015). There is no cure for celiac disease and to-date the only treatment is life-long adherence to a gluten-free diet. As this is the only solution for celiac patients, the quality and variety of gluten-free products must improve.

2.2 Gluten-free

Intuitively, as the prevalence of celiac disease diagnoses have increased, the gluten-free market has shown tremendous growth. In the United States, 30% of adults are interested in partially or fully omitting gluten from their diets (McLynn, 2013) indicating the demand for gluten-free is evident. In 2013, the United States Food and Drug Administration officially defined "gluten-free" with the Final Rule of Food Labeling; Gluten-free Labeling of Foods. In order for products to bear the claim "gluten-free", food must not include a gluten-containing grain (e.g., spelt wheat); an ingredient derived from a gluten-containing grain (e.g., wheat flour); or an ingredient derived from a gluten-containing grain that has been processed to remove gluten (e.g., wheat starch) (FDA, 2014). The use of any of those ingredients must result in the presence of less than 20 parts per million (ppm) of gluten in the food product to be deemed gluten-free. Additional claims such as "no gluten", "free of gluten" and "without gluten" are considered synonymous with "gluten-free" and therefore, are required to adhere to the standards of the FDA definition.

In conjunction with regulatory standards, quality standards are critically important to the food industry. The gluten-free segment of the food market has shown tremendous growth. In 2014, the gluten-free market was valued at $1.77 billion and is projected to grow to $23.9 billion by 2020 (Statista, 2016). However, in spite of the growing success of this niche market there is still a large push to improve products in terms of taste, texture, aroma and shelf-life. Gluten-free products pose a difficult challenge, as omitting gluten proteins from products creates a large functional void which needs to be replaced by other ingredients. Gluten provides both elasticity and extensibility to a dough which in turn allows for gas retention and leavening. It is also unique in that it plays a significant role in structure, moisture retention and shelf-life stability (Cornish et al., 2006). Due to gluten's diverse functionality, there is not a single ingredient that can replace gluten in a baking system. Often, hydrocolloids and isolated starches are incorporated to maintain structure and retain moisture. Isolated starch products such as potato starch, tapioca starch and corn starch are most commonly formulated in gluten-free products. Starch sources such as these are capable of binding large amounts of water, aiding in crumb quality and freshness. In addition, hydrocolloids such as xanthan gum, locust bean gum and hydroxypropyl methylcellulose (HPMC) are incorporated into gluten-free products. Xanthan and locust bean gums are well-suited for fermented bread applications due to their salt tolerance and stability over a range of pH and temperatures. Surface-active hydrocolloids such as HPMC are also well adapted for gluten-free bread; providing emulsion stabilization by dispersing and retaining air cells within the batter, resulting in a uniform crumb structure and in some cases, improved volume.

2.3 Sorghum

Sorghum (*Sorghum bicolor (L.) Moench*) has been extensively researched for its applications in gluten-free baking, feed manufacturing, and ethanol production. Grain sorghum belongs to the grass

family, Poaceae, and is most comparable in composition to maize. Sorghum contains a high degree of genetic diversity in addition to considerable variation which occurs naturally across germplasm collections.

Currently, sorghum is grown on five of the seven continents and ranks fifth among the most important cereal crop in the world (The United Sorghum Checkoff, 2012). This abundant, durable crop readily grows in semi-arid climates, such as those found in Africa, India, parts of Asia, and the Midwestern United States, because of its natural tolerance to drought, insects and heat. These unique attributes allow sorghum to thrive in regions uninhabitable for other crops, while remaining economical to producers. Furthermore, sorghum maintains an impressive nutritional profile, containing high amounts of vitamins, minerals, dietary fiber, antioxidants, and phytochemicals (Lemlioglu, 2014). Although protein digestibility of sorghum is poor upon cooking, the proteins found in sorghum are not harmful to individuals with celiac disease thus making it a promising wheat alternative for gluten-free food applications, given methods can be implemented to improve digestibility.

2.3.1 Plant Structure

As mentioned previously, sorghum exhibits tremendous diversity. Both phenotypic and genotypic expressions vary between cultivars, however the general plant structure is fundamentally the same. The plant consists of roots, stem, leaves, and panicle or seed head. The seed head consists of multiple sets of paired spikelets. Sorghum develops in stages, reaching physiological maturity between 80 to 120 days after emergence (Vanderlip, 1993). When maturity and optimum moisture content (20-35%) are reached, stalks range in height from 2 to 4 feet with seed heads containing roughly 500-2000 kernels.

The sorghum kernels or caryopses are the most diverse component of the plant. Caryopsis size and weight vary widely based upon accumulation of dry matter during stage three of

development. Typical weight of an individual caryopsis at maturity averages between 20 to 30 mg (Delcour & Hoseney, 2010a). An assortment of pigmentations are possible for pericarp color. While only three genetic colors exist based on gene expression the caryopsis may appear white, yellow, red, brown, bronze or black (Rooney & Awika, 2004).

The sorghum caryopsis is generally spherical in shape and comprised of the germ, endosperm, and bran or pericarp (Figure 2.2). The germ is the reproductive portion of the caryopsis. Within this portion of the kernel, densely packed B-vitamins, minerals, lipids and proteins are contained to provide a nutrient source for a germinating kernel. The endosperm constitutes the bulk of the kernel at approximately 70%. Vitreous and floury endosperm are dispersed within the kernel and surrounded by the aleurone layer. Vitreous endosperm is distinguishable by the high-molecular-weight proteins and number of disulfide bonds present (Ioerger et al., 2007). Inversely, floury endosperm is comprised of low concentrations of proteins, fewer cross linkages and a greater concentration of starch. The aleurone layer is tightly bound to the vitreous and floury endosperm sectors, and is mainly comprised of lipids yet contains some starch. Winger et al. (2014) noted endosperm color as a defining characteristic of sorghum flours; claiming it provides some indication of the flavor and consistency of the final baked good product. The bran is the outer most layer of the kernel and is comprised of sublayers to include the testa, pericarp, mesocarp, epicarp, and others (Rooney & Miller, 1982, Delcour & Hoseney, 2010a). The pericarp serves as a physical protective barrier between environmental elements and the kernel. Within the pericarp resides starch, fiber, and low concentrations of vitamins. The testa is a sublayer contained by the pericarp, which also provides an additional level of fortification. Varieties with a pigmented testa contain polyanthocianins or condensed tannin, which are phenolic compounds that impart a bitter taste used as a defense mechanism by the crop to ward off birds and other predators (Delcour & Hoseney, 2010a).

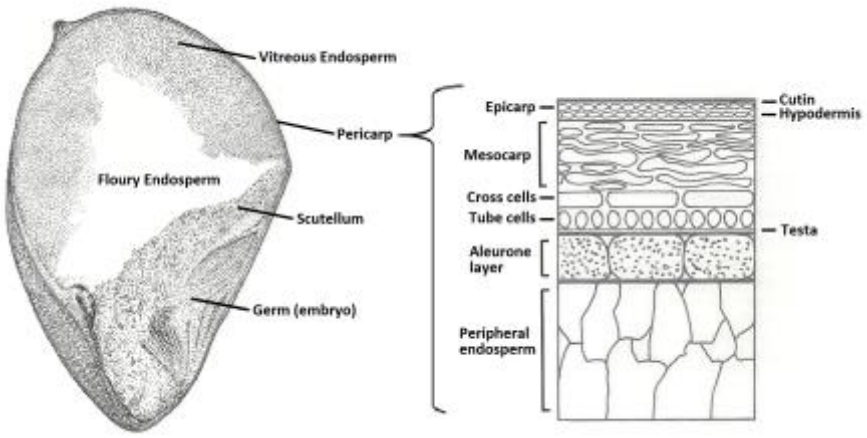


Figure 2.2: Sorghum grain caryopsis structure, showing the pericarp, endosperm (vitreous and floury), scutellum and the germ. Adapted from Delcour and Hoseney (2010a).

The pericarp and testa layers are unique in that they determine the classification of the sorghum grain. A common reference system categorizes sorghum variety based on genotypic expression in relation to tannin content, which is housed in the testa and pericarp. These genetic categories are outlined as Types I, II, and III (Earp et al., 2004,Price et al., 1978). Type I sorghum varieties exhibit no pigmentation in the testa layer, contain no tannins, and have a low degree of phenols. Type II varieties contain tannin deposits in vesicles within the testa layer. Lastly, Type III varieties display tannin deposits in the cell walls of the testa and pericarp layers. It should be noted that grain color is not a direct indicator to sorghum type. To accurately and definitively categorize a sorghum cultivar, DNA and spectroscopic analyses must be conducted (Dykes et al., 2014).

2.3.2 Composition

Macromolecules such as carbohydrates, proteins, and lipids represent the majority of sorghum flour composition, while minor constituents such as vitamins, minerals, phytochemicals

and inorganic materials are also present. The intricate roles and interactions of macromolecules dictate the reactions of sorghum flour during hydration, mixing, cooking and staling processes.

2.3.2.1 Carbohydrates

Carbohydrates are the most essential component of any cereal grain due to the amount of energy stored within. The primary carbohydrates found in sorghum are amylose and amylopectin. Located predominantly in the endosperm, the total starch content of a kernel is 55.6-75.8%, with an average of 70% db (Taylor et al., 2010). Amylose accounts for roughly 24-33% of the total starch in normal, non-waxy sorghum varieties. The average degree of polymerization (DP) of amylose lies between 1330-1390, while amylopectin has an average DP of 8900 (Gaffa et al., 2004). Both amylose and amylopectin are physically bound by the storage proteins; trapped within by a complex starch-protein matrix.

2.3.2.2 Protein

Sorghum generically has an average protein content of 11% but can range between 6 to 18% (Lásztity, 1996). Non-prolamin proteins such as albumins, globulins and glutelins are present within the endosperm and account for approximately 30% of the protein content (de Mesa-Stonestreet et al., 2010). The major fraction of protein within sorghum are the storage prolamin proteins known as kafirins. These proteins are most widely known for forming indigestible protein matrices with starch granules (Figure 2.3). Various classifications of kafirins exist and are distinguishable based on molecular weight and solubility characteristics.

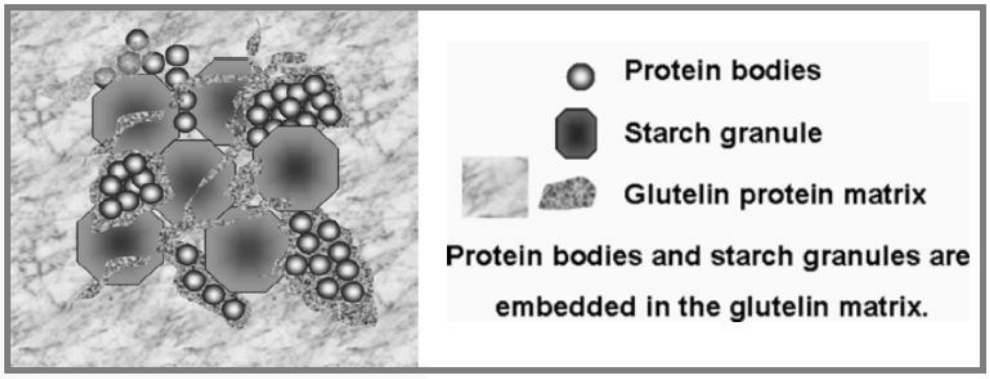


Figure 2.3: Sorghum protein body in relation to starch and the glutelin matrix (de Mesa-Stonestreet et al., 2010).

Kafirins exist in four subunits; α, β, γ and δ. Occurring in the greatest concentration, α-kafirins account for approximately 66 to 71% of the total kafirins within the floury endosperm and 80 to 84% in vitreous endosperm (Watterson et al., 1993). The α-kafirins exist in one of two polypeptide groups; molecular weight (M_w) 25 kDa or 23 kDa. Found primarily as monomers or oligomers, these polypeptides are comprised of the nonpolar amino acids proline, leucine, and alanine. The α-kafirins only form intramolecular disulfide bonds thus do not exhibit extensive crosslinking. The β-kafirins make up 10–13% of the total kafirins within floury endosperm and 7–8% in vitreous endosperm tissue (Watterson et al., 1993). They are categorized by an approximate M_w 18 kDa. The β-kafirins exist as both monomers and polymers containing amino acids methionine and cysteine, which are rich in sulfur. Due to the high number of sulfur groups present in this polypeptide, more opportunities for disulfide bonding exist. Consequently, β-kafirins are extensively crosslinked via intramolecular and intermolecular disulfide bonds. The γ-kafirins are identified by M_w 20 kDa and constitute 19–21% of the total kafirin fraction in floury endosperm and 9–12% in vitreous endosperm, according to Watterson et al. (1993). Similar to β-kafirins, the γ-kafirins are rich in sulfur-containing amino acids such as cysteine, proline and histidine. This fraction

of kafirins are largely found as polymers or oligomers. Furthermore, γ-kafirins are highly crosslinked with intramolecular and intermolecular disulfide bonds making them highly indigestible by protease enzymes (Belton et al., 2006,de Mesa-Stonestreet et al., 2010). Additionally, due to the frequent occurrence of proline, these polypeptides are the most hydrophobic of the kafirins. Lastly, δ-kafirins are present in minute concentrations. Identified by Mw 13 kDa, this fraction of kafirins are rich in methionine. To-date little is known about the participation of δ-kafirins in intramolecular or intermolecular disulfide bonds.

2.3.3 Food Application

For centuries throughout African and Asian cultures, sorghum has been a dietary staple due to its availability and unique ability to grow in semi-arid climates. These cultures manufacture an assortment of products such as popped sorghum, porridge, fermented and unfermented flatbreads, rolls, couscous, and malted beverages (Rose et al., 2014). Traditional products, while providing much needed sustenance, typically lack in favorable sensory attributes namely flavor and texture, as well as nutritional quality (Taylor et al., 2010).

The United States is the largest producer of grain sorghum, with Kansas being the number one contributing state as of 2014 (U.S. Department of Agriculture National Statistics Service, 2015), (Figure 2.4). Although the U.S. is the leading cultivator of sorghum, the use of sorghum is almost extensively for livestock feed and biofuel production. Research has been accumulating since the 1970's to determine the utilization of sorghum in food applications. Undeniably, with the continued increase in wheat related illnesses, allergies and sensitivities, sorghum continues to grow in popularity. Although the research has been on-going for many years, the struggle to make sorghum-based baked goods comparable to wheat-based baked goods still exists. Fully understanding the chemical composition and molecular interactions will aid in developing better baked good products.

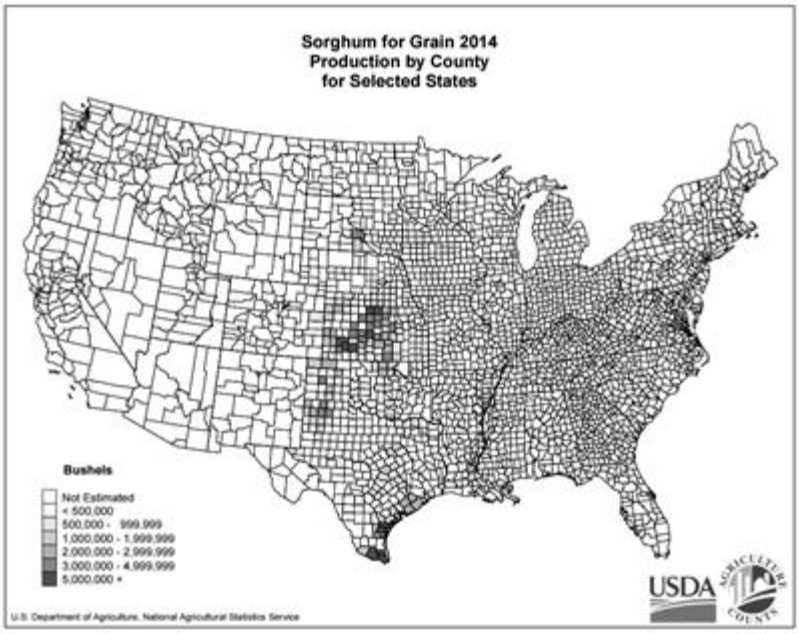


Figure 2.4: Domestic map of sorghum for grain production per acre by county for 2014 (U.S. Department of Agriculture National Statistics Service, 2015)

There are boundless benefits of grain sorghum. Not only is it globally available in great quantities but it also possesses a robust nutritional profile. Previous studies have linked individual constituents of sorghum to a variety of health benefits, such as cardiovascular health, colon health, and diabetes (Awika & Rooney, 2004). Despite the availability and contingent health benefits, sorghum proteins do not form cohesive bonds with other constituents such as starch or water to the extent needed to construct a viscoelastic dough (Oom et al., 2008). For this reason it is difficult to produce sorghum bread using a traditional dough method. Instead, sorghum is more suited for products made by a batter method, similar to cakes. Through hypothetical, chemical modification of the inherent kafirin proteins of sorghum may attribute some degree of viscoelasticity. In a study accomplished by Goodall et al. (2012) showed a composite dough made with 30 to 60% of a high digestibility, high-lysine (HDHL) sorghum variety flour and wheat flour had significantly improved

extensibility compared to normal sorghum-wheat composite dough. The HDHL sorghum was selectively bred to obtain a folded protein body shape. This morphology allows α-kafirins to be more accessible for interactions when in a flour form, ultimately improving the protein network of the dough to possess viscoelastic property. If the alterations of protein bodies through breeding techniques can improve viscoelastic properties of dough, then there is promise that chemical modification with reducing agents can produce similar results.

2.4 Reducing Agents

On a biochemical level, reducing agents are catalysts for catabolic reaction between proteins. Large conglomerations of proteins, previously referred to as a protein body, are held together by disulfide bonds and ionic charges which create a tertiary structure. Reducing agents target the disulfide (–S-S-) bonds within or between proteins, reducing them to sulfhydryl group or thiol group (-SH), shown in Figure 2.5 as the sulfhydryl-disulfide exchange. Thus, when a reducing agent is introduced to a protein body, the protein structure is catabolized into smaller subunits or polypeptides as the disulfide bonds are cleaved.

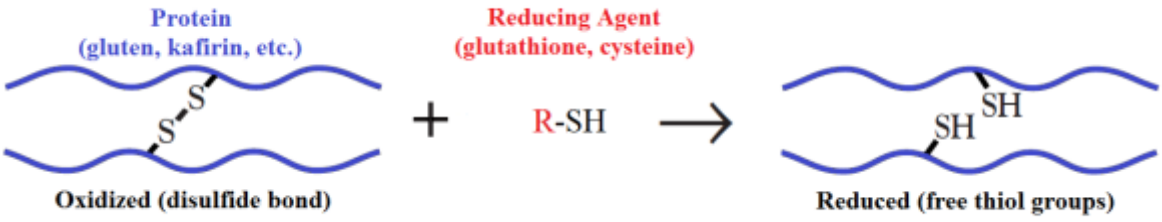


Figure 2.5: Sulfhydryl-disulfide exchange reduction reaction of protein with reducing agents. Adapted from Lallemand (2011).

Traditionally reducing agents are utilized in wheat bread formulations as dough conditioners. Reducing agents such as glutathione, L-cysteine and sodium metabisulfite cleave the disulfide bonds between gluten proteins. This action causes a reduction in dough strength and is often used to increase the extensibility of the dough system. Known benefits of reducing agents in wheat dough are decreased mix time, reduced dough elasticity, shorter proofing period and improved handling properties for high protein or strong dough.

In contrast to wheat proteins, sorghum kafirin proteins do not bear the same viscoelastic properties, therefore the effect of reducing agents in a sorghum bread formulation is unknown. As previously mentioned, kafirins are highly crosslinked and exist within the grain as tightly compacted protein bodies. It is known that the basic function of reducing agents is to break disulfide bonds. Since sorghum proteins have a high level of disulfide bonds, in theory, reducing agents would be able to break down the large protein bodies into smaller protein conglomeration or individual proteins and free the embedded starch granules trapped within the protein matrix. As a result, the liberated starch granules may have a greater chance of becoming hydrated, and gelatinizing during baking. Also the reduced kafirin proteins may be more apt to form a cohesive dough in addition to being more readily digestible.

