EFFECT OF SORGHUM FLOUR TREATED WITH OZONE AND HEAT ON THE QUALITY OF GLUTEN-FREE BREAD AND CAKE

by

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ABSTRACT

In order to improve the quality of products available for consumers who require a gluten-free diet, this study examined the effects of heat and ozone treatments on sorghum flour functionality in gluten-free bread and cake. In the ozone treatment experiment, commercially milled food-grade sorghum flour was subjected to ozone at the rate of 0.06 L/min for 15, 30, and 45 minutes. In the heat treatment experiment, commercially milled food-grade sorghum flour was subjected to dry-heat at two temperatures (95ºC and 125º) for 15, 30, and 45 minutes. Characterization of flour from each treatment included measurements of flour pH, color, and pasting properties. Evaluation of bread quality from each treatment included measurements of specific volume, color, crumb properties, and crumb firmness. Evaluation of cake quality from each treatment included measurements of specific gravity, volume, symmetry, uniformity, color, crumb structure, and crumb firmness.

Bake testing using ozonated sorghum flour in a high-ratio white layer cake formulation showed that volume significantly increased ($p<0.05$) as ozonation time increased. Additionally, longer ozonation exposure times increased cells per slice area, lightness, and slice brightness values in gluten-free cakes while reducing crumb firmness. Despite improving lightness and slice brightness values, ozonation did not significantly increase ($p>0.05$) the specific volume of gluten-free batter based bread.

In the heat treatment experiment, the optimum time and temperature relationship for improving sorghum flour was 125ºC for 30 minutes. This treatment level produced bread with the highest specific volume (3.08 mL/g) and the most cells

per slice area (50.38 cells/cm^2). This treatment level also produced cakes with the highest volume (72.17 cc) and most cells per slice area (79.18 cells/cm^2). Additionally, cake and bread made from this heat treatment was deemed more acceptable in comparison to the control during consumer testing. The control sorghum flour in both studies produced breads and cakes with low volume, poor crumb properties, and dense textures. These results can assist in the product development process in advancing the quality of sorghum-based gluten-free foods for the consumers who require a gluten-free diet.

TABLE OF CONTENTS

List of Figures

Chapter 1

Chapter 2

List of Tables

Chapter 1

Chapter 2

Appendices

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Chapter 1:

Literature Review

CELIAC DISEASE

Introduction

Celiac disease is an ailment caused by an abnormal immune response to gluten proteins in wheat, rye, barley, and possibly oats products (Sollid and Lundin 2009). According to Rewers (2005), the classic definition of celiac disease includes the following criteria:

1) Abnormal gastrointestinal manifestations including chronic diarrhea, failure to grow, weight loss, vomiting, bloating, distention, constipation, or abdominal pain.
2) Confirmation by a small bowel biopsy finding atrophy to the villi: the absorption surface of the small intestine.
3) Crypt hyperplasia: the enlargement of crypts in response to stimuli by injury or perceived threat of invasion to the body.
4) Normalization of the finger-like villi after treating with a gluten-free diet.

Simply put, this disease stimulates an immune reaction in the small intestine of allergy sufferers affecting the absorption of certain nutrients from foods. This ailment distresses approximately 3 million Americans making it roughly as common as type I diabetes (Rubio-Tapia et al 2009). While there is currently no medication to correct this

disorder, patients can reverse symptoms and health problems from the disease by adapting to a strict gluten-free diet.

Mode of Action

Celiac disease occurs when predisposed individuals with immune, genetic, and environment factors ingest gluten. This protein found in wheat, barley, and rye consists of glutamine and proline which are poorly digested in the upper gastrointestinal tract (Green and Cellier 2007). Gliadin is the alcohol-soluble fraction of gluten which contains the majority of toxic components to celiac patients. Molecules of undigested gliadin are resistant to degradation by gastric, pancreatic, and intestinal proteases in the intestinal tract. These remnants can pass through the epithelial barrier of the intestine during intestinal infections and interact with antigen-presenting cells in the lamina propria (Weiser and Koehler 2008).

Celiac patients suffer from an inflammatory reaction between the gliadin fractions and an immune response in the upper small intestine. This reaction is characterized by these gluten fractions infiltrating the lamina propria and epithelium causing chronic inflammatory cells and villous atrophy (Figure 1) (Green and Cellier 2007, Rewers 2005). The adaptive response entails bound proteinases and other tissue-damaging mechanisms causing crypt hyperplasia and injury to the villi. These gliadin peptides also activate an innate immune response by increasing the expression of interleukin-15 and activate intraepithelial lymphocytes. These activate cells become cytotoxic, and the loss of epithelial cells occurs (Wieser and Koehler 2008).