2.4.1 Glutathione

Glutathione is a tripeptide consisting of amino acids glutamate, cysteine and glycine (Figure 2.6). This tripeptide is a metabolic by-product of yeast, namely from nonviable cells, which occur at higher concentrations in dry yeasts (Pyler & Gorton, 2009). Furthermore, glutathione is endogenous in low levels within the wheat germ. The presence of glutathione in wheat dough is known to increase extensibility of the gluten protein matrix. The sulfhydryl (-SH) present on the amino acid, cysteine, allows glutathione to behave as a reducing agent. By utilizing a process known as the

sulfhydryl-disulfide interchange, glutathione is continuously able form and rearrange disulfide bonds that occur intermolecularly and intramolecularly within a protein structure.

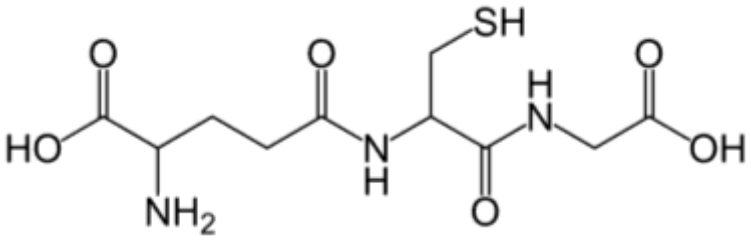


Figure 2.6: Chemical structure of glutathione

Glutathione is recognized as a safe food additive by the FDA (Yano, 2010), yet it is relatively expensive and may not be suitable for industrial applications. It was selected for this research because it has been reported to reduce disulfide bonds and aid in gluten-free baking products containing rice flour (Yano, 2010). Moreover, successfully using glutathione in a second gluten-free baking application could increase use of brewer's spent yeast (a natural and flavorful source of glutathione), ultimately creating a value-added product for another segment of the grain industry.

2.4.2 L-cysteine

L-cysteine is a synthetic version of the essential amino acid cysteine, which is naturally occurring in animal proteins. L-cysteine is the most common and extensively utilized reducing agent for wheat dough in commercial settings. It is employed for its ability to reduce mix time, increase dough extensibility and improve pan flow. For use in bread systems, L-cysteine can be used in concentrations ranging from 10-90 ppm but most commonly added at 20 to 30 ppm (fwb). As previously mentioned, L-cysteine possesses a thiol group (-SH) which provides the reducing capability. When L-cysteine participates in the sulfhydryl-disulfide interchange reaction, the number of reduced protein subunits is proportional to the number of cysteine molecules added.

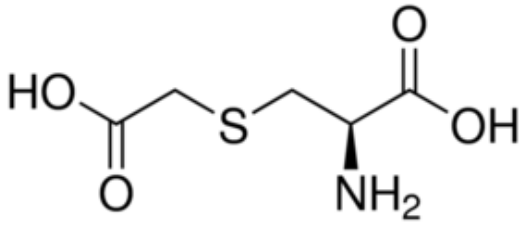


Figure 2.7: Chemical structure of L-cysteine

2.4.3 Sodium Metabisulfite

Sodium metabisulfite (SMB) is most commonly used for its antioxidant capacity in food preservation for baked goods, wine, dried fruit and jams. Most frequently, sodium metabisulfite is used as a reducing agent in cookie and cracker production. When hydrated during mixing, sodium bisulfite is formed from the sodium metabisulfite (Dow 2015), while sulfur dioxide is released and free to interact with proteins (Equation 2.1). Unlike L-cysteine or glutathione, sodium metabisulfite does not perform as a reducing agent through sulfhydryl-disulfide interchange. The active component of sodium metabisulfite is the bisulfite ion. When suspended in water, bisulfite anions within sodium metabisulfite generate a sulfurous acid which equilibrates (Shandera et al., 1995). The sulfurous acid subsequently reacts with the cysteine amino acids, creating S-sulfocysteine residues within the protein structure. These residues inhibit the restoration of disulfide bonds. Essentially, sodium metabisulfite acts as a cap, covering the reactive thiol group on cysteine so it is unavailable to reform a disulfide bond. This reaction sequence makes sodium metabisulfite uniquely different from glutathione and L-cysteine. At equal quantities of each reducing agent, sodium metabisulfite has the greatest reducing capacity, due to the two sulfur atoms available for reactions.

$$Na_2S_2O_5 + H_2O \underset{Dehyration}{\overset{Hydration/}{\longleftrightarrow}} 2\ NaHSO_3$$

Equation 2.1: Reversible conversion of sodium metabisulfite ($Na_2S_2O_5$) into sodium bisulfate ($NaHSO_3$) through hydration or dehydration reaction (Dow, 2015).

The reaction of sodium metabisulfite with disulfide bonds in wheat is extensive; cleaving nearly all the disulfide bonds. Incidentally, there is far greater danger of over-dosing a formulation with sodium metabisulfite compared to other reducing agents. This reducing power is one reason the industry currently prefers alternatives reducing agents. Precautions should be taken when using sodium metabisulfite. Even though the FDA recognizes it as a GRAS ingredient, some individuals may present a sensitivity especially asthmatics. Sulfites characteristically present an unpleasant aftertaste in finished products and it must be declared on the label if more than 10 ppm (fwb) is present in the final product. Irrespective of the negative attributes, past research has shown promising use of sodium metabisulfite as a reducing agent in sorghum. Elkhalifa et al. (1999) found, after cooking with sodium metabisulfite, there was an increase in sorghum starch digestibility. It was selected for this research due to its strong reducing power, low cost and effectiveness in past research

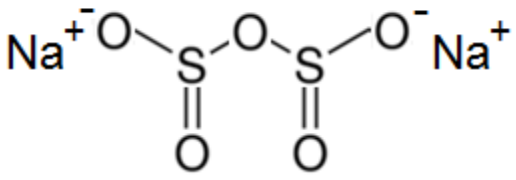


Figure 2.8 Chemical structure of sodium metabisulfite

CHAPTER 3

Materials and Methods

3.1 Materials

Commercially available, tannin-free, pearled white sorghum flour was purchased from Nu Life Market (Scott City, KS). Other ingredients included unmodified potato starch (Bob's Red Mill, Milwaukee, WI), emulsified vegetable shortening (HYMO Stratas Foods LLC, Memphis, TN) iodized salt (Kroger, Cincinnati, OH), granulated sugar (Kroger, Cincinnati, OH), hydroxypropyl methylcellulose (HPMC) (Methocel K4M, E 464, Dow Chemical Co., Midland, MI), instant nonfat dry milk (NFDM) (Honeyville, Honeyville, UT), and instant yeast (Lesaffre Yeast Corporation, Milwaukee, WI). Flour and yeast were stored at 30°F until ready to use. The reducing agents, glutathione and sodium metabisulfite were acquired from Fisher Scientific (Fair Lawn, NJ), while L-cysteine was acquired from Sigma Aldrich Co. (St. Louis, MO).

3.2 Flour Analyses

3.2.1 pH Measurement

The pH of the sorghum flour was determined using AACCI Method 02-52.01. Equipment was calibrated with standard buffers prior to analysis. A 10 g portion of sorghum flour was combined with 100 mL of distilled water and continuously agitated for 15 min using a magnetic stirrer. Once flour was fully suspended, it was allowed 10 min to settle before decanting the supernatant. The pH measurement was acquired by fully submerging the calibrated electrode in the supernatant fluid and reading the potentiometer.

3.2.2 Moisture Content

Moisture content of the sorghum flour was measured using AACCI Method 44-15.02. The weight loss of the sample during heating was measured and used to calculate moisture as a percentage of the initial sample weight. A sample of approximately 2.0 g of flour was weighed into an aluminum moisture dish. The dishes were left uncovered and placed in an air oven to bake for 60 min at 54.4°C. Immediately after heating, the aluminum moisture dishes were covered then transferred to a desiccator to cool to room temperature (24°C) (~ 45 to 60 min). The moisture content was computed using calculator provided in AACCI Method 44-15.02.

3.2.3 Protein Content

The crude protein content of the sorghum flour was acquired following the AACCI Method 46-30.01, Combustion Method. In pure oxygen, at temperatures in excess of 510°C, nitrogen contained within the flour sample is freed and measured by thermal conductivity detection. The volatile nitrogen content was multiplied by a factor of 6.25 to determine the equivalent crude protein content (%) within the sample, as shown in Equation 3.1.

a) $$\boldsymbol{Cruded\ Protein}\ (\%, \boldsymbol{as-is}) = \left(\frac{\boldsymbol{Weight\ of\ Residue}}{\boldsymbol{Weight\ of\ Sample}} \boldsymbol{x\ 100}\right) \boldsymbol{6.25}$$

b) $$\boldsymbol{Crude\ Protein}\ (\%, \boldsymbol{14\%\ mb}) = \left(\frac{(100-14)}{(100-\boldsymbol{Sample\ MC}\%)}\right) \boldsymbol{x\ Crude\ Protein\ as-is\ value}$$

Equation 3.1: Calculation for sample protein content on "as-is" basis (a) and adjusted for 14% moisture basis (b).

3.2.4 Ash Content

The ash content of the sorghum flour was measured using AACCI Method 08-01.01. Approximately 4.0 g of sorghum flour was scaled into a porcelain crucible and placed in a temperature controlled muffle furnace heated to 575°C. Samples remained in the furnace overnight

(≥16 h) then crucibles were transferred to a desiccator to cool. Once cooled to room temperature (24°C), the remnants in the crucibles were weighed. Ash content was reported as a percentage of the whole sample (Equation 3.2).

c) $$\boldsymbol{Ash}\ (\%, \boldsymbol{as-is}) = \frac{\boldsymbol{Weight\ of\ Residue}}{\boldsymbol{Weight\ of\ Sample}} \boldsymbol{x\ 100}$$

d) $$\boldsymbol{Ash}\ (\%, \boldsymbol{14\%\ mb}) = \frac{(\boldsymbol{100-14})}{(\boldsymbol{100-Sample\ MC\%})} \boldsymbol{x\ ash\ as-is\ value}$$

Equation 3.2: Calculation for sample ash content on "as-is" basis (a) and adjusted for 14% moisture basis (b).

3.2.5 Starch Damage

Starch damage of sorghum flour sample was determined using AACCI Method 76-33.01, Amperometric Method by SDmatic (CHOPIN Technologies, Villeneuve-la-Garenne Cedex, France). The SDmatic utilizes an amperometric probe to measure the speed and capacity of iodine absorption by sorghum flour in an acid solution at 35°C. The test solution was prepared in a glass reaction bowl using 120 mL of distilled water, 3.0 ± 0.2 g of boric acid and 3.0 ± 0.2 g of potassium iodine. A single drop of 0.1N sodium thiosulfate solution was added to the test solution prior to sample loading. Once the glass reaction bowl containing the test solution was loaded onto the apparatus, the head of the apparatus was lowered to submerge the measuring probe, stirrer and heating resistor. Next, 1.0 ± 0.100 g of sorghum flour was scaled on to an SDmatic plastic sample holder, which was then inserted into the vibrating bed. Flour sample weight, moisture content and protein content were recorded into the software prior to test initiation. Test solution and sodium thiosulfate were heated and mixed thoroughly to ensure accurate iodine production, followed by the addition of sorghum flour and continued mixing to facilitate iodine absorption. Upon test completion, the SDmatic display provided iodine absorption rate (AI%) or starch damage of the

sorghum flour in Chopin Dubois units (UCD) and AACCI Method 76-31.01 units (%, fwb). Due to the settings of the SDmatic, the output values given were adjusted for sorghum flour using the calibrated equation developed by Wilson et al. (2016)(Equation 3.3).

$$\textbf{Starch Damage (sorghum flour)} = \mathbf{0.168(Ai\%)^2 + -30.123(Ai)\% + 1349.648}$$

Equation 3.3: Calibrated linear regression model (r=0.95) for sorghum starch damage using the SDmatic (Wilson et al., 2016).

3.3 Bread Method

Bake tests of sorghum bread containing reducing agents were conducted to study bread baking potential. The batter bread formulation was adapted from previous sorghum research described by Schrober et al. (2005), and is listed in Table 3.1. Ingredient proportions are reported on a flour weight basis (fwb), where sorghum flour and potato starch comprise the total flour weight.

Table 3.1: Sorghum bread formulation

Ingredients	Flour Basis (%)
Sorghum flour [a]	70.0
Potato starch [a]	30.0
Sucrose [b]	6.0
Emulsified shortening	3.0
HPMC	2.0
Active dry yeast	2.0
Salt [b]	1.5
Non-fat dry milk (NFDM)	1.0
Distilled water	105.0

[a] Sorghum flour and potato starch comprise the total flour weight; all percents based on flour weight basis.

[b] Ingredient added in solution (Appendix A)

Batters were produced in a Hobart stand mixer model N-50 (The Hobart Mfg., Troy, OH) using a flat paddle attachment. Sorghum flour, potato starch, HPMC, NFDM, instant yeast, and

emulsified shortening were scaled and blended together for 1 min on speed 2 to fully homogenize ingredients. Liquid ingredients were then added to include sugar–salt solution, reducing agent solution and distilled water (Appendix 1). Dependent on concentration treatment, different aliquot volumes of stock reducing agent solutions were added to the formula. A complementary volume of distilled water was added to the formula last to achieve a final water content of 105% (fwb).

All ingredients were mixed for 30 sec on speed 1. The bowl and paddle attachment were scraped with a rubber spatula then mixing continued for an additional 2.5 min on speed 2. Following mixing, 250 g of batter were scaled into a greased loaf pan (9 cm x 15 cm x 5.5 cm), labeled and placed in the fermentation cabinet (National Manufacturing Co., Lincoln, NE) to proof for 35 min at 30°C with 95% relative humidity (RH). Baking pans were directly transferred from the fermentation cabinet into a rotary baking oven (National Manufacturing Co., Lincoln, NE) to bake at 204°C for 30 min. Beakers of water were placed on alternate shelves inside the oven to add steam into the oven. After baking, loaves were de-panned and allowed to cool for 1.5 to 2.0 h on wire racks prior to further analyses or packaging.

3.4 Bread Analyses

After baking, bread was allowed to cool to room temperature (24°C) before further testing occurred. This ensured moisture and temperature equilibriums were attained and samples provided accurate, representative values during analysis. Once the weight and volume measurements were recorded, bead loaves proceeded to further analyses.

3.4.1 Specific Loaf Volume

Bread loaves were weighed (g) and respective volumes (cm^3) were measured by rapeseed displacement in accordance with AACCI Method 10-05.01. Specific volume (cm^3/g) was calculated by dividing loaf volume by loaf weight.

3.4.2 Internal Crumb Structure

The C-Cell (Calibre Control International Ltd., Appleton, Warrington, United Kingdom) was used to evaluate the internal crumb structure. The instrument uses dedicated image analysis software (C-Cell Software Version 2.0) to quantify cell characteristics. The characteristics of interest for this study were cell number, cell diameter and cell wall thickness. For the analysis, two 35 mm slices were analyzed from each bread loaf and photographed using the C-Cell imaging system to obtain high-definition images.

3.4.3 Staling Study

Texture analysis of the sorghum bread crumb was measured over a period of time to accomplish a staling study. The analyses were performed using a TA.XT*Plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) in accordance with AACCI Method 74-10.02 Measurement of Bread Firmness—Compression Test.

For each treatment, three batters were made, each yielding four loaves of bread. Bread loaves produced from the same batter were analyzed on different days. After baking, weights and volumes were recorded for each loaf. One hour after baking, loaves were double packaged in polyethylene bread bags and stored at room temperature (24°C). On each test day, three loaves of each treatment were cut into six 25 mm thick slices using a slice regulator. The two end slices were discarded, while three of the middle slices were chosen at random for texture analysis. The TA.XT*Plus* was outfitted with a TA-4 acrylic cylinder (38-mm diameter, 35-mm tall) with a 5 kg load cell. The analysis was carried out at constant speed of 1.0 mm/s with pre-test and post-test speeds of 10.0 mm/s over a distance of 10.0 mm. A trigger force of 5.0 g was used to compress the center point of the slice to 40% (10 mm) of the 25 mm bread slice. The force to compress 40% of the slice is taken as the firmness of the crumb and is an indication of freshness and quality. The elasticity of the crumb is

calculated as a percent of the force at 30 sec of relaxation and the peak force multiplied by 100, and is an estimate of bread cohesiveness or gumminess in cakes.

3.4.4 Protein Digestibility

Protein digestibility was assessed using the *in-vitro* pepsin digestibility (IVPD) method as described by Mertz et al. (1984). Bread samples containing reducing agent treatments, were frozen and lyophilized then subsequently ground using the Udy Cyclone Sample Mill (Udy Corporation, Fort Collins, CO) equipped with 0.5 mm screen. A 200 mg portion of ground bread sample was suspended in 35 mL of pepsin solution (1.5mg/mL) in 0.1 M phosphate buffer (pH 2.0). The mixture was incubated with gentle agitation at 37°C for 2 h using an Incu-Shaker 10L (Benchmark Scientific, South Plainfield, NJ). After incubation, 2 mL of 2.0 M sodium hydroxide was added to stop the digestion reaction. The suspension was then centrifuged (3220xg) for 15 min at 4°C. The supernatant fluid was decanted while the residue was washed in 10 mL of 0.1 M phosphate buffer (pH 2.0) and centrifuged a second time under the same conditions. Washing cycle was repeated once more, followed by centrifugation. The supernatant was again decanted and the remaining sample sediment was placed into a -80°F freezer. After being lyophilized and dried, the protein contents of digested and undigested breads were determined by nitrogen combustion using a Leco nitrogen determinator (Leco, St. Joseph, MO) according to AACCI Method 46-30.01. Protein digestibility was calculated using Equation 3.3 and expressed as a percentage of total protein in bread sample.

$$\textbf{Protein Digestibility}\ (\%) = \frac{(\textbf{N in raw sample} - \textbf{N in digested sample})}{\textbf{N in raw sample}} \textbf{x 100}$$

Equation 3.4: Equation for protein digestibility calculated from nitrogen content of raw and digested samples of treated breads.

3.5 Batter Analyses

3.5.1 Batter Consistency

The batter consistency was analyzed using a Rapid Visco™ Analyser (RVA 4500, Perten Instruments AB, Hägersten, Sweden) according to AACCI Method 76-21.03. Prior to analysis, the moisture contents of the sorghum flour and partial formula blend samples were determined. To ensure samples were comparable on a 14% (mb), 3.36 g of sorghum flour and 3.41 g of the partial formula blend, based on respective moisture contents, was added to 25 mL of distilled water.

For treatment samples, a 25 mL volume of reducing agent solution was added to a new aluminum sample can. The sample can was fitted with a polycarbonate stirring paddle then mounted onto the machine. When the test was initiated, the sample was gently mixed while subjected to the Standard 1 temperature profile (Table 3.2). The parameters analyzed during the RVA test include peak viscosity, holding strength, final viscosity, peak time and pasting temperature. The generated curve (Figure 3.1) shows the viscosity transition in relation to time and temperature. The viscosity measurements were recorded in centipoise (cP) units (1 cP= 1 mPa/sec).

Table 3.2: Standard 1 RVA temperature profile [a]

Stage	Temperature/ Speed	STD1
1	50°C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 10 sec
4	50°C	1 min, 0 sec
5	95°C	4 min, 42 sec
6	95°C	7 min, 12 sec
7	50°C	11 min, 0 sec
End of test		13 min, 0 sec
Time between readings		4 sec

* Idle temperature: 50 ± 1 °C

[a] Adapted from Perten Instruments (2013)

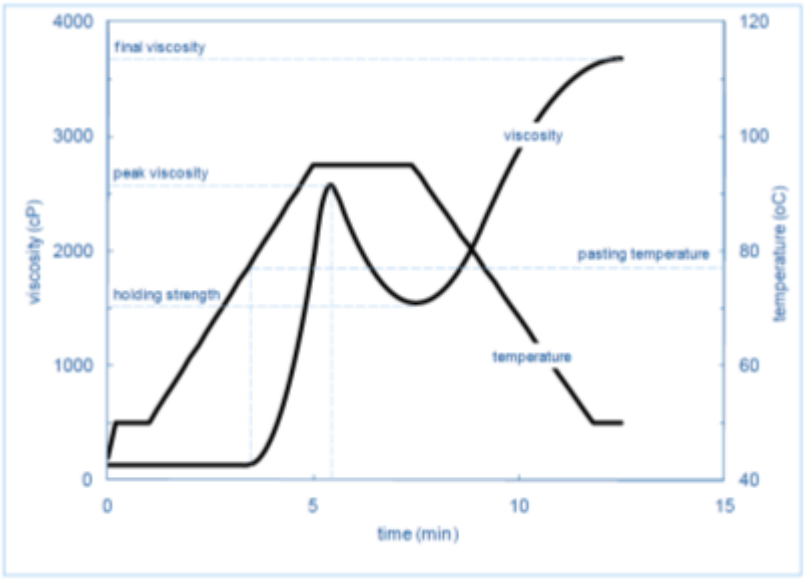


Figure 3.1: Typical RVA pasting profile showing the commonly measured parameters (Perten Instruments, 2013).

3.5.2 Protein Analysis

Protein analysis was accomplished following the method previous described by Bean et al. (2011). Reversed-phase high performance liquid chromatography (RP-HPLC) was performed with an Agilent 1100 series instrument (Agilent, Santa Clara, CA) using surface porous Poroshell 300 SB columns (2.1 x 75 mm) with C18 as the stationary phase (Agilent, Santa Clara, CA). Mobile phase A was deionized water plus 0.1% trifluoroacetic acid (TFA) (w/v) and mobile phase B was acetonitrile (ACN) plus 0.07% (w/v) TFA.

Chemicals and Samples. RP-HPLC sampling was completed at the ARS-CGAHR USDA facility in Manhattan, KS. HPLC-grade β-mercaptoethanol (BME) and 4-vinylpyridine (4-VP) were obtained from Sigma Aldrich Co. (St. Louis, MO). Reducing agent solutions were prepared using distilled water plus glutathione, L-cysteine or sodium metabisulfite at several concentrations (Appendix A).

Preliminary Sample Preparation. Samples were prepared using 100 mg pearled white sorghum flour and 100 µL distilled water or 100 µL of 0.5%, 1.0%, 2.5% or 5.0% (w/v) of reducing agent solution. BME was used as a positive control while no reducing agent was used as a negative control. Samples were continuously stirred by hand for 3 min using a metal spatula then transferred immediately into a -80°F freezer and lyophilized.

1ST Stage Sample Preparation. Samples were prepared using 100 mg pearled white sorghum flour and 100 µL distilled water or 100 µL of reducing agent solution (0.5%, 1.0%, 2.5%, 5.0% (w/v) of L-cysteine in distilled water; 100 ppm, 250 ppm, 500 ppm, 1000 ppm (w/v) of sodium metabisulfite in distilled water. BME was used as a positive control while no reducing agent was used as a negative control. Samples were continuously stirred by hand for 3 min using a metal spatula. Once mixed, samples were simultaneously heated and agitated using a VorTemp™ 1550 Shaking Incubator (Labnet International, Inc., Edison, NJ) set at 30-32°C for 35 min. Samples were then transferred immediately into a -80°F freezer and lyophilized.

2ND Stage Sample Preparation. Samples were prepared using 100 mg of the partial sorghum bread formulation, consisting of 70% (fwb) sorghum flour, 30% (fwb) potato starch and 2% (fwb) HPMC, and 100µL distilled water or 100 µL of 0.5%, 1.0%, 2.5% or 5.0% (w/v) of reducing agent solution. BME was used as a positive control while no reducing agent was used as a negative control. Samples were continuously stirred by hand for 3 min using a metal spatula. Once mixed, samples were simultaneously heated and agitated using a VorTemp™ 1550 Shaking Incubator (Labnet International, Inc., Edison, NJ) set at 30-32°C for 35 min. Samples were then transferred immediately into a -80°F freezer and lyophilized.

Kafirin Extraction Procedure. Kafirins were extracted from lyophilized samples using two cycles of rinsing with 1.0 mL of 6.65% (v/v) 4-vinylpyridine (4-VP) alkylating solution followed by homogenization using a Vortex-Genie 2 (Scientific Industries, Bohemia, NY) at approximately 3000

rpm for 30 sec. Samples were then centrifuged (Eppendorf™ 5424 Microentrifuge, Fisher Scientific, New Lawn, NJ) for 4 min at a speed of 10,000 rpm. A third cycle of 1.0 mL 4-VP rinse was accomplished followed by a 60 min vortex stage before entering the centrifuge for 4 min at 10,000 rpm. After centrifugation, the supernatant was decanted into a waste container while the sediment material was subjected to further extraction procedures. Two extraction cycles were accomplished consisting of 1.0 mL 60% (v/v) t-butanol (t-buOH) rinse, 5 min vortex and 4 min centrifuge at the previously stated speeds. Between cycles a 0.5 mL aliquot of the supernatant was transferred to a clean microtube, while the remaining supernatant was decanted to waste. After the two cycles, each sample existed as a 1.0 mL aliquot of pooled extract. Next, a 20 µL of 2% BME (v/v) was added to the sample and vortexed for 15 min at 3000 rpm. The samples were alkylated a final time using 66.7 µL of 4-VP followed by 10 min of vortex. Lastly, alkylated extracts were transferred to clean HPLC vials to be injected into the RP-HPLC for analysis.

3.5.3 pH Measurement

The pH of the batter was determined using AACCI Method 02-52.01. The pH meter and electrode were calibrated with standard buffer solutions prior to analysis. A 10 g portion of batter was combined with 100 mL of distilled water and continuously agitated for 15 min using a magnetic stirrer. Once batter was fully suspended, it was allowed 10 min to settle before decanting the supernatant. The pH measurement was acquired by fully submerging the electrode in the supernatant fluid.

3.5.4 Yeast Activity

The yeast activity of the batter was determined by measuring CO_2 gas production using a Risograph (National Manufacturing Co., Lincoln, NE) following AACCI Method 89-01.01. Batters were prepared in accordance with the previously described bread method. A 50 g portion of batter

was scaled into a stainless steel chamber, placed into a water bath set at 30°C and allowed 5 min to acclimate before test was initiated. Just prior to testing, the stainless steel chamber was attached to a gas-measuring device. Yeast activity was determined by the volume of gas evolved over a period of 90 min at standard barometric pressure.

3.6 Statistical Design and Analyses

In this study, reducing agents, glutathione and L-cysteine were tested at four concentrations, 0.5%, 1.0%, 2.5% or 5.0% (fwb), while sodium metabisulfite was tested at 100 ppm 250 ppm, 500 ppm, and 1000 ppm (fwb). Triplicate readings of each chemical, physical and textural test were performed.

All data from the chemical, physical and textural tests were analyzed using JMP, Software Release 12.0 (SAS, Institute Inc., Cary, NC, 2016). When treatment effects were found to be significantly different, the least square means with Tukey-Kramer groupings were used to differentiate treatment means at a level of significance of $\alpha < 0.05$.

CHAPTER 4

Results and Discussion

4.1 Prologue to Study

For this study a flour from a white, non-waxy sorghum variety, *Jowar*, was selected. Non-waxy varieties retain a starch profile with a normal distribution of amylose and amylopectin. Furthermore, white sorghum possesses a pericarp with less than 2% pigmented testa. Implying, once decorticated, the majority of any confounding effects imparted by the pericarp and corresponding chemical components are significantly reduced. Decorticated white sorghum flour was procured through Nu Life Market and was utilized for all experimentation.

4.2 Physiochemical Flour Composition

The physiochemical composition of the flour is summarized in Table 4.1. Ash determination was performed to define the mineral residue content and correlate flour performance. Since sorghum bran in rich in tannins and other antioxidants, a sorghum flour containing bran particles, indicated by a high ash content, would be expected to decrease flour performance. The sorghum flour had an average ash content of 0.926 ± 0.002% (fwb). Flour moisture content was determined to control storage conditions, rheological properties of batter and control final product characteristics. Moisture content average was 10.95 ± 0.08% (fwb). Protein determination was performed using a Leco TruSpec CN. The protein content of the flour was approximately 7.73 ± 0.04% (fwb). Due to the rigidity of the protein body structures, protein content is not directly tied to the functional properties of the flour, yet the potential remains to contribute to flour functionality if released from those structures (Hamaker & Bugusu, 2003). The pH of flour was also determined and was found to be consistent with other cereal grains, ranging from 6.00 to 6.20.

Table 4.1: Summary of physiochemical properties of pearled white sorghum flour

Parameter	Amount (fwb)
Moisture	10.95 ± 0.08%
Ash	0.926 ± 0.002%
Protein	7.73 ± 0.04%

Starch damage is an important factor in determining dough performance during mixing and directly influences dough behavior during fermentation. Damaged starch granules are more susceptible to swelling and degradation by α-amylase than intact starch granules (Delcour & Hoseney, 2010b). Subsequently, starch damage affects the volume of finished products as well as color. As previously mentioned in the materials and methods section, the rate of starch damaged was measured by an amperometric method where an electrode generates iodine and the device continually measures an electric current that is proportional to the amount of free iodine in the solution. Once flour is added, iodine binds to the damaged starch granules. The greater the iodine absorption, the greater the proportion of starch damaged. Sorghum flour samples were tested in triplicate, and adjusted using Wilson et al. (2016) calibrated equation to provide an average iodine absorption of 96.76% (± 0.34%), and average starch damage was 7.87% (± 0.79%) (AACCI Method 76-31.01).

4.3 Bread and Batter Results

A pH determination was accomplished to gauge if the batter environment was suitable for a yeast leavened bakery product in addition to facilitating the intended reduction reaction. The rate of the sulfhydryl-disulfide interchange reaction is pH dependent. At a pH of 7, sulfhydryl groups are predominantly in a protonated state. As pH increases so will the reaction rate until the sulfhydryl group is deprotonated (Nagy, 2013). Therefore, batter must maintain a pH 6.0 - 8.0 to facilitate the

cleaving of disulfide bonds. The pH of batter for the control as well as reducing agent treatments were determined to be a suitable environment to enable the reaction, ranging between 6.00 to 6.80.

4.3.1 Protein Analysis Results

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to separate kafirin proteins and provide analysis for classification and quantification of individual or total peak area for protein fractions. Kafirins have a distribution of molecular weights; a difference of 3 to 12 kDa separates one subclass from the next. Therefore, the separation of proteins can provide information relative to functional properties based on amount, presence of specific proteins, effect of processing, and/or differences between cultivars (Bean et al., 2011).

Total kafirin extraction was made possible by first removing the salt-soluble globulins proteins followed by remaining water-soluble components including albumins. After extraction procedures were complete, samples were transferred to a clean HPLC vial and loaded for analysis. Samples were injected into a pre-equilibrated column at time 0 min. The eluent streams were measured at multiple wavelengths, but 214 nm was used for peak integration and data collection.

Opposite to traditional high performance liquid chromatography, RP-HPLC uses a nonpolar stationary phase and a polar mobile phase. RP-HPLC is routinely used in the analysis of cereal proteins. During the extraction procedure, solutes are retained within the column by hydrophobic interactions with the stationary phase. Kafirins are known for their hydrophobicity. The hydrophobic portions present on kafirin proteins bind to the nonpolar, hydrophobic C18 chains that comprise the stationary phase. As the mobile phase gradient shifts from predominantly polar (mobile phase A) to nonpolar (mobile phase B), solutes elute in order of decreasing polarity thus increasing hydrophobicity (Reuhs & Rounds, 2010). The resulting data is a curve consisting of varying peaks which describe the different protein fractions. Oddly, γ-kafirins elute first, followed by β-kafirins and eventually α-kafirin fractions. Peaks belonging to β-kafirins and α-kafirin often blend

together due to the overlapping elution. The greater the peak area, the greater the concentration of the corresponding protein is present in the sample.

The reducing agent, β-mercaptoethanol (BME) was used at 2.0% (v/v) in distilled water as a positive control to provide a reference chromatogram of kafirin peaks after the reduction reaction. In contrast, the lack of a reducing agent was used as a negative control to generate a baseline chromatogram, illustrating the quantity of kafirins in sorghum flour and partial formula blend samples when the reduction reaction is absent. By comparing the treatment samples to the positive and negative controls, it was clear to determine if a reduction of protein body structure occurred. Both concentration and type of reducing agent are influential to the extraction of sorghum proteins (Park et al., 2006). When protein body structures are reduced, larger quantities of individual kafirin fractions elute, resulting in large peaks on the chromatogram, which are decipherable when compared to a chromatogram of the negative control. The total peak area correlates to the quantity of free proteins, therefore an increase in peak area corresponds to an increase in the amount of proteins reduced. From 0 to 3 min the resulting peak was not captured as part of the total peak area as it consisted of residual molecules contained in the column which were not relevant for analysis. Total peak area (mAU) was calculated for each sample run using the Agilent software.

Preliminary RP-HPLC testing uncovered L-cysteine and sodium metabisulfite at 0.5 % to 5.0% (fwb) exhibited significant reduction of protein structures, indicated by large total peak areas. Unfortunately, glutathione treatments at all concentrations produce low total peak areas, similar to the negative control, signifying protein reduction did not adequately occur during the given reaction time. Due to the lack of protein reduction, glutathione was omitted from the remainder of the study (Appendix B). Subsequent baking trials revealed the tested levels of L-cysteine produced good bread loaves, yet sodium metabisulfite at the same concentrations, produced inadequate bread loaves

(Appendix B). Thus, sodium metabisulfite levels were reduced to 100 ppm, 250 ppm, 500 ppm and 1000 ppm (fwb).

1ST Stage Analysis. RP-HPLC analysis of 100 mg sorghum flour samples treated with 100 µL distilled water or reducing agent solution (0.5%, 1.0%, 2.5%, 5.0% (w/v) of L-cysteine in distilled water; 100 ppm, 250 ppm, 500 ppm, 1000 ppm (w/v) of sodium metabisulfite in distilled water, were submitted to a 3 min mixing period then held at 32°C for an additional 35 min simulated proofing during the bread making process. For all sample treatments, total peak area significantly increased when subjected to simulated proofing compared with the 3 min holding time. The increase in total peak area is likely the result of longer reaction time and elevated temperature. The additional 35 min at 32°C provided samples the necessary time and conditions to facilitate further protein reduction. Sorghum flour treated with 2.5% (fwb) and 5.0% (fwb) of L-cysteine along with BME generated the greatest total peak areas (Figure 4.1a). Due to a lack of significant difference in total peak area between 2.5% (fwb) and 5.0% (fwb), and supporting bake test results, 2.5% (fwb) was deemed the optimum concentration for L-cysteine (Table 4.2).

Samples treated with all levels of sodium metabisulfite did not produce total peak areas significantly different from one another (Figure 4.1b). Yet when used at a concentration of 1000 ppm, sodium metabisulfite produced a greater total peak area (2662 mAU) than the negative control (896 mAU). As with the L-cysteine treatment, an optimum concentration of sodium metabisulfite was determined based on the amount of kafirin protein reduction and performance during bake tests. Sodium metabisulfite at 500 ppm (fwb) was considered the optimum concentration.

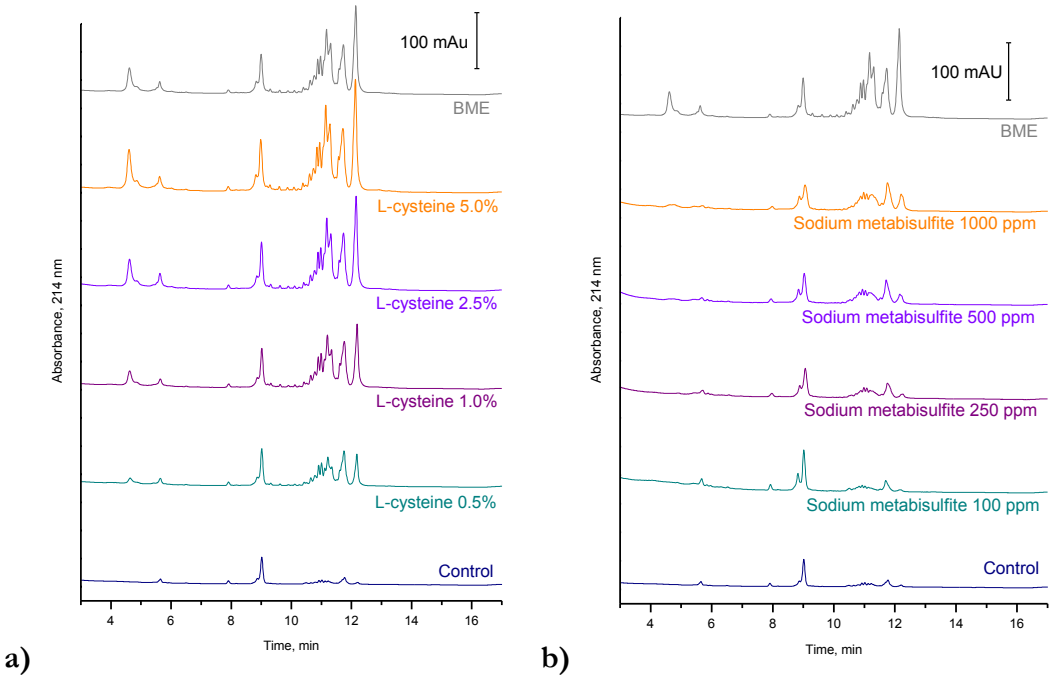


Figure 4.1: RP-HPLC chromatograms for 1st stage analysis with sorghum flour and simulated proofing conditions for L-cysteine (a) and sodium metabisulfite (b) treatments.

2ND Stage Analysis. The final RP-HPLC analysis was of a partial formula blend of 70% (fwb) sorghum flour, 30% (fwb) potato starch and 2% (fwb) HPMC with L-cysteine and sodium metabisulfite solutions and subjected to a 3 min mixing period followed by a 35 min holding periods in a simulated proofing environment (32°C). The partial formula blend was tested to determine if other major ingredients influenced the behavior of the reducing agents. Total peak area of the partial formula blends (Figure 4.2 and Table 4.2) were significantly lower in contrast to the total peak areas of sorghum flour for all treatments (Figure 4.3). Significantly lower total peak area across all treatments insinuates the addition of potato starch and HPMC influence reducing agent effectiveness. Speculatively, there are two potential reasons total peak areas were reduced in the partial formula compared to the sorghum flour, for all treatments. First, sample size was consistently 100 mg, thus, treated samples using a partial formula blend only contained approximately 67.3 mg of

sorghum flour whereas samples using strictly sorghum contained 100 mg of sorghum flour. A difference in the initial protein content of the partial formula blend samples could be the cause of lower total peak area. Secondly, nearly a third of the partial formula blend is comprised of potato starch, which is capable of retaining large amounts of water. Because the reducing agent is suspended in solution, perhaps when the potato starch granules absorb water, the mobility of reducing agent is decreased. If this were the case, limited mobility would make it difficult for the reducing agents to reach and interact with the intended kafirin proteins.

Regardless of the potential hindrance from other ingredients, samples treated with 2.5% (fwb) and 5.0% (fwb) L-cysteine displayed the greatest total peak areas (4107 mAU and 4205 mAU, respectively), along with BME (4038 mAU). Samples treated with sodium metabisulfite produced lower total peak areas compared to those treated with L-cysteine. Within sodium metabisulfite treatments, 500 ppm and 1000 ppm (fwb) produced the greatest total peak areas, 1774 mAU and 1881 mAU, respectively.

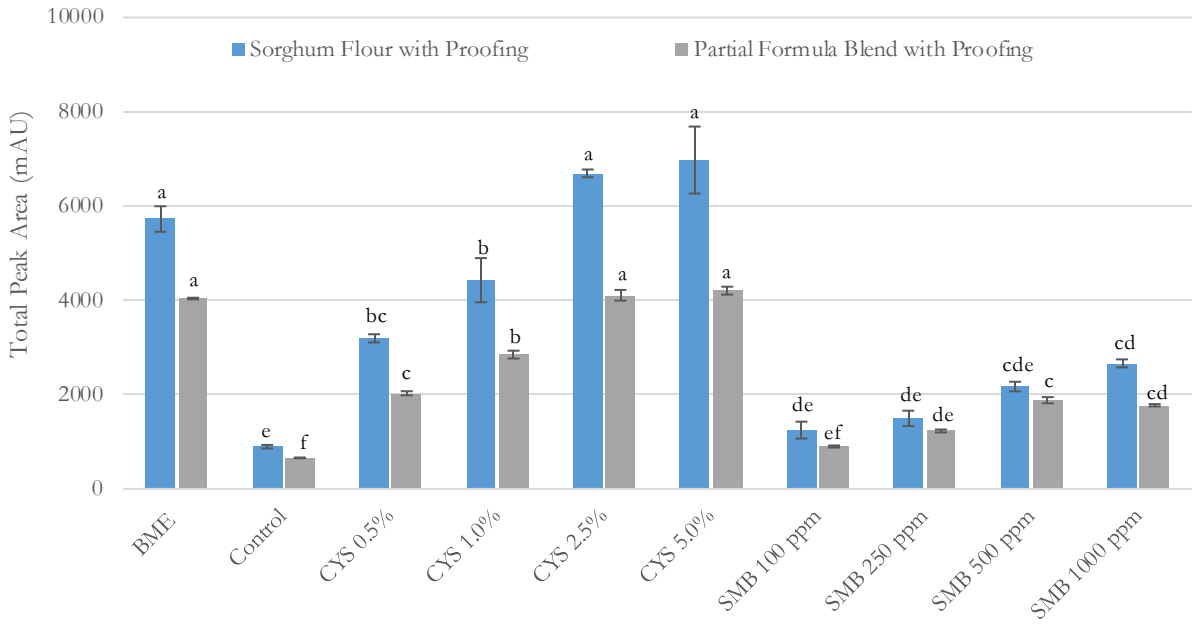


Figure 4.2: Total peak area (mAU) of sorghum flour samples and partial blend formula samples containing for L-cysteine (CYS) and sodium metabisulfite (SMB subjected to simulate proofing conditions.

*Error bars are standard error for each treatment mean.

Table 4.2: RP-HPLC total peak area (mAU) of extracted kafirins from sorghum flour and partial formula blend samples[a] treated with reducing agents and simulated proofing

Treatment	Sorghum flour	Partial formula blend
BME	5725a	4038a
Control	896e	659f
L-cysteine 0.5%	3194bc	2028c
L-cysteine 1.0%	4428b	2849b
L-cysteine 2.5%	6693a	4107a
L-cysteine 5.0%	6976a	4206a
Sodium metabisulfite 100 ppm	1247de	900ef
Sodium metabisulfite 250 ppm	1496de	1229de
Sodium metabisulfite 500 ppm	2171cde	1881c
Sodium metabisulfite 1000 ppm	2662cd	1774cd

* Values followed by different letters within a column are significantly different ($p < 0.05$)

[a] Partial formula blend samples: 70% (fwb) sorghum flour, 30% (fwb) potato starch and 2% (fwb) HPMC

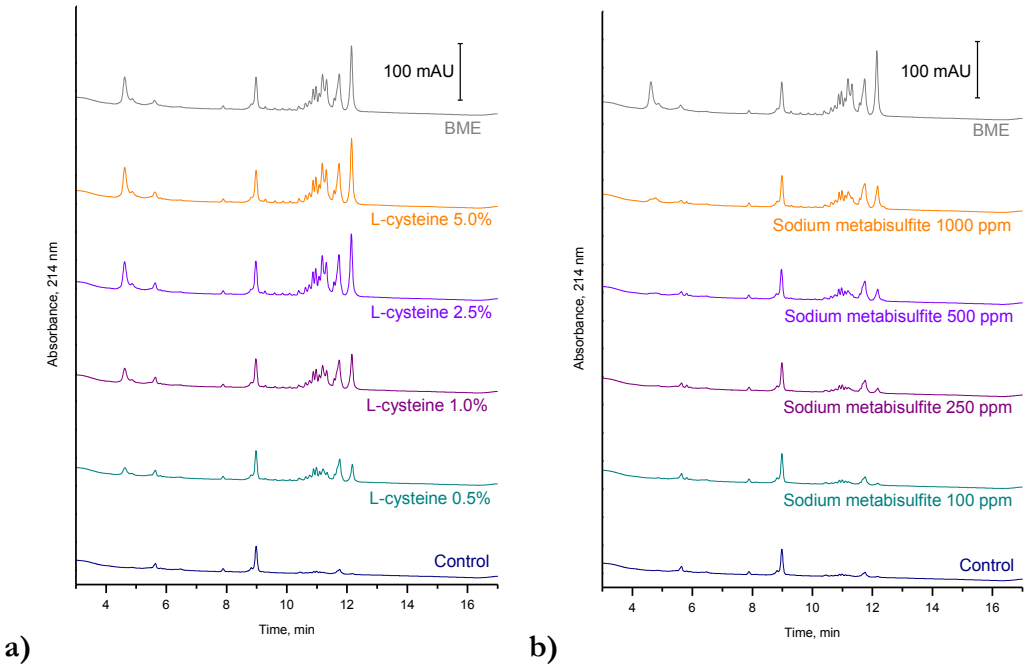


Figure 4.3: RP-HPLC chromatograms for 2nd analysis with partial formula blend and simulated proofing conditions for L-cysteine (a) and sodium metabisulfite (b) treatments.

4.3.2 Bread Baking Results

Bake tests were conducted to determine the impact of reducing agents on bread volume, while C-Cell imaging was used to investigate crumb grain characteristics. L-cysteine was tested at 0.5%, 1.0% and 2.5% (fwb). Bread was not baked using 5.0% (fwb) L-cysteine because there was not a significant difference in protein reduction (shown previously in RP-HPLC analysis) between 2.5% (fwb) and 5.0% (fwb) L-cysteine. Sodium metabisulfite was evaluated at 100 ppm, 250 ppm and 500 ppm (fwb). Bread containing 1000 ppm (fwb) of sodium metabisulfite was omitted due to lack of a significant difference in protein reduction between 500 ppm (fwb) and 1000 ppm (fwb) concentrations. When incorporated at higher levels (0.5% to 2.5% fwb) sodium metabisulfite produced loaves with poor volume and dense crumb (Appendix B). Glutathione was not baked due to the lack of protein reduction found with RP-HPLC analysis.

As shown in Table 4.3, bread loaf volume and specific loaf volume between breads formulated with L-cysteine and sodium metabisulfite were similar to the control treatment. Bread containing 2.5% (fwb) L-cysteine had significantly lower loaf volume than breads containing 500 ppm (fwb) sodium metabisulfite. Yet there was not a difference in specific loaf volume between any treatments.

Table 4.3: Physical characteristics of sorghum bread at optimum concentrations

Treatment	Actual Volume (cm^3)	Specific Volume (cm^3/g)	Number of Cells (mm)	Cell Wall Thickness (mm)	Cell Diameter (mm)
Control	561.7ab	2.74a	3695a	0.524a	4.155ab
L-cysteine 0.5%	558.3ab	2.80a	3403a	0.542a	3.416bc
L-cysteine 1.0%	561.7ab	2.76a	3643a	0.524a	3.316bc
L-cysteine 2.5%	548.3b	2.68a	3589a	0.518a	2.764c
SMB 100 ppm	580.0ab	2.81a	3720a	0.525a	4.553a
SMB 250 ppm	580.0ab	2.81a	3699a	0.529a	4.266ab
SMB 500 ppm	586.7a	2.82a	3770a	0.520a	3.663abc

* Values followed by different letters are significantly different, within a column ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)

The internal crumb grain characteristics also exhibited differences between reducing agents. The diameter of the gas cells within the crumb were statistically greater for breads containing 100 ppm (fwb) sodium metabisulfite compared to breads containing any level of L-cysteine. Except for 2.5% (fwb) L-cysteine, all treatments exhibited the same cell diameter as the control bread crumb. The cell wall thickness and the number of gas cells present within the bread crumb were not statistically different between treatments (Table 4.3).

The optimum concentrations were based on bake quality characteristics. In Figure 4.4, C-Cell images provide a clear illustration of the crumb grain characteristics in addition to some indication of bread loaf symmetry and volume. Between breads treated with sodium metabisulfite, 500 ppm (fwb) was deemed the optimum concentration. Likewise between breads treated with L-cysteine, 2.5% (fwb) was considered the optimum concentration.

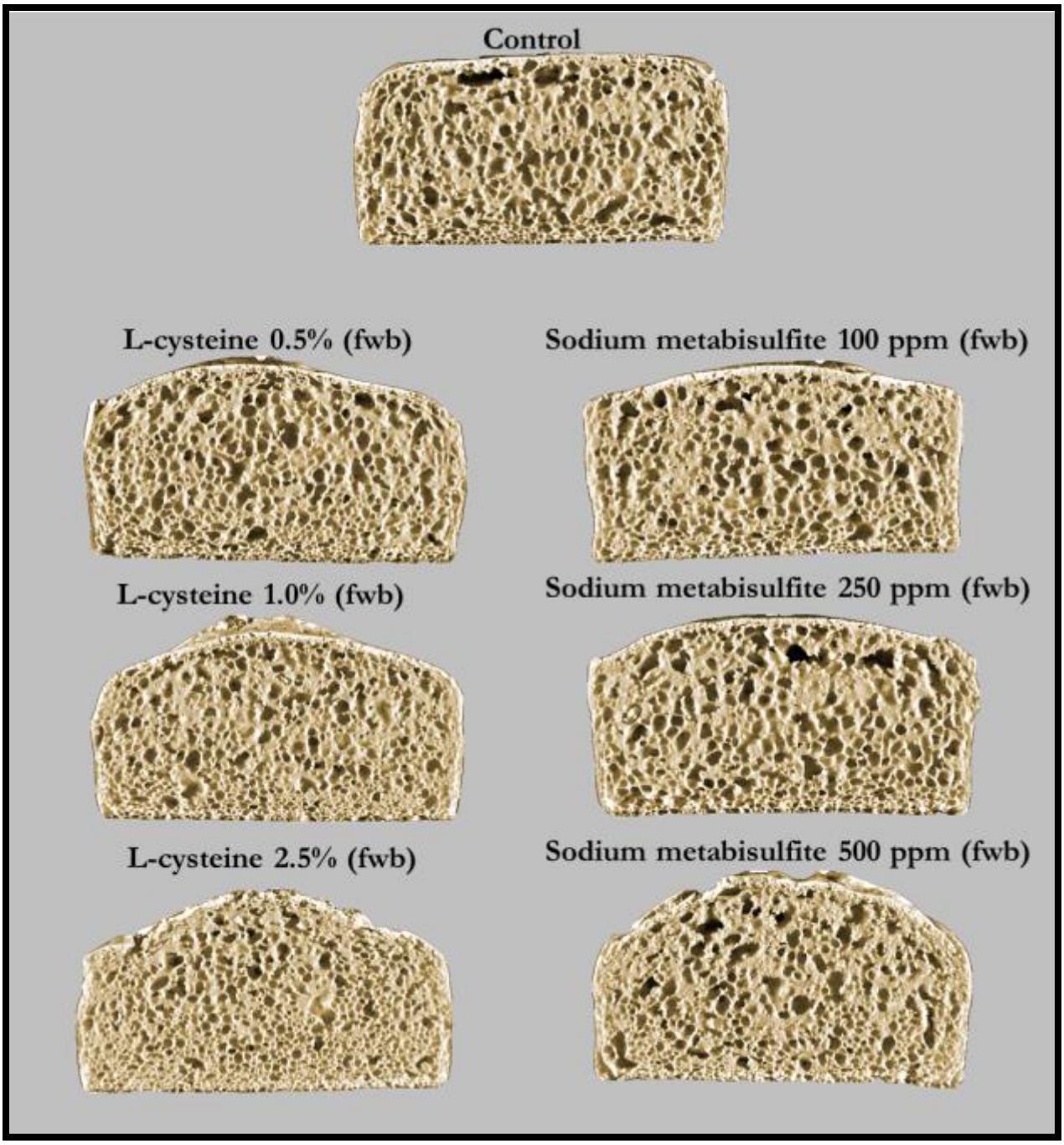


Figure 4.4: C-Cell images of bread slices taken from the center of breads treated with 0.5%, 1.0% and 2.5% (fwb) of L-cysteine and 100 ppm, 250 ppm, and 500 ppm (fwb) of sodium metabisulfite.

Based on the volume contour of the bread loaves, an argument could be made that the lack of water level optimization played an important role in attaining or preventing the maximum loaf volume for each treatment (. Since the implemented bread method closely resembles a traditional cake method, it is intuitive that water level optimization may be determine in the same fashion as high-ratio cakes. In Figure 4.4, the C-Cell image of breads containing 100 ppm (fwb) sodium metabisulfite or 0.5% (fwb) L-cysteine along with the control bread, exhibited a nearly-flat top without cracks. The lack of contour in the bread indicates the bread formulation contained excess water. Inversely, bread containing sodium metabisulfite at 500 ppm (fwb) or L-cysteine at 2.5% (fwb) presented a rounded top with minimal to severe surface cracks, which is a standard indication the bread formulation contained an insufficient amount of water. In order to confidently and conclusively state reducing agents do not significantly affect loaf volume, further investigation needs to occur in which water levels are optimized for each reducing agent treatment.

4.3.3 Yeast Activity and Gas Production

The yeast activity of batters were evaluated using a Risograph. Batters were prepared in accordance with the previously described bread method. A 50 g portion of batter was scaled into a stainless steel chamber, placed into a water bath. Just prior to testing, the stainless steel chamber was attached to a gas-measuring device. Yeast activity was determined by the volume of gas evolved over a period of 90 min at standard barometric pressure. The selected concentration of L-cysteine (2.5% fwb), sodium metabisulfite (500 ppm fwb) and the control batters were tested.

Although the analysis lasted for 90 min, the activity occurring between 3 and 40 min of analysis was of most importance for the implemented bread method (Schrober et al., 2005), which has a 35 min proofing period. The analysis captured both the rate of CO_2 gas evolution at each minute (Figure 4.5), and the cumulative total of CO_2 gas produced (Figure 4.6), illustrating yeast activity rate and the gas production capability, respectively.

Yeast activity in batters treated with 500 ppm (fwb) sodium metabisulfite was similar to the control batters throughout the 40 min testing period. Sodium metabisulfite and the control reached a peak yeast activity rate at 35 min and maintained that rate for the remained of the analysis. Batters treated with 2.5% (fwb) of L-cysteine exhibited yeast activity similar to the control and sodium metabisulfite treatments during the first 15 min, then at 20 min showed a significantly lower rate (Table 4.4). In comparison to the control and sodium metabisulfite at 500 ppm (fwb), L-cysteine at 2.5% (fwb) reached peak yeast activity at 20 min, before it began to decline at 35 min until the end of testing. This suggests when utilizing L-cysteine at 2.5% (fwb) the ideal proofing period would be 15 to 20 min to achieve peak yeast activity.

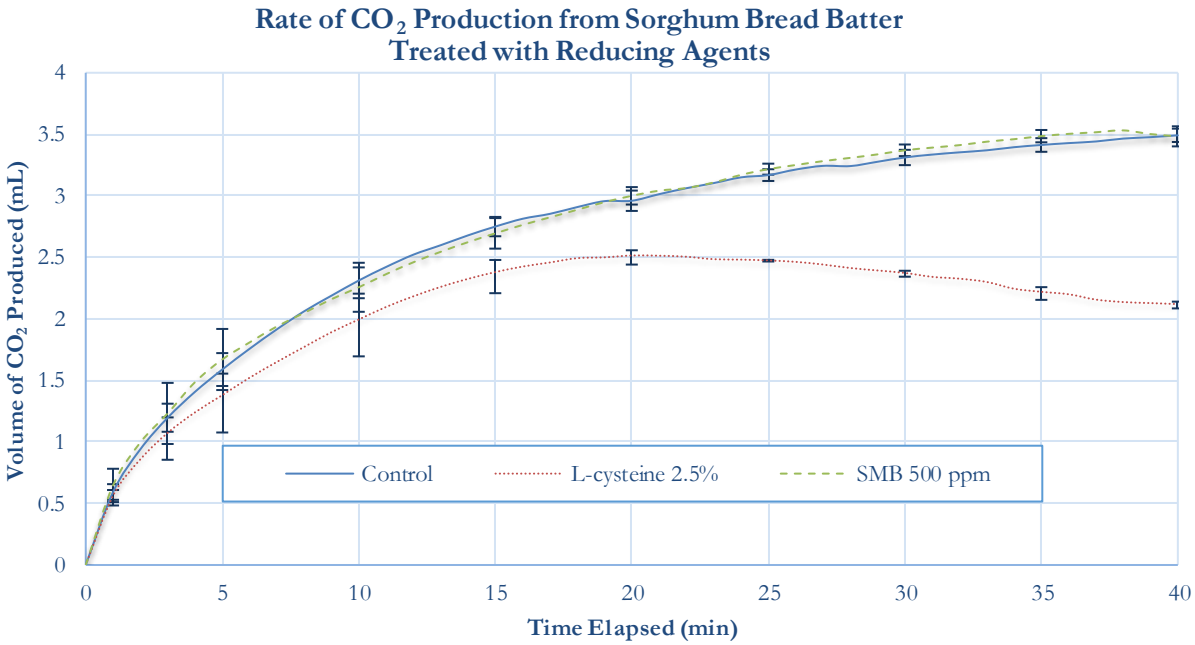


Figure 4.5: Rate of CO_2 gas production from sorghum bread batters treated with optimum concentration of L-cysteine (2.5%, fwb) and sodium metabisulfite (500 ppm, fwb).
* Error bars represent standard error associated with treatment means.

Table 4.4: Rate of CO_2 production (mL/min) from sorghum bread batters [a]

	Time Elapsed (min)									
Treatment	1	3	5	10	15	20	25	30	35	40
Control	0.59a	1.20a	1.59a	2.31a	2.75a	2.96a	3.17a	3.31a	3.41a	3.49a
L-cysteine 2.5%	0.53a	1.07a	1.38a	2.00a	2.38a	2.52b	2.48b	2.37b	2.22b	2.12b
SMB 500 ppm	0.65a	1.23a	1.67a	2.26a	2.70a	3.00a	3.22a	3.37a	3.49a	3.48a

* Values followed by different letters within a column are significantly different ($p < 0.05$)
[a] Samples were treated with optimum concentrations of L-cysteine and sodium metabisulfite

Cumulative total volume was of obtained in conjunction with yeast activity rate, to quickly evaluate if the same volume of gas was generated by each treatment batter (Figure 4.6). Throughout the 40 min duration of the analysis, significant differences were not observed between any treatments (Table 4.5). Additional yeast activity data related to higher concentration of sodium metabisulfite provided in Appendix B.

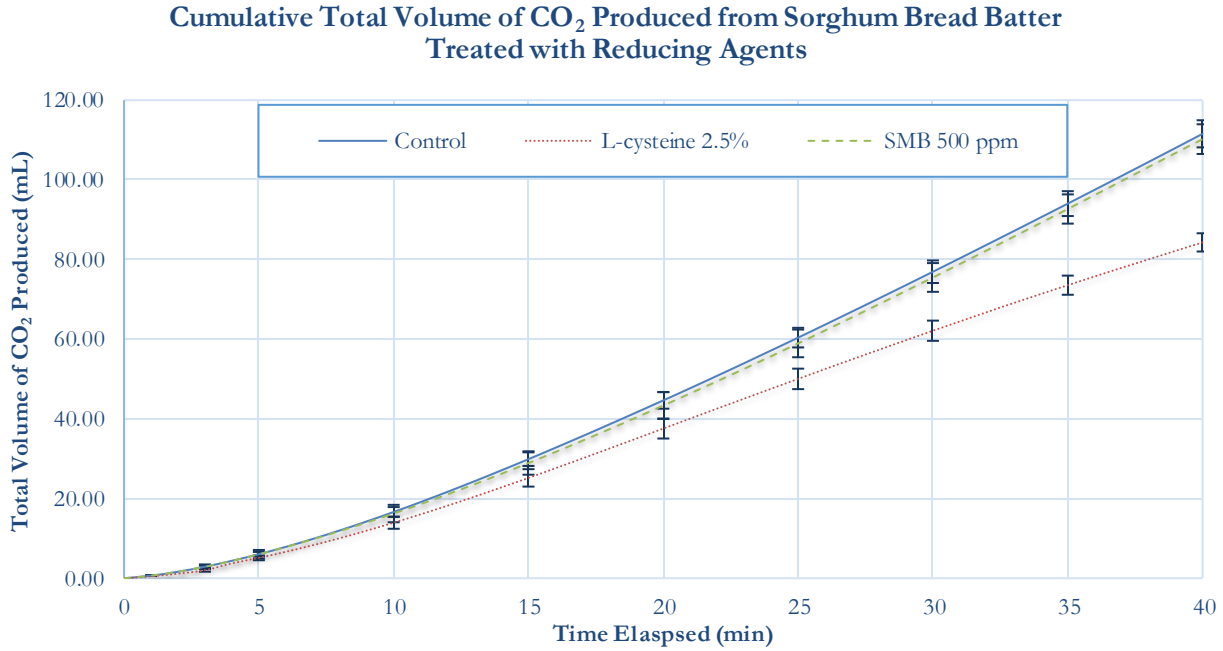


Figure 4.6: Cumulative total CO_2 gas production of sorghum bread batters treated with optimum concentration of L-cysteine (2.5%, fwb) and sodium metabisulfite (500 ppm, fwb).
* Error bars represent standard error associated with treatment means.

Table 4.5: Cumulative total volume of CO_2 gas production for sorghum bread batters [a]

	Time Elapsed (min)									
Treatment	1	3	5	10	15	20	25	30	35	40
Control	0.6a	2.9a	6.0a	16.6a	29. 9a	44.6a	60.4a	76.9a	94.0a	111.6a
L-cysteine 2.5%	0.5a	2.0a	5.1a	14.0a	25.2a	37.6a	50.0a	62.1a	73.6a	84.3a
SMB 500 ppm	0.7a	2.9a	6.0a	16.2a	28.9a	43.4a	59.0a	75.5a	92.7a	110.2a

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Samples were treated with optimum concentrations of L-cysteine and sodium metabisulfite

4.3.4 Bread Staling

The staling study was conducted using a TA.XT*Plus* Texture Analyzer to detect changes in firmness and elasticity of sorghum bread crumb over a period of 5 days. The purpose of this study was to gauge crumb firmness, by measuring a fixed distance, as well as crumb elasticity, by computing crumb recovery after compression, over a period of 5 days. The force required to compress the crumb 10 mm (40%) is taken as the firmness of the crumb and is an indication of freshness and quality. The elasticity of the crumb is determined by the force at 30 sec of relaxation and the peak force and is an estimate of bread cohesiveness or gumminess in cakes.

For initial texture analysis, breads designated as day 0 were analyzed 2 hours after baking. The control bread had the firmest crumb texture with a firmness of 710 ± 25 g (Table 4.6). The crumb from breads containing 2.5% (fwb) L-cysteine had a significantly softer texture (555 ± 16 g) compared to the control breads, while breads containing 500 ppm (fwb) of sodium metabisulfite had the significantly softest crumb texture overall (415 ± 84 g).

Over the 5 day period, all breads displayed a consistent increase in crumb firmness as bread aged from 0 to 3 days (Table 4.6), yet bread crumb firmness on day 5 was not greater on day 3.

Table 4.6: Crumb firmness of sorghum bread treated with 2.5% (fwb) L-cysteine and 500 ppm (fwb) sodium metabisulfite over 5 days

	Crumb Firmness (g)			
	Day 0	Day 1	Day 3	Day 5
Control	710 ± 25a	964 ± 64a	1243 ± 123a	1408 ± 13a
L-cysteine 2.5%	555 ± 16b	946 ± 94a	1220 ± 115a	1447 ± 118a
SMB 500 ppm	415 ± 84c	841 ± 52a	893 ± 81b	1040 ± 54b

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)

The ability of the bread crumb to recover after compression is expressive of the cohesiveness and spring of the bread. Significant differences in elasticity were not observed between treatments, on any given day of analysis (Table 4.7). However, within a given treatment, significant differences in elasticity were seen over time. The control bread crumb, with an initial elasticity of 42.6%, exhibited significantly less elasticity by day 3 (38.0 %). Breads treated with 2.5% (fwb) of L-cysteine and 500 ppm (fwb) if sodium metabisulfite produced crumb textures with similar crumb elasticity on day 0 and day 1 however on day 3 the crumb grains were significantly less elastic. On day 5 of analysis, was not interpreted due to subjective observations made during analysis. Breads containing sodium metabisulfite were fragile and crumbled easily. Also after compression, indentions produced by the probe were evident; showing very little recovery which would indicate less cohesion and suggest lower elasticity.

Table 4.7: Crumb firmness of sorghum bread treated with 2.5% (fwb) L-cysteine and 500 ppm (fwb) sodium metabisulfite over 5 days

	Crumb Elasticity (%)			
	Day 0	Day 1	Day 3	Day 5
Control	42.6 ± 2.2a	36.9 ± 4.5a	38.0 ± 0.8a	28.7 ± 5.9a
L-cysteine 2.5%	40.9 ± 1.2a	39.8 ± 2.8a	28.2 ± 5.7a	31.6 ± 4.2a
SMB 500 ppm	43.4 ± 0.4a	37.8 ± 2.8a	30.1 ± 6.0a	34.4 ± 3.5a

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)

4.3.5 Batter Consistency

The parameters investigated during the RVA analyses provided insight into batter consistency. The batter consistency or viscosity measurements were recorded in centipoise (cP) units (1cP= 1 mPa sec-1). The objective of this analysis was to observe and quantify the batter viscosity of the sample as it transitioned through a temperature gradient in excess water. When starch granules are heated to a specific temperature in excess water, the crystalline structure of the granule begins to dissipate, resulting in the loss of birefringence (Perten Instruments, 2013). This irreversible process is known as gelatinization. The curve produced by the RVA (shown previously in Figure 3.1) describes a phenomenon which occurs post gelatinization. Pasting encompasses a series of events starting with granular swelling, leaching of amylose and amylopectin from the structure, and ending with the total disintegration of the starch granule. From the generated curve, the parameters most representative of the events occurring during analysis are peak viscosity, pasting temperature, peak time, holding strength and final viscosity.

As the sample temperature increases from 50°C to 95°C, normal sorghum starch will undergo gelatinization between 66.4°C and 70.2°C (Taylor et al., 2010). As the sample temperature surpasses the gelatinization temperature of the starch, intact granules swell and amylose is leached (Delcour & Hoseney, 2010c). When the volume fraction occupied by the swollen granules increases so will viscosity (Guan et al., 2007). Pasting temperature is determined based on the corresponding temperature when the sample viscosity begins to increase (Perten Instruments, 2013). During the next stage of analysis, greater shear force is applied by mixing the sample at a rate of 960 rpm. In the presence of increasing temperature and shear force, when swelling and exudation of starch polymers is in equilibrium with granule rupture and polymer alignment, this is the maximum viscosity which is marked as the peak viscosity (PV). Subsequently, when peak viscosity is obtained, the corresponding time and temperature are then defined as peak temperature (Perten Instruments, 2013).

As the analysis continues, high shear force and high temperature conditions are held constant. Under these conditions the starch granules proceed to exudate amylose, and some amylopectin. Solubilized polymers will progressively orient themselves in the direction of the shear force (Delcour & Hoseney, 2010b). This alignment creates a low degree of internal friction thus a reduction in viscosity occurs. The minimum value is recorded as holding strength (HS).

When the analysis enter the cooling stage, the temperature gradually decreases to 50°C. The cooling stage results in an increase in viscosity as starch polymers begin to re-associate through hydrogen bonding and entanglement (Delcour & Hoseney, 2010b). The consequent increase in viscosity is labeled as the final viscosity (FV). Although starch pasting properties and the intrinsic chemistry is important to the overall understanding of sorghum flour in a baked product, for this study RVA was used as a means to measure viscosity of the sorghum flour or partial formula blend during heating.

The optimum treatment combinations, 2.5% (fwb) of L-cysteine, 500 ppm (fwb) of sodium metabisulfite and the control batters were subjected RVA testing to reveal changes to the viscosity profile occurring during heating. Two sets of experimentation were conducted. In the first set, sorghum flour was analyzed in the presence of reducing agent solutions to determine the effect of reducing agents on sorghum flour. In the second set, a partial formula blend of 70% (fwb) sorghum flour, 30% (fwb) potato starch and 2% (fwb) HPMC was analyzed to determine if the addition of starch and hydrocolloids significantly influenced the effect of the reducing agents.

Sorghum Flour Analysis. The first round of experimentation utilizing strictly sorghum flour did reveal significant differences between treatments (Table 4.8). The peak viscosity (PV) was significantly greater when reducing agents were introduced to the batter compared to the control batter containing only water and sorghum flour. As mentioned previously, the peak viscosity is suggestive of the water holding capacity of starch. A greater peak viscosity indicates the starch has a

greater water holding capacity. Perhaps, the smaller protein fractions are capable of binding water, aiding in a higher viscosity. From the RP-HPLC data, it was shown reducing agents increased the amount of freed kafirin protein subunits, represented by a larger total peak area compared to the negative control treatment. Therefore, it could be postulated that because reducing agents successfully affected the compact protein body structures, starch granules previously bound by the protein matrix are potentially less restricted and possess a greater ability to swell or participate in gelatinization. Perhaps with a greater concentration of unrestricted starch granules, an increase in viscosity would be observed.

Corresponding to the peak viscosity, the peak time and pasting temperature also displayed significant changes between treatments. Samples treated with L-cysteine at 2.5% (fwb) exhibited lower pasting temperature compared to other treatments. Sodium metabisulfite when added at 500 ppm (fwb) appeared to delay the peak time (5.62 min) compared to the control and 2.5% (fwb) L-cysteine treatments, which peaked earlier (5.4 min and 5.33 respectively).

The thinnest consistency of the samples were captured as holding strength (HS). Samples treated with sodium metabisulfite maintained a greater holding strength (1943.3 cP) compared to both the control (1656.5 cP) and L-cysteine (1649.0 cP) treatment.

Final viscosity (FV) of samples revealed the greatest diversity. Samples containing 2.5% (fwb) of L-cysteine possessed the lowest final viscosity (3639.3 cP). The control treatment had a significantly different final viscosity (4632.5 cP) than either reducing agent treatments. Samples containing 500 ppm (fwb) of sodium metabisulfite displayed the greatest final viscosity (5485.3 cP).

Table 4.8: RVA Consistency of sorghum flour samples treated with optimum concentration of L-cysteine and sodium metabisulfite

Treatment:	PV[a] (cP)	HS[b] (cP)	FV[d] (cP)	Peak Time (min)	Pasting Temp (°C)
Control	2658.0b	1656.5b	4632.5b	5.40b	80.25a
L-cysteine 2.5%	2868.7a	1649.0b	3639.3c	5.33b	76.17b
SMB 500 ppm	2964.7a	1943.3a	5485.3a	5.62a	80.67a

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Peak viscosity; [b] holding strength; [c] final viscosity

Partial Formula Blend Analysis. The second round of experimentation utilized a partial formula blend of 70% (fwb) sorghum flour, 30% (fwb) potato starch and 2% (fwb) HPMC to identify viscosity differences between treatments in the presence of the other major ingredients. The peak viscosity was significantly greater for samples treated with 500 ppm (fwb) of sodium metabisulfite (4015.3 cP) compared to the control (3742.3 cP) and 2.5% (fwb) of L-cysteine (3643.3 cP) treated samples (Table 4.9).

Peak times were altered in a manner consistent with the sorghum flour samples. Samples treated with L-cysteine reached peak viscosity earlier than the control, however were not significantly different from those treated with sodium metabisulfite. Differences in pasting temperatures were not seen between treatments.

The holding strength or minimum viscosity of samples presented variation. Those treated with 2.5% (fwb) L-cysteine reached a minimum viscosity of 2250.7 cP, which was lower than the control's minimum viscosity (2533.0 cP). Samples containing 500 ppm (fwb) of sodium metabisulfite maintained a greater minimum viscosity (2651.3 cP) compared to either treatments.

The final viscosity of the samples again showed significant differences between treatments. Samples treated with 2.5% (fwb) L-cysteine exhibited a lower final viscosity (4542.3 cP), while those treated with 500 ppm (fwb) of sodium metabisulfite (5341.7 cP) or the control (5431.3 cP) maintained a greater final viscosity.

Due to the larger concentration of starch and the presence of HPMC within the sample, viscosities displayed less variation between treatments. Possibly, the indirect effect of reducing agents on the sorghum starch granules is hindered by the high quantity of potato starch.

Table 4.9: RVA consistency of partial formula blend samples treated with reducing agents at optimum concentrations

Treatment:	PV[a] (cP)	HS[b] (cP)	FV[d] (cP)	Peak Time (min)	Pasting Temp (°C)
Control	3742.3b	2533.0b	5431.3a	5.42a	71.22a
L-cysteine 2.5%	3643.3b	2250.7c	4542.3b	5.25b	70.45a
SMB 500 ppm	4015.3a	2651.3a	5341.7a	5.33ab	71.45a

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Peak viscosity; [b] holding strength; [c] final viscosity

Comparison of flour and partial blend within treatments: Sorghum flour and partial formula blend samples treated with the control, 2.5% (fwb) L-cysteine and 500 ppm (fwb) sodium metabisulfite treatments, saw a significant difference in peak viscosity, holding strength and final viscosity. Samples using a partial formula blend had consistently higher viscosities. Greater viscosity values were probable due the high water holding capacity of potato starch, which is capable of holding 7-8 times its weight in water, and HPMC. Peak times and pasting temperatures were altered by the addition of potato starch and HPMC for 2.5% (fwb) L-cysteine and 500 ppm (fwb) sodium metabisulfite (data provided in Appendix C).

Additionally, potato starch had a peak temperate of 68.3°C, while the pasting temperature of sorghum flour was found to be 80.25°C. When potato starch is blended with sorghum flour, to comprise 30% of the total flour, pasting temperature of the sample is affected; decreasing to 71.2°C. Furthermore, HPMC is a surface-active substance which could stabilize the paste with its many hydrophobic (hydroxyl propyl and methyl ester) side groups and the hydrophilic cellulose backbone (Gallagher 2009). By creating a more stable paste, HPMC could have also contributed to the increase in viscosity.

4.3.6 Digestibility of Bread

Although slightly outside the scope of the stated objectives, bread digestibility was of interest after seeing promising results from bake tests and RP-HPLC data. Sample digestibility was determined using the method previously described by Mertz et al. (1984) and are expressed as a percentage of the total protein (determined from nitrogen content) in the raw treatment sample. Breads treated with L-cysteine at 2.5% (fwb), exhibited the highest protein digestibility (51.75%) of any treatment; achieving a level of digestibility comparable only to uncooked control flour, shown in Table 4.10. Breads containing 500 ppm (fwb) of sodium metabisulfite had a lower protein digestibility (30.45%) relative to the 2.5% (fwb) L-cysteine treatment, however it was similar to that of the control bread. The remaining samples were not statistically different from the control bread. These findings slightly contrast those of Hamaker et al. (1987), Oria et al. (1995) and Elkhalifa et al. (1999), which determined reducing agents, such as BME, sodium bisulfite and L-cysteine, improved protein digestibility in cooked sorghum flour. A potential explanation for this inconsistency is the differences in product application. In the study conducted by Hamaker et al. (1987) and Oria et al. (1995), cooked sorghum flour was subjected to protein digestion, whereas this study evaluated protein digestion of sorghum bread. Sorghum bread contains a multitude of ingredients which could confound the digestion of proteins.

Regardless, the weight of one finding is substantial. Extensive research has proven sorghum protein digestibility drastically decreases upon cooking (reviewed by Duodu et al., 2003). Through the addition of reducing agent, L-cysteine at 2.5% (fwb), the level of digestible protein was restored to the original level detected in untreated, uncooked sorghum flour. Furthermore, bread treated with 2.5% L-cysteine improved protein digestibility by 18.8% compared to the control bread. By recouping a substantial percentage of digestible protein, the nutritional value of sorghum is considerably improved.

Table 4.10: Protein digestibility of sorghum bread treated with L-cysteine and sodium metabisulfite

Treatment	Protein Digestibility (%)
Control Flour	59.42 ± 2.57a
Control Bread	32.98 ± 0.44bc
L-cysteine 0.5%	32.82 ± 0.42bc
L-cysteine 1.0%	28.84 ± 2.13c
L-cysteine 2.5%	51.75 ± 1.26a
Sodium metabisulfite 100 ppm	38.20 ± 7.18bc
Sodium metabisulfite 250 ppm	37.84 ± 1.94bc
Sodium metabisulfite 500 ppm	30.45 ± 1.18bc
Sodium metabisulfite 0.5%	40.55 ± 0.71b

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)

CHAPTER 5

Conclusion

The first objective of this study was to determine the effect of reducing agents on the structure of the primary prolamin proteins, kafirins, which exists in condensed protein bodies. Based on the results given by RP-HPLC, reducing agents L-cysteine and sodium metabisulfite successfully reduced protein body structure at concentrations 0.5-5.0% (fwb) and 500-500,000 ppm, respectively. After subjecting sorghum flour and partial formula blend samples to RP-HPLC analysis, proofing was discovered to facilitate greater protein reduction in sorghum flour but to a lesser degree in the partial formula blend. Regardless of starting material, L-cysteine at 2.5% (fwb) and 5.0% (fwb) generated the largest total peak areas corresponding to greater elution of kafirin protein fractions. Sodium metabisulfite when used at 500 ppm (fwb) and 1000 ppm (fwb) produced the greatest protein reduction within the sodium metabisulfite treatments yet was significantly lower than L-cysteine at 2.5% (fwb) or 5.0% (fwb).

The second objective was to investigate the influence of reducing agents on the consistency of sorghum bread batter. From the rapid viscosity analysis, the addition of reducing agents affected batter consistency. Sorghum flour samples treated with 2.5% (fwb) L-cysteine expressed higher peak viscosity and a lower final viscosity. Additionally, L-cysteine at 2.5% (fwb) lowered the peak time and increased the pasting temperature of sorghum flour compared to the control treatment. When using a partial formula blend, L-cysteine at 2.5% (fwb) did not significantly alter peak viscosity, however holding strength and final viscosity decreased. The peak time was slightly delayed while pasting temperature remained consistent with other treatments.

When sorghum flour samples were treated with 500 ppm (fwb) of sodium metabisulfite, an increase in peak viscosity, holding strength and final viscosity were observed. There was not a

noticeable effect of sodium metabisulfite at 500 ppm (fwb) on pasting temperature but peak time was delayed compared to the control treatment. A partial formula blend treated with sodium metabisulfite at 500 ppm (fwb) maintained a higher peak viscosity and holding strength but final viscosity was similar to the control treatment. Also the previous delay in peak time was not witnessed for partial blend formula samples treated with sodium metabisulfite at 500 ppm.

Yeast activity was also evaluated for its implications on batter consistency based on CO_2 production. Yeast activity of batter containing 500 ppm (fwb) of sodium was equal to that of the control treatment. L-cysteine at 2.5% (fwb) produced a similar volume of gas compared to sodium metabisulfite at 500 ppm and the control up to 20 min, yet after 35 min L-cysteine batters experienced a decrease in yeast activity to produce lower volume of CO_2 gas.

The last objective was to evaluate the effect of reducing agents on the physical characteristics of sorghum bread. Breads produced using the optimum concentrations, 2.5% (fwb) of L-cysteine and 500 ppm of sodium metabisulfite, exhibited statistically similar loaf volumes. However, L-cysteine treated breads presented a noticeable sulfurous aroma and yellow-gold crust color. When added at 2.5% (fwb), L-cysteine produced bread with a softer initial crumb texture compared to the control. Over the duration of 5 days, bread crumb texture of L-cysteine treated breads was similar in firmness to control crumb texture. Breads treated with 500 ppm (fwb) of sodium metabisulfite exhibited the softest initial crumb texture and although firmness values significantly increased during storage, breads treated with sodium metabisulfite retain a softer crumb texture compared to L-cysteine and control treatments.

Sorghum bread samples were subjected to *in-vitro* pepsin digestion (IVPD). In general, sodium metabisulfite at the designated concentrations did not have an impact on protein digestibility of sorghum bread. Bread containing 500 ppm (fwb) of sodium metabisulfite possessed a similar protein digestibility value as the control bread, averaging 30.45%, but was significantly lower

compared to bread treated with 2.5% (fwb) L-cysteine. Contrariwise, L-cysteine when incorporated at 2.5% (fwb) drastically improved protein digestibility. In fact, when treated with 2.5% (fwb) L-cysteine the IVPD value returned to original range for uncooked sorghum flour, as captured by the control flour.

CHAPTER 6

Future Work

This study provided further insight into the functionality of reducing agents in sorghum bread application, however there are extensions and related areas of this research where further exploration is needed.

- For this study concentrations were added at significant and wide range in order to determine if effects could be observed in batter and/or bread characteristics. Concentration range was selected based on past published literature. Although these concentrations did produce viable results, illustrating that reducing agents can reduce protein body structure, alter batter viscosity, and still produce a loaf of bread consistent to controls, L-cysteine and glutathione were not optimized to the same extent as sodium metabisulfite. Future work to determine the optimum concentrations of L-cysteine and possibly glutathione, in the form of brewer's spent yeast, would provide a more balanced assessment of effects imparted by reducing agents.
- Additionally, in regards to optimization, further investigation into the effects of reducing agents on bread loaf volume should be accomplished at optimum water levels for each reducing agent treatment. Based on the results of RP-HPLC which illustrated significant reductions in protein structure with the addition of select reducing agent treatments, it would be expected a greater amount of water could bind with the newly available protein. Like-wise, as supported by the C-Cell images of treated and untreated breads, water levels likely impeded or facilitated the achievement of maximum loaf volume. Reevaluating using optimized water levels would be worth-while to definitively describe the effects of reducing agents on bread loaf volume.
- A yeast leavened method incorporating 70% sorghum flour with 30% potato starch was tested in this study. The addition of potato starch may have affected the influence of reducing agents

on batter and bread. For future studies, the evaluation of reducing agents in a 100% sorghum flour bread, using a yeast leavened formula could provide more accurate results relating to true functionality of specific reducing agents on batter and bread characteristics. Additionally, because some concentrations had detrimental effects on the yeast, future studies could mimic the outlined experiments using a chemically leavened sorghum bread.

- Moreover, the order of ingredient addition may be worth investigating to prove further illumination of functional properties. The implemented method incorporated reducing agent solutions into the formula after dry ingredients were blended together. From the RVA and RP-HPLC results, potato starch influenced the changes imparted by the reducing agents. If reducing agent solutions were blended with the sorghum flour initially then followed by the remaining dry and liquid ingredients, this would provide the greatest opportunity for reducing agents to interact with sorghum kafirins, without competing or being immobilized by other ingredients.
- Reducing agents L-cysteine and sodium metabisulfite were found to improve specific attributes of sorghum batter and/or bread. Further studies could utilize response surface methodology to develop formulations specific to reducing agents to optimize end-product characteristics such as texture, volume, digestibility and/or shelf-life.
- Similar to the previous point, further investigation into the effects of reducing agents on final product could prove interesting if other baked products were examined. Leavened breads are a complex and difficult to recreate using gluten-free grains. By testing products which do not rely as heavily on leavening, such as a flatbread or English muffin, effects may be more prominent. Because reducing agents L-cysteine and sodium metabisulfite provoked unique changes in either batter consistency or bread characteristics, it would be intriguing to examine different products.
- To fully understand the events occurring during RVA analysis, additional work should be accomplished to test optimum concentrations, 2.5% (fwb) L-cysteine and 500 ppm (fwb)

sodium metabisulfite, of reducing agents to determine the gelatinization properties of the batter. Using differential scanning calorimetry (DSC), gelatinization temperature as well as the enthalpy of the treated batters could be evaluated. This would allow for the quantification of gelatinized starch and provide valuable insight to any potential indirect effects reducing agents may have on starch gelatinization and subsequent pasting properties.

- Lastly, the RP-HPLC analysis was oriented to quantify the total kafirin protein content. This approach was appropriate as the goal was to confirm the reductive effect of reducing agents on kafirin structure. However, this analysis did not provide the necessary information to gauge the degree of protein reduction that occurred. For future studies interested in the degree of protein reduction, Ellman's Test should be implemented. Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid), allows for the quantification of free thiol groups present in a sample permitting researcher additional insight into the biochemical reactions and reduction yields that occur with different reducing agent treatments.

References

AACC International. Approved Methods of Analysis, 11th Ed. Methods 02-52.01, 08-01.01, 10-05.01, 44-15.02, 46-30.01, 74-10.02, 76-21.01, 76-31.01, 76-33.01 and 89-01.01. AACC International: St. Paul, MN. http://dx.doi.org/10.1094/AACCIntMethod-02-52.01.

Awika, J.M., Rooney, L.W., 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65, 1199-1221.

Bean, S.R., Ioerger, B.P., Blackwell, D.L., 2011. Separation of kafirins on surface porous reversed-phase high-performance liquid chromatography columns. Journal of Agricultural and Food Chemistry 59, 85.

Belton, P.S., Delgadillo, I., Halford, N.G., Shewry, P.R., 2006. Kafirin structure and functionality. Journal of cereal science 44, 272-286.

Beta, T., Corke, H., Rooney, L.W., Taylor, J.R., 2001. Starch properties as affected by sorghum grain chemistry. Journal of the science of food and agriculture 81, 245-251.

Carson, L., Setser, C., Sun, X.S., 2000. Sensory characteristics of sorghum composite bread *. International Journal of Food Science & Technology 35, 465-471.

Cornish, G.B., Békés, F., Eagles, H.A., Payne, P.I., 2006. Chapter 8 Prediction of Dough Properties for Bread Wheats. In: Wrigley, C.W., Békés, F. and Bushuk, W. (Eds.), Gliadin and Glutenin: The Unique Balance of Wheat Quality. St. Paul, Minn.: AACC International, St. Paul, Minn., pp. 243-280.

de Mesa-Stonestreet, N.J., Alavi, S., Bean, S., 2010. Sorghum Proteins: The Concentration, Isolation, Modification, and Food Applications of Kafirins. Journal of Food Science 75, no-no.

De Vincenzi, M., Luchetti, R., Peruffo, A.D., Curioni, A., Pogna, N.E., Gasbarrini, G., 1996. In vitro assessment of acetic- acid- soluble proteins (glutenin) toxicity in celiac disease. Journal of Biochemical Toxicology 11, 205.

Delcour, J.A., Hoseney, R.C., 2010a. Sorghum. In: Hoseney, R.C. (Ed.), Principles of Cereal Science and Technology, 3rd edition. St. Paul, Minn. : AACC International, St. Paul, Minn., pp. 17.

Delcour, J.A., Hoseney, R.C., 2010b. Starch. In: Anonymous Principles of Cereal Science and Technology, 3rd ed. AACC International, Inc., 3340 Pilot Knob Road, St. Paul, Minnesota 55121, U.S.A., pp. 34-39.

Delcour, J.A., Hoseney, R.C., 2010c. Structure of Cereals. In: Anonymous Principles of Cereal Science and Technology, 3rd ed. AACC International, Inc., 3340 Pilot Knob Road, St. Paul, Minnesota 55121, U.S.A., pp. 1-22.

Dow, 2015. Differences between sodium metabisulfite and sodium bisulfite. dow answer center 2016, 1.

Duodu, K.G., Taylor, J.R.N., Belton, P.S., Hamaker, B.R., 2003. Factors affecting sorghum protein digestibility. Journal of cereal science 38, 117-131.

Dykes, L., Hoffmann, O., Portillo Rodriguez, W., Rooney, L., Rooney, 2014. Prediction of total phenols, condensed tannins, and 3-deoxyanthocyanidins in sorghum grain using near-infrared (NIR) spectroscopy. Journal of cereal science 60, 138-142.

Earp, C.F., McDonough, C.M., Rooney, L.W., 2004. Microscopy of pericarp development in the caryopsis of Sorghum bicolor (L.) Moench. Journal of cereal science 39, 21-27.

Elkhalifa, E.O., Chandrashekar, A., Mohamed, B.E., El Tinay, A.H., 1999. Effect of reducing agents on the in vitro protein and starch digestibilities of cooked sorghum. Food Chemistry 66, 323-326.

Fasano, A., Berti, I., Gerarduzzi, T., Not, T., Colletti, R.B., Drago, S., Elitsur, Y., Green, P.H.R., Guandalini, S., Hill, I.D., Pietzak, M., Ventura, A., Thorpe, M., Kryszak, D., Fornaroli, F., Wasserman, S.S., Murray, J.A., Horvath, K., 2003. Prevalence of celiac disease in at- risk and not-at- risk groups in the United States: a large multicenter study. Archives of Internal Medicine 163, 286.

FDA, 2014. 'Gluten-Free' Now Means What It Says. FDA 2016, 1.

Gaffa, T., Yoshimoto, Y., Hanashiro, I., Takeda, Y., Honda, O., Kawasaki, S., 2004. Physicochemical properties and molecular structures of starches from millet (pennisetum typhoides) and sorghum (sorghum bicolor l. moench) cultivars in nigeria. Cereal Chemistry 81, 255-260.

Goodall, M.A., Campanella, O.H., Ejeta, G., Hamaker, B.R., 2012. Grain of high digestible, high lysine (HDHL) sorghum contains kafirins which enhance the protein network of composite dough and bread. Journal of cereal science 56, 352-357.

Green, P.H., Lebwohl, B., Greywoode, R., 2015. Celiac disease. Journal of Allergy and Clinical Immunology 135, 1099-1106.

Guan, L., Seib, P.A., Shi, Y.C., 2007. Morphology changes in waxy wheat, normal wheat and waxy maize sharch granules in relation to their pasting properties. Cereal Foods World 52, A19.

Guo, Y., Tu, K., Pan, L., Zhang, W., Zhang, Y., 2012. Effects of three reducing agents on pasting properties of stored rice. Starch - Stärke 64, 198-206.

Hamaker, B.R., Bugusu, B.A., 2003. Overview: sorghum proteins and flood quality. Belton PS, Taylor JRN, .

Hamaker, B.R., Kirleis, A.W., Butler, L.G., Axtell, J.D., Mertz, E.T., 1987. Improving the in vitro Protein Digestibility of Sorghum with Reducing Agents. Proceedings of the National Academy of Sciences of the United States of America 84, 626-628.

Ioerger, B., Bean, S.R., Tuinstra, M.R., Pedersen, J.F., Erpelding, J., Lee, K.M., Herrman, T.J., 2007. Characterization of polymeric proteins from vitreous and floury sorghum endosperm. Journal of Agricultural and Food Chemistry 55, 10232.

Kagnoff, M.F., Austin, R.K., Johnson, H.C.L., Bernardin, J.E., Dietler, M.D., Kasarda, D.D., 1982. Celiac sprue: Correlation with murine T cell responses to wheat gliadin components. Journal of Immunology 129, 2693-2697.

Lallemand, 2011. A guide to reducing agents. Lallemand Baking Update 1.

Lásztity, R., 1996. The Chemistry of cereal proteins, 2nd edition. Boca Raton : CRC Press, Boca Raton.

Lemlioglu, A.D., 2014. Sorghum: Obliging Alternative and Ancient Grain. Cereal Foods World 59, 12-20.

McLynn, K., 2013. Percentage of U.S. Adults Trying to Cut Down or Avoid Gluten in Their Diets Reaches New High in 2013, Reports NPD. 2016.

Mertz, E.T., Hassen, M.M., Cairns-Whittern, C., Kirleis, A.W., Tu, L., Axtell, J.D., 1984. Pepsin Digestibility of proteins in sorghum and other major cereals. Proceedings of the National Academy of Sciences of the United States of America 81, 1-2.

Miller, C., 2013. Biochemical studies of prolamins from sorghum and wheat (doctoral dissertation). http://krex.k-state.edu/dspace/handle/2097/16937

Mkandawire, N.L., 2013. Harnessing the benefits of sorghum: In vitro starch digestion, in vitro fermentation and processing of tannin and tannin-free sorghums. ProQuest Dissertations and Theses.

Nagy, P., 2013. Kinetics and mechanisms of thiol- disulfide exchange covering direct substitution and thiol oxidation- mediated pathways. Antioxidants and Redox Signaling 18, 1623-1641.

Oom, A., Pettersson, A., Taylor, J.R.N., Stading, M., 2008. Rheological properties of kafirin and zein prolamins. Journal of cereal science 47, 109-116.

Oria, M.P., Hamaker, B., Shull, J., 1995. Resistance of sorghum alpha- kafirins, beta- kafirins, and gamma- kafirins to pepsin digestion. Journal of Agricultural and Food Chemistry; J.Agric.Food Chem. 43, 2148-2153.

Park, S., Bean, S., Wilson, J., Schober, T., 2006. Rapid Isolation of Sorghum and Other Cereal Starches Using Sonication. Cereal Chemistry 83, 611-616.

Perten Instruments, 2013. Rapid viso analyser RVA 4500 installation and operations manual. , 65-68.

Price, M.L., Butler, L.G., Van Scoyoc, S., 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum- grain. Journal of Agricultural and Food Chemistry 26, 1214-1218.

Pyler, E.J., Gorton, L.A., 2009. Bakery Ingredients part b: minor ingredients. In: Anonymous Baking Science & Technology, 4th Ed., Vol. 1Sosland Publishing Co., Kansas City, MO, pp. 295.

Reuhs, B.L., Rounds, M.A., 2010. High-performance liquid chromatography. In: Nelson, J. (Ed.), Food Analysis, fourth ed. Springer US, New York and London, pp. 499-512.

Rooney, L.W., Awika, J.M., 2004. Specialty Sorghums for Healthy Foods.

Rooney, L.W., Miller, F.R., 1982. Variation in the structure and kernel characteristics of sorghum. , 25-35.

Rose, D., Williams, E., Mkandawire, N., Weller, C., Jackson, D., 2014. Use of whole grain and refined flour from tannin and non-tannin sorghum (sorghum bicolor (l.) moench) varieties in frybread. Food Science and Technology International 20, 333-339.

Sang, Y., Bean, S., Seib, P.A., Pedersen, J., Shi, Y., 2008. Structure and functional properties of sorghum starches differing in amylose content. Journal of Agricultural and Food Chemistry 56, 6680.

Schober, T., Messerschmidt, M., Bean, S., Park, S., Arendt, E., 2005. Gluten-free bread from sorghum: Quality differences among hybrids. Cereal Chemistry 82, 394-404.

Shandera, D.L., Parkhurst, A., Jackson, D., 1995. Interactions of sulfur-dioxide, lactic-acid, and temperature during simulated corn wet-milling. Cereal Chemistry; Cereal Chem. 72, 371-378.

Spurkland, A., Knutsen, G., Sollid, E.S., Thorsby, I., Ingvarsson, L.M., Falk, E., Spurkland, E., 1997. Dermatitis herpetiformis and celiac disease are both primarily associated with the HLA-DQ (α1*0501, β1*02) or the HLA- DQ (α1*03, β1*0302) heterodimers. Tissue antigens 49, 29-34.

Statista, 2016. Statistics and facts on the gluten-free foods market in the U.S. 2015, 1.

Subramanian, V., Hoseney, R.C., Bramelcox, P., 1994. Factors affecting the color and appearance of sorghum starch. Cereal Chemistry; Cereal Chem. 71, 275-278.

Taylor, J.R.N., Emmambux, M.N., Emmambux, M.N., 2010. REVIEW: Developments in our understanding of sorghum polysaccharides and their health benefits. Cereal Chemistry 87, 263-271.

The United Sorghum Checkoff, 2012. Sorghum 101.

Tjon, J., Bergen, J., Koning, F., 2010. Celiac disease: how complicated can it get? Immunogenetics 62, 641-651.

U.S. Department of Agriculture National Statistics Service, 2015. Sorghum for grain 2014 production by county for selected states. AS-PR14-RGBChor.

Vader, W., Kooy, Y., van Veelen, P., de Ru, A., Harris, D., Benckhuijsen, W., Peña, S., Mearin, L., Drijfhout, J.W., Koning, F., 2002. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. Gastroenterology 122, 1729-1737.

Vanderlip, R.L., 1993. How a Sorghum Plant Develops. In: Anonymous Kansas State University, pp. 1.

Wal, V.D., Kooy, Y.M.C., Van Veelen, P., Vader, W., August, S.A., Drijfhout, J.W., Peña, S.A., Koning, F., 1999. Glutenin is involved in the gluten-driven mucosal T cell response. European Journal of Immunology 29, 3133-3139.

Watterson, J., Shull, J., Kirleis, A., 1993. Quantitation of alpha-karafins, beta-karafins, and gamma-kafirins in vitreous and opaque endosperm of sorghum- bicolor. Cereal Chemistry; Cereal Chem. 70, 452-457.

Wilson, J.D., Kaufman, R.C., Seabourn, B.W., Galant, A.L., Herald, T., 2016. A Rapid method to determine starch damage in sorghum. International Journal of Food Properties 19, 154-158.

Winger, M., Khouryieh, H., Aramouni, F., Herald, T., 2014. Sorghum flour characterization and evaluation in gluten-free flour tortilla. Journal of Food Quality 37, 95-106.

Yano, H., 2010. Improvements in the bread-making quality of gluten-free rice batter by glutathione. Journal of Agricultural and Food Chemistry 58, 7949-7954.

APPENDIX A

Preparation of Solutions

Reducing Agent Solutions

Reducing solutions were suspended in solution to achieve equal dispersion within a sample. For concentrations added as a percentage (0.5%, 1.0%, 2.5% and 5.0%, fwb) of the formula, the amount of reducing agent needed for solutions was used to calculate using the steps outlined in Example 1.

Example 1: RP-HPLC extraction solution preparation for 100 mg flour sample size

Step 1: Create stock solution based on amount of reducing agent required per flour weight basis.

$$\mathbf{5.0\%}\ \boldsymbol{L-cysteine} = \frac{\mathbf{5.0}\ \boldsymbol{g\ of\ CYS}}{\mathbf{100}\ \boldsymbol{g\ flour}} = \frac{\mathbf{0.005}\ \boldsymbol{g\ CYS}}{\mathbf{100}\ \boldsymbol{mg\ flour}}$$

Step 2: Designate solution concentration.

$$\frac{\mathbf{0.005}\ \boldsymbol{g\ CYS}}{\mathbf{100}\ \boldsymbol{\mu L\ H2O}} = \frac{\mathbf{0.5}\ \boldsymbol{g\ CYS}}{\mathbf{10}\ \boldsymbol{mL\ H20}}$$

Step 3: Dilute stock solution to create remaining solutions.

Stock solution: **5.0% L-cysteine = Reserve 10 mL**
2.5% L-cysteine = 5 mL stock solution + 5 mL H20
1.0% L-cysteine = 2 mL stock solution + 8 mL H20
0.5% L-cysteine = 1 mL stock solution + 9 mL H20

For concentrations added as parts per million (100 ppm, 250 ppm, 500 ppm, and 1000 ppm, fwb) into the formula, the amount of reducing agent needed for solutions was used to calculate using the steps outlined in Example 2.

Example 2: Test bake requiring 3 bread loaves per 4 concentration.

Step 1: Determine amount of reducing agent required per 100 g flour .

$$\frac{\mathbf{100}\ \boldsymbol{part\ SMB}}{\mathbf{1000000}\ \boldsymbol{part\ flour}} = \frac{\mathbf{0.0001}\ \boldsymbol{g\ of\ SMB}}{\mathbf{100}\ \boldsymbol{g\ flour}}$$

Step 2: Designate solution concentration.

$$\frac{\mathbf{0.0001}\ \boldsymbol{g\ SMB}}{\mathbf{10}\ \boldsymbol{mL}} = \frac{\mathbf{100}\ \boldsymbol{ppm\ SMB}}{\mathbf{10}\ \boldsymbol{mL}}$$

Step 3: Scale-up solution volume based on batch size.

$$30\ \text{mL}_{\ 100\ \text{ppm}} + 75\ \text{mL}_{\ 250\ \text{ppm}} + 150\ \text{mL}_{\ 000\ \text{ppm}} + 300\ \text{mL}_{\ 1000\ \text{ppm}} = 555\ \text{mL}_{\ \text{Total solution needed}}$$

555 mL solution required at a concentration of 100 ppm/10 mL

Sugar-Salt Solution

Modified from AACCI Method 10-10.03, these preparations provide stock solution of sugar and salt suspension of such strength that 10 ml of solution contain required quantities of ingredients per 100 g flour. Solution contains 6 g sugar and 1.5 g salt in 10 ml solution. Weigh 1200 g sugar and 300 g NaCl and place in 2-liter volumetric flask. Add distilled water to cover solids and mix thoroughly. Continuously stir while adding water until sugar and salt are dissolved and 2-liter mark is reached. Volume displacement for sugar and salt combination is approximately 0.039 ml. Solution will keep for several weeks at room temperature, however, discard if cloudiness is noted.

Total Water Formula

The amount of water required by the sorghum bread formula after the addition of reducing agent solution and sugar salt solution was determined using the following equation. Due to the displacement caused by sugar and salt when in solution, the amount of sugar-salt solution must be multiplied by a factor of 0.61.

$$\text{Water Added}(\text{mL}) = 1.05(\text{flour weight}, \text{g}) - (\text{reducing agent solution}, \text{mL}) - 0.61(\text{sugar} - \text{salt solution}, \text{mL})$$

APPENDIX B

Sodium Metabisulfite Preliminary Results

Protein Analysis Methods

Protein analysis was accomplished following the method previous described by Bean et al.(2011). Reversed-phase high performance liquid chromatography (RP-HPLC) was performed with an Agilent 1100 series instrument (Agilent, Santa Clara, CA) using surface porous Poroshell 300 SB columns (2.1 x 75 mm) with C18 as the stationary phase (Agilent, Santa Clara, CA). Mobile phase A was deionized water plus 0.1% trifluoroacetic acid (TFA) (w/v) and mobile phase B was acetonitrile (ACN) plus 0.07% (w/v) TFA.

Chemicals and Samples. RP-HPLC sampling was completed at the ARS-CGAHR USDA facility in Manhattan, KS. HPLC-grade β-mercaptoethanol (BME) and 4-vinylpyridine (4-VP) were obtained from Sigma Aldrich Co. (St. Louis, MO). Reducing agent solutions were prepared using distilled water plus 0.5%, 1.0%, 2.5% or 5.0% (w/v) of glutathione, L-cysteine or sodium metabisulfite.

1ST Stage Sample Preparation. Samples were prepared using 100 mg pearled white sorghum flour and 100 µL distilled water or 100 µL of 0.5%, 1.0%, 2.5% or 5.0% (w/v) of reducing agent solution. BME was used as a positive control while no reducing agent was used as a negative control. Samples were continuously stirred by hand for 3 min using a metal spatula then transferred immediately into a -80°F freezer and lyophilized.

2ND Stage Sample Preparation. Samples were prepared using 100 mg pearled white sorghum flour and 100 µL distilled water or 100 µL of 0.5%, 1.0%, 2.5% or 5.0% (w/v) of reducing agent solution. BME was used as a positive control while no reducing agent was used as a negative control. Samples were continuously stirred by hand for 3 min using a metal spatula. Once mixed,

samples were simultaneously heated and stirred using a VorTemp™ 1550 Shaking Incubator (Labnet International, Inc., Edison, NJ) set at 30-32°C for 35 min. Samples were then transferred immediately into a -80°F freezer and lyophilized.

Protein Analysis Results

1ST Stage Analysis. During the first stage of RP-HPLC testing, sorghum flour with reducing agent treatments were evaluated. Samples underwent a 3 min mix period prior to being lyophilized and extracted. This trial was designed to quantify the amount of reduction occurring just after the mixing stage in the bread method. In Table 4.7 and Figure 4.6a, the results show flour treated with 5.0% of L-cysteine exhibited the greatest total peak area (4684 mAU) of all treatments; over seven times the total peak area of the negative control (638.1 mAU), where reducing agent was not added. The total peak area from the 5.0% L-cysteine treated flour was only comparable to that of the positive control, BME (3727 mAU), indicating a similar degree protein reduction was achieved by both. Also, sorghum flour treated with 2.5% of L-cysteine displayed a relatively high total peak area (2727 mAU) compared to the glutathione and sodium metabisulfite treatments. Although the total peak area for 2.5% (fwb) was lower than 5.0% (fwb) treatment of L-cysteine, both treatments were comparable to the BME control treatment peak area, representing the desired reduction had occurred.

Inversely, flours treated with glutathione at any of the four concentrations produced low total peak areas (Table 4.7 and Figure 4.6b). Glutathione treatments were similar to the negative control treatment, implying the desired reduction of kafirin proteins did not occur.

When sorghum flour was treated with sodium metabisulfite, concentrations of 0.5% (fwb) and 1.0% (fwb) produced the greatest total peak areas, 1918 mAU and 2323 mAU, respectively, but not as high as the L-cysteine treatments. These treatments were significantly different from both the

positive and negative control treatments, indicating a relatively moderate degree of protein reduction occurred within flour samples.

Table 6.1: Stage 1 analysis RP-HPLC total peak area (mAU) of extracted kafirins: sorghum flour treated with reducing agents and 3 min mix period [a]

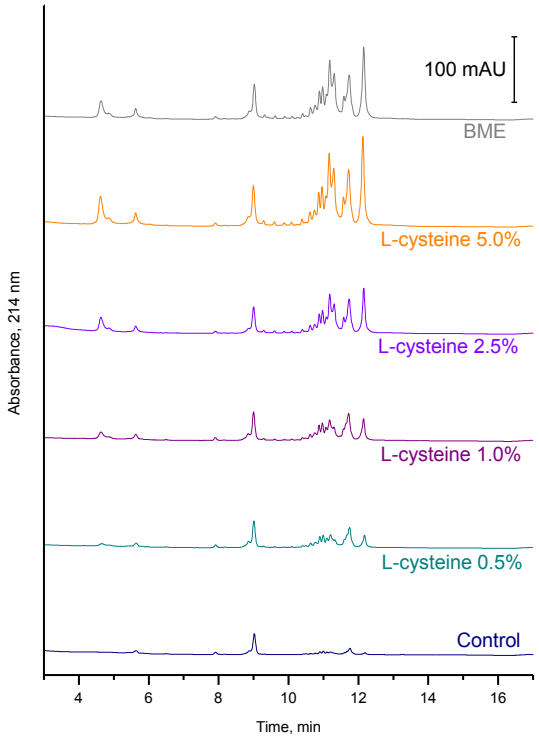
Treatment	Concentration (%, fwb)				
	--	0.50%	1.00%	2.50%	5.00%
BME[b]	3727ab	--	--	--	--
Control[c]	638f	--	--	--	--
L-cysteine	--	1346def	2152cd	2727bc	4684a
Glutathione	--	831f	734f	691f	703f
Sodium metabisulfite	--	1918cde	2323cd	1474def	1019ef

* Values followed by different letters are significantly different ($p < 0.05$)

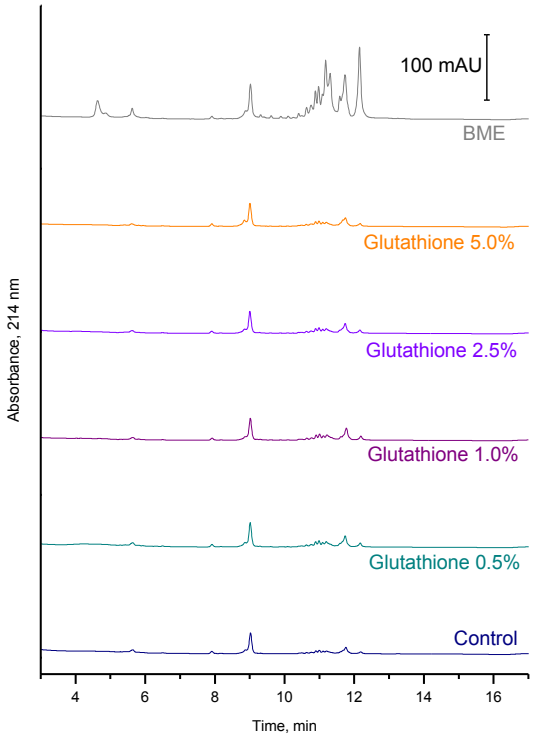
[a] Samples were sorghum flour treated with reducing agents at various concentrations subjected to mixing for 3 min.

[b] Positive control, 2.0 % (v/v) β-mercaptoethanol in distilled water

[c] Negative control, no reducing agent added



a)



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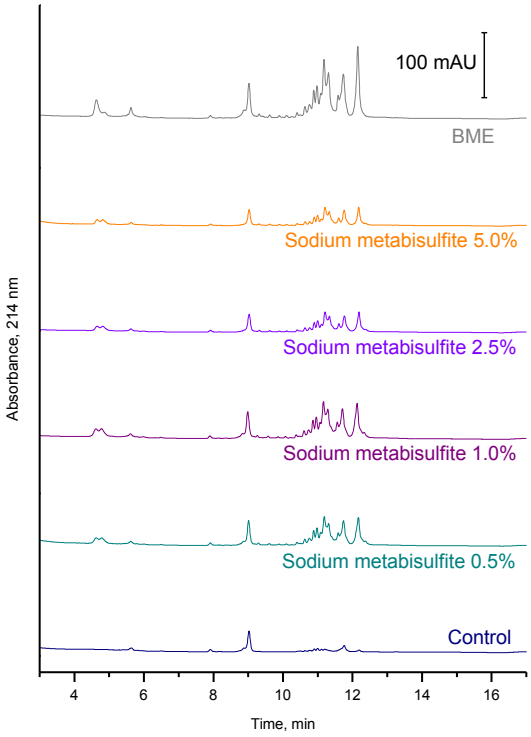


c)

Figure 6.1: Stage 1 analysis RP-HPLC chromatograms of extracted kafirins from sorghum flour treated with reducing agents and 3 min mix period

2ND Stage Analysis. RP-HPLC analysis of sorghum flour with reducing agent treatments were submitted to a 3 min mixing period then held at 32°C for an additional 35 min simulated proofing during the bread making process For all sample treatments, total peak area significantly increased when subjected to simulated proofing compared with the 3 min mixing time. The intensification in total peak area is likely the result of longer reaction time. The additional 35 min at 32°C provided samples the necessary time and conditions to facilitate further protein reduction.

By using the simulated proofing environment, more treatments were able to achieve a similar degree of reduction as the positive control, BME (5725.5 mAU). L-cysteine treatments at 5.0% (fwb) and 2.5% (fwb) (Table 4.8 and Figure 4.7a) displayed total peak areas of 6976.3, and 6692.7 mAU, respectively, while sodium metabisulfite treatments at 2.5% (fwb) and 1.0% (fwb) (Figure 4.7b) presented total peak areas of 5904.2 and 6099.9 mAU, respectively (Table 4.8). Unfortunately,

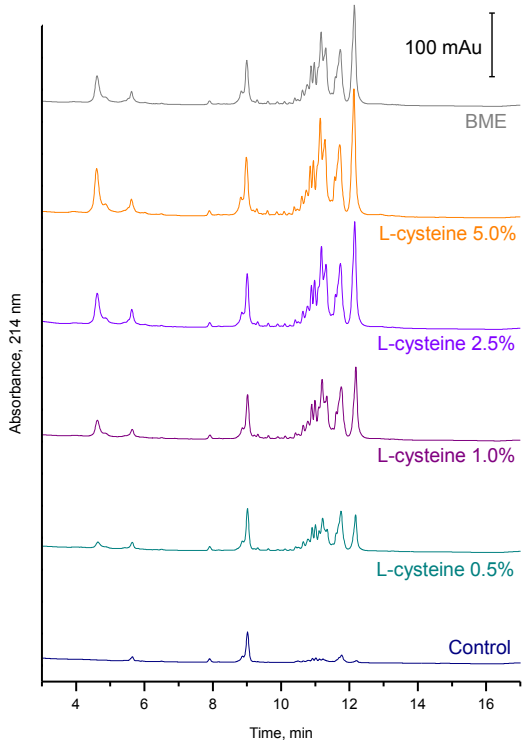
even with the extended reaction time, glutathione treatments at all concentrations continued to produce low total peak areas (≤1904.6 mAU) (Figure 4.7 c). Due to the lack of protein reduction, glutathione was omitted from the remainder of the study.

Table 6.2: Stage 2 analysis RP-HPLC total peak area (mAU) of extracted kafirins [a]

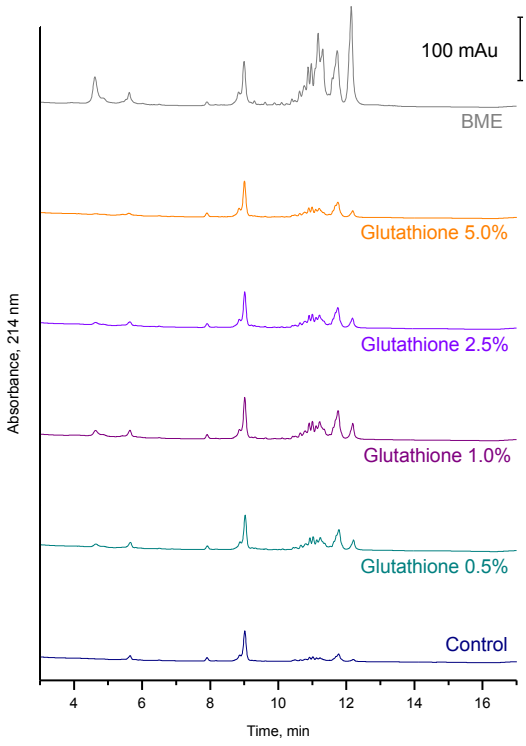
		Concentration (%, fwb)			
Treatment	**--**	**0.50%**	**1.00%**	**2.50%**	**5.00%**
BME	5725abc	--	--	--	--
Control	896f	--	--	--	--
L-cysteine	--	3194de	4428cd	6693ab	6976a
Glutathione	--	1741ef	1904ef	1586ef	1269f
Sodium metabisulfite	--	4953bcd	6100abc	5904abc	4674cd

* Values followed by different letters are significantly different ($p < 0.05$)

[a] Samples were sorghum flour treated with reducing agents at various concentrations subjected to simulated proofing conditions



a)



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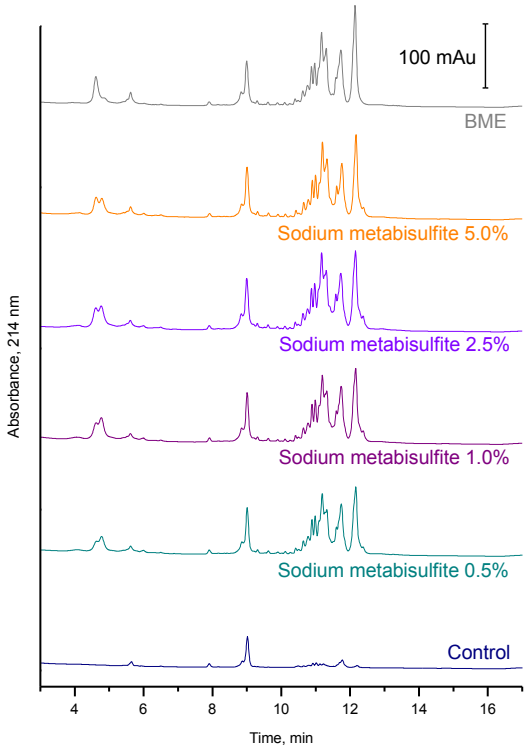

c)

Figure 6.2: Stage 2 analysis RP-HPLC chromatograms of extracted kafirins from sorghum flour treated with reducing agents subjected to 3 min mix and 35 min proofing period.

Bake Test

The external bread characteristics between breads formulated with L-cysteine and sodium metabisulfite were notably different. Breads containing L-cysteine, at any of the three concentrations, was significantly greater than breads containing sodium metabisulfite. Yet there was not a difference in volume between the control bread and the L-cysteine treated breads. The internal crumb grain characteristics also exhibited differences between reducing agents. The cell wall thickness and diameter of the gas cells within the crumb were statistically greater for breads containing L-cysteine compared to breads containing sodium metabisulfite. Yet, the number of gas cell present within the crumb were the same across all treatments.

Table 6.3: Physical characteristics of sorghum bread treated with L-cysteine and sodium metabisulfite

Treatment	Actual Volume (cm^3)	Specific Volume (cm^3/g)	Number of Cells (mm)	Cell Wall Thickness (mm)	Cell Diameter (mm)
Control	578.3a	2.85a	3945a	0.509a	3.749a
L-cysteine 0.5%	558.3a	2.80a	3403a	0.542a	3.416a
L-cysteine 1.0%	561.7a	2.76a	3643a	0.524a	3.316a
L-cysteine 2.5%	548.3a	2.68a	3589a	0.518a	2.764ab
SMB 0.5%	288.3b	1.36b	3731a	0.373b	1.888b
SMB 1.0%	293.3b	1.44b	3841a	0.379b	1.801b
SMB 2.5%	268.3b	1.31b	3662a	0.369b	1.693b

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* Percents are expressed on a flour weight basis (fwb)

Breads formulated with sodium metabisulfite were extremely dense compared to L-cysteine breads and the control bread. C-Cell images of the control, L-cysteine and sodium metabisulfite treated breads illustrate the differences in volume. The data indicates breads formulated at 0.5% to 5.0% (fwb) of sodium metabisulfite were overdosed. As mentioned, bread systems are easily overdosed with sodium metabisulfite due to its' extensive reducing power. Due to the poor loaf volume, further bake tests were performed to determine an optimum concentration from sodium metabisulfite and the threshold concentration.

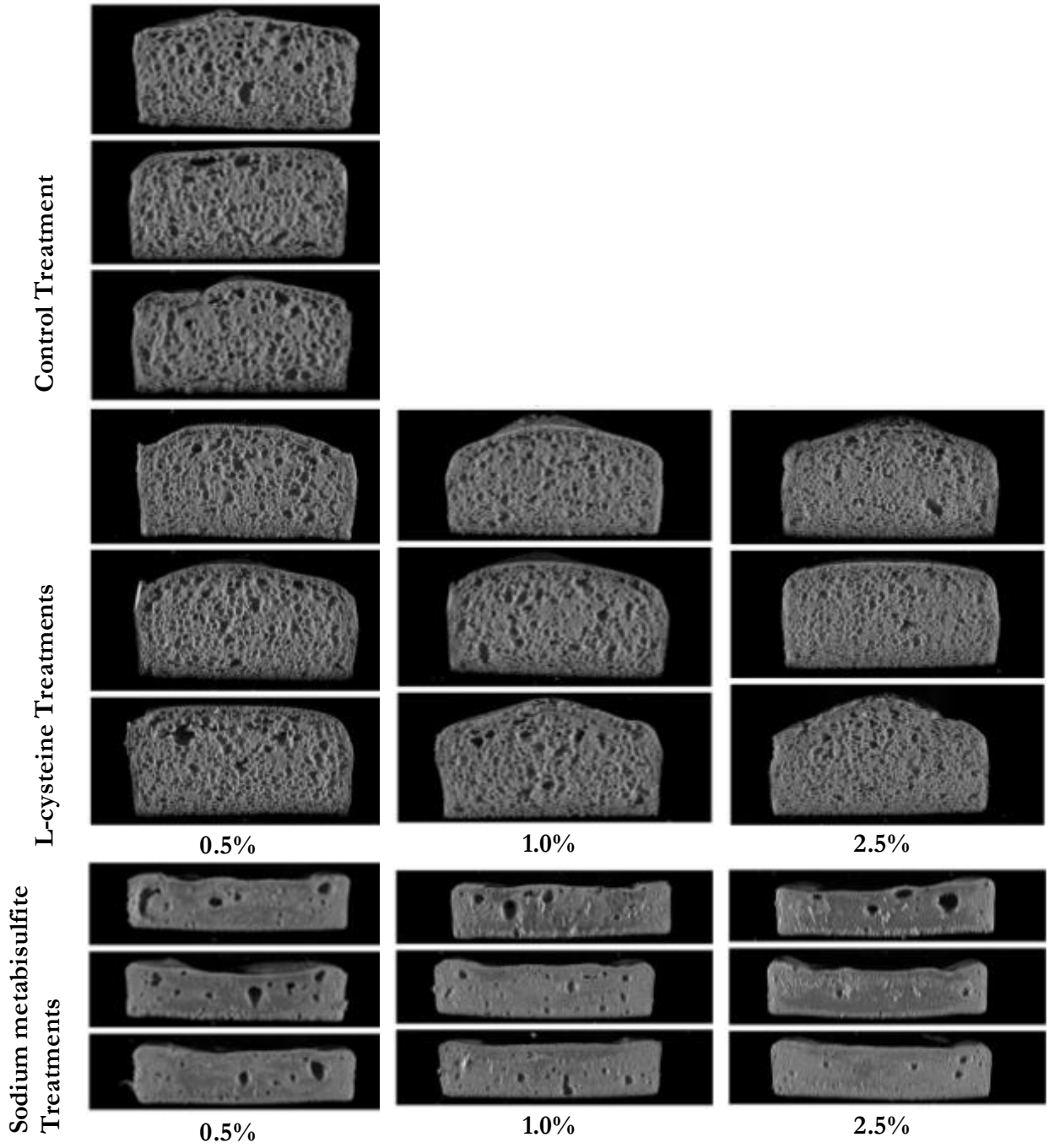

Figure 6.3: C-Cell images of bread crumb treated with reducing agents at 0.5%, 1.0% and 2.5% (fwb).

Yeast Activity

The yeast activity of batters were evaluated using a Risograph. Batters were prepared in accordance with the previously described bread method. A 50 g portion of batter was scaled into a stainless steel chamber, placed into a water bath. Just prior to testing, the stainless steel chamber was attached to a gas-measuring device. Yeast activity was determined by the volume of gas evolved over a period of 90 min at standard barometric pressure. The selected concentration of L-cysteine (2.5% fwb), sodium metabisulfite (500 ppm fwb) and the control batter were tested.

Although the analysis lasted for 90 min, the activity occurring between 3 and 40 min of analysis was of most importance for the implemented bread method (Schrober et al., 2005), which has a 35 min proofing. The analysis captured both the rate of CO_2 gas evolution at each minute (Figure 4.5), and the cumulative total of CO_2 gas produced (Figure 4.6), illustrating yeast activity rate and the gas production capability, respectively.

As predicted, batters containing 3000 ppm (fwb) of sodium metabisulfite produced significantly lower volumes of CO_2 gas at all time intervals (Table 4.11), indicating yeast activity was severely stifled or absent. From the generated curves in Figure 4.11 and Figure 4.12, it seems volume of CO_2 gas began to rise slightly, however during the 90 min proofing a peak in CO_2 gas production was not observed. In contrast, batters treated with sodium metabisulfite at 500 ppm (fwb) produced similar volumes of CO_2 gas as the control batter, signifying yeast activity was not hindered but in fact performed just as well as in the absence of a reducing agent. The time when yeast activity was greatest for batters treated with 500 ppm (fwb) of sodium metabisulfite appears to fall between 35 to 45 min, yet after that period yeast activity does not taper significantly. This implies longer fermentation periods can be applied to batters treated with sodium metabisulfite at 500 ppm (fwb) without negatively impacting the volume of CO_2 gas generated by the yeast. Batters treated with 2.5% (fwb) of L-cysteine displayed an intermediate level of yeast activity. L-cysteine treated batters

produced larger volumes of CO_2 gas compared to batters treated with the threshold concentration (3000 ppm) of sodium metabisulfite. Although after 20 min L-cysteine treated batters produced lower volumes of CO_2 gas in comparison to the control and sodium metabisulfite at 500 ppm. The greatest volume of CO_2 gas was generated around 20 min for batters treated with 2.5% (fwb) of L-cysteine. This suggests when utilizing L-cysteine at 2.5% (fwb) the ideal proofing period would be 15 to 20 min to achieve peak yeast activity.

Table 6.4: Rate of CO_2 production (mL/min) for sorghum bread batters [a]

	Time Elapsed (min)									
Treatment	1	3	5	10	15	20	25	30	35	40
Control	0.59a	1.20a	1.59a	2.31a	2.75a	2.96a	3.17a	3.31a	3.41a	3.49a
L-cysteine, 2.5%	0.53a	1.07a	1.38a	2.00a	2.38a	2.52b	2.48b	2.37b	2.22b	2.12b
SMB, 500 ppm	0.65a	1.23a	1.67a	2.26a	2.70a	3.00a	3.22a	3.37a	3.49a	3.48a
SMB, 3000ppm	0.14b	0.19b	0.18b	0.14b	0.14 b	0.13c	0.14c	0.15c	0.18c	0.19c

* Values followed by different letters within a column are significantly different ($p < 0.05$)
[a] Samples were treated with optimum concentrations of L-cysteine and sodium metabisulfite in addition to a threshold concentration of 3000 ppm sodium metabisulfite.

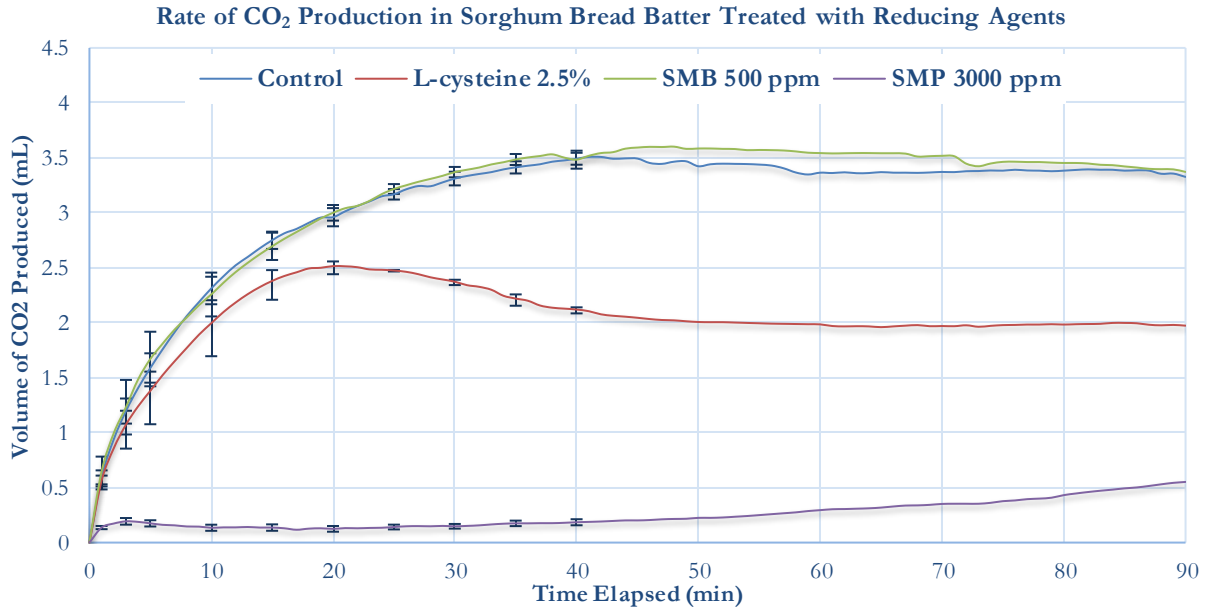

Figure 6.4: Rate of CO_2 gas production for optimum concentrations and threshold concentration.

Table 6.5: Cumulative total volume of CO_2 gas production for sorghum bread batters [a]

	Time Elapsed (min)									
Treatment	1	3	5	10	15	20	25	30	35	40
Control	0.6a	2.9a	6.0a	16.6a	29. 9a	44.6a	60.4a	76.9a	94.0a	111.6a
L-cysteine 2.5%	0.5a	2.0a	5.1a	14.0a	25.2a	37.6a	50.0a	62.1a	73.6a	84.3a
SMB 500 ppm	0.7a	2.9a	6.0a	16.2a	28.9a	43.4a	59.0a	75.5a	92.7a	110.2a
SMB 3000 ppm	0.1b	0.5b	0.9a	1.6b	2.3b	2.9b	3.6b	4.4b	5.2b	6.0b

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Samples were treated with optimum concentrations of L-cysteine and sodium metabisulfite in addition to a threshold concentration of 3000 ppm sodium metabisulfite.

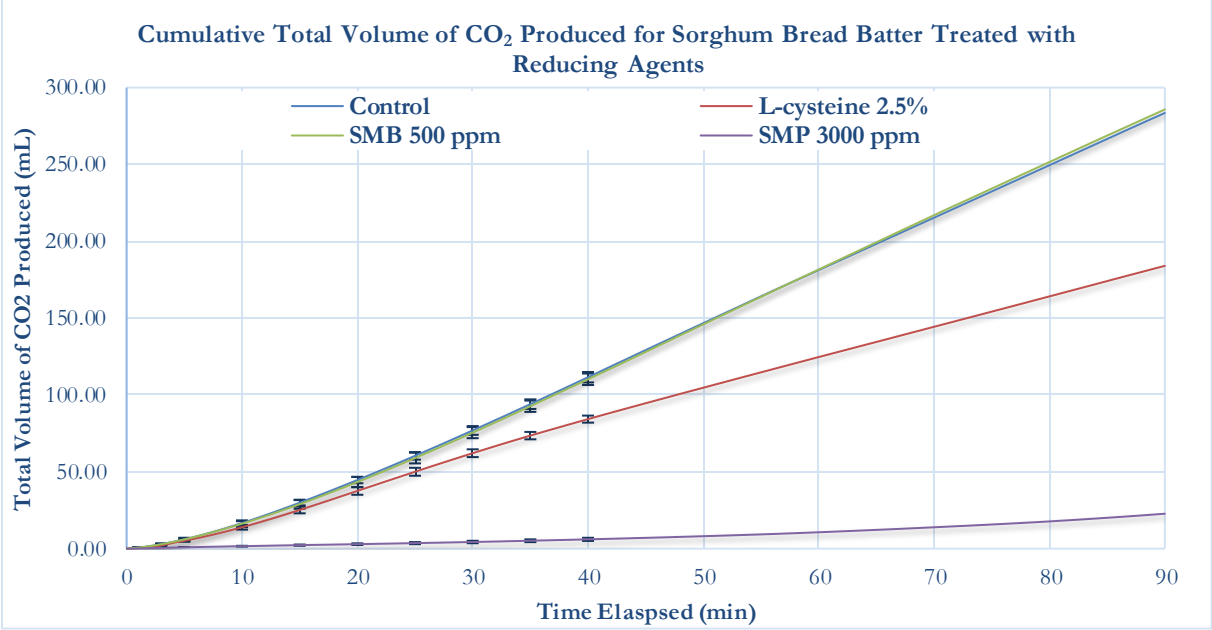

Figure 6.5: Cumulative total CO_2 gas production for optimum concentrations and threshold concentration

Yeast is sensitive to osmotic pressure. High osmotic pressure will decrease the fermentation rate. Salt at normal formula concentrations around 1.5-2.0% (fwb), negatively impacts yeast fermentation. For this reason, bread containing 6.5% (fwb) salt was tested to determine if the additional sodium contributed by sodium metabisulfite was increasing the osmotic pressure to a level that was inhibiting yeast fermentation. Bread formulated with 6.5% (fwb) total sodium, did show a decrease in volume but not to the same severity of the results seen when formulating with 0.5%-2.5% (fwb) sodium metabisulfite. The corresponding bake test indicated osmotic pressure was not the sole factor contributing to decreased yeast activity and poor loaf volume.

APPENDIX C

Supplemental Data and Figures

Supplement to Batter Consistency Data (pages 45-50)

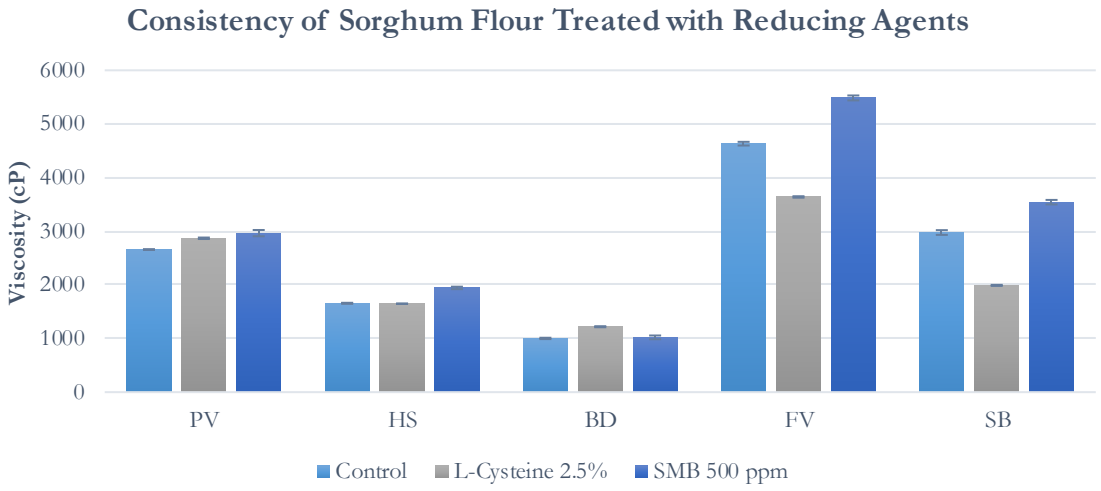

Figure 6.6: Consistency of sorghum flour treated with reducing agents at optimum concentrations.

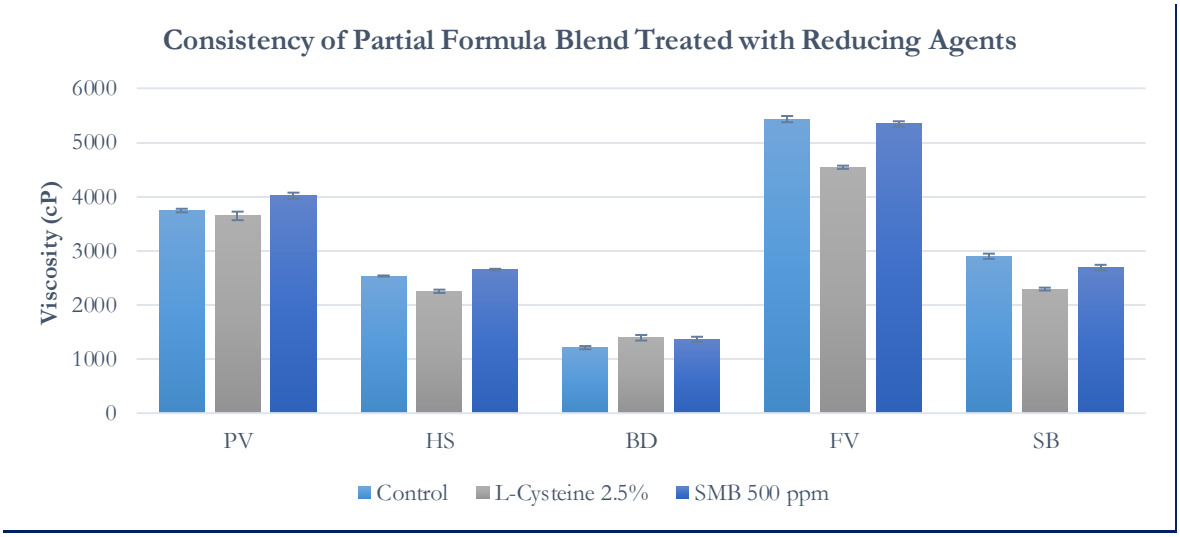

Figure 6.7: Consistency of partial formula blend treated with reducing agents at optimum concentrations.

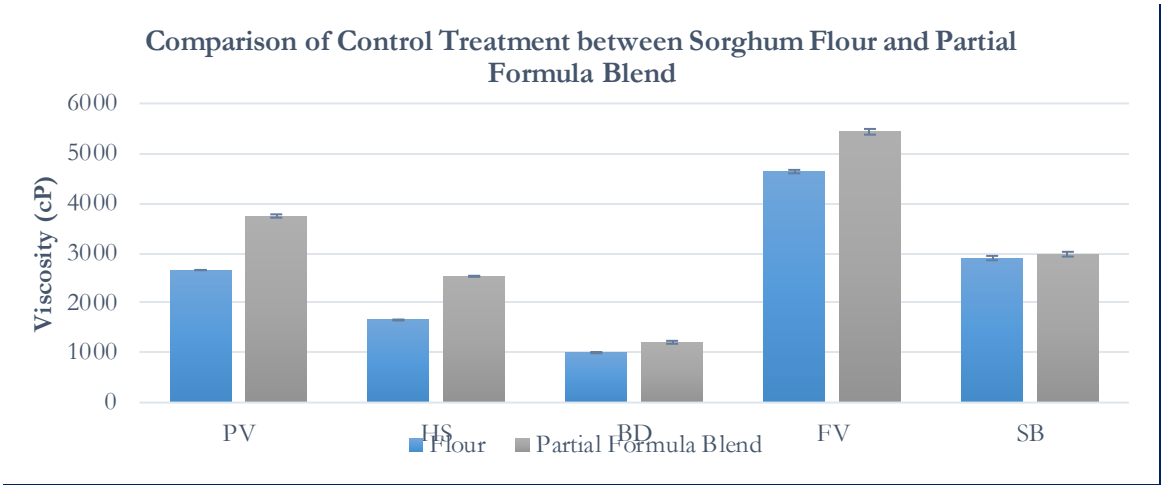

Figure 6.8 Comparison of sorghum flour and partial blend with control treatment.

Table 6.6: Comparison of sorghum flour and partial formula blend samples for control

Treatment:	PV[a] (cP)	HS[b] (cP)	FV[d] (cP)	Peak Time (min)	Pasting Temp (°C)
Flour	2658b	2533.0b	4633a	5.40a	80.25a
Partial Formula Blend	3742a	2250.7c	5431b	5.42a	71.22a

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Peak viscosity; [b] holding strength; [c] final viscosity

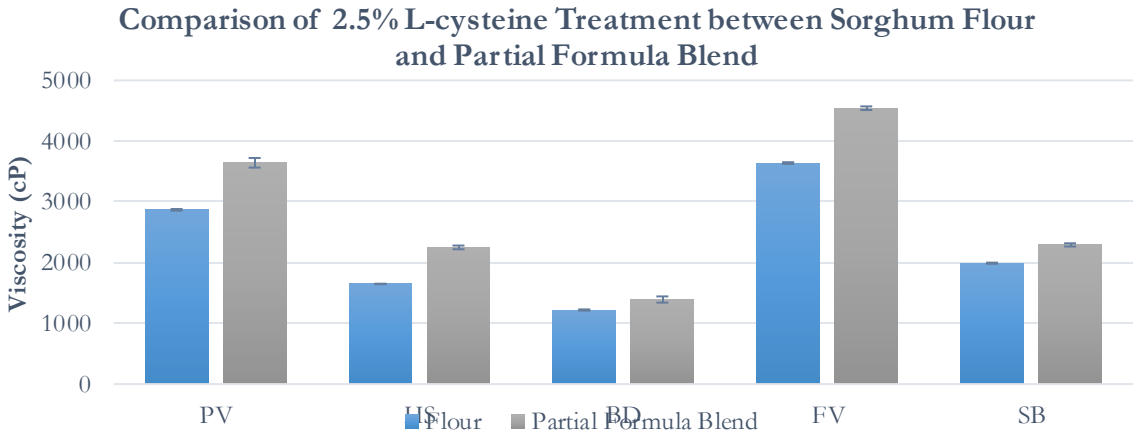

Figure 6.9: Comparison of sorghum flour and partial blend with 2.5% L-cysteine treatment.

Table 6.7: Comparison of sorghum flour and partial formula blend samples treated with 2.5% L-cysteine

Treatment:	PV[a] (cP)	HS[b] (cP)	FV[d] (cP)	Peak Time (min)	Pasting Temp (°C)
Flour	2869a	1649a	1220a	3639a	1990a
Partial Formula Blend	3643b	2251b	1393b	4542b	2292b

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Peak viscosity; [b] holding strength; [c] final viscosity

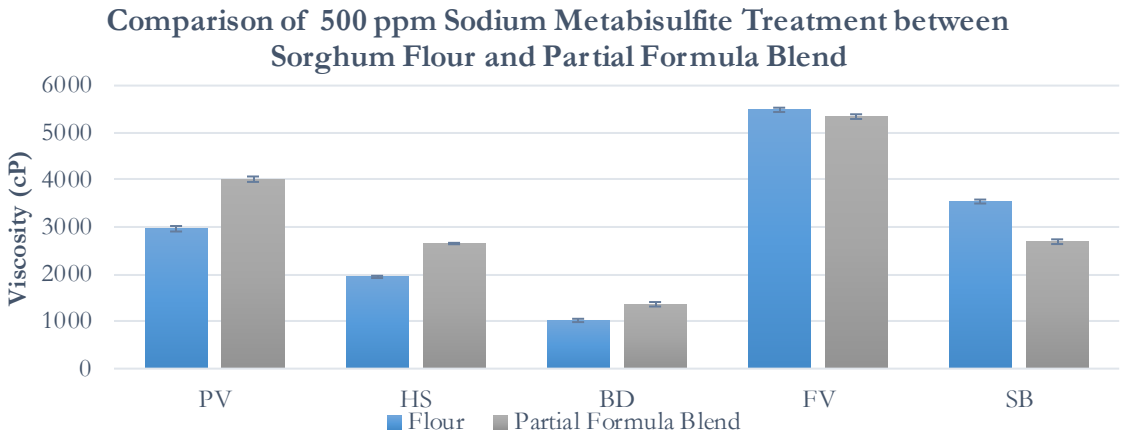

Figure 6.10: Comparison of sorghum flour and partial blend with 500 ppm (fwb) sodium metabisulfite treatment.

Table 6.8: Comparison of sorghum flour and partial formula blend samples treated with 500 ppm sodium metabisulfite

Treatment:	PV[a] (cP)	HS[b] (cP)	FV[d] (cP)	Peak Time (min)	Pasting Temp (°C)
Flour	2965a	1943a	1021a	5485a	3542a
Partial Formula Blend	4015b	2651b	1364b	5342b	2690b

* Values followed by different letters within a column are significantly different ($p < 0.05$)
* All concentrations are expressed on flour weight basis (fwb)
[a] Peak viscosity; [b] holding strength; [c] final viscosity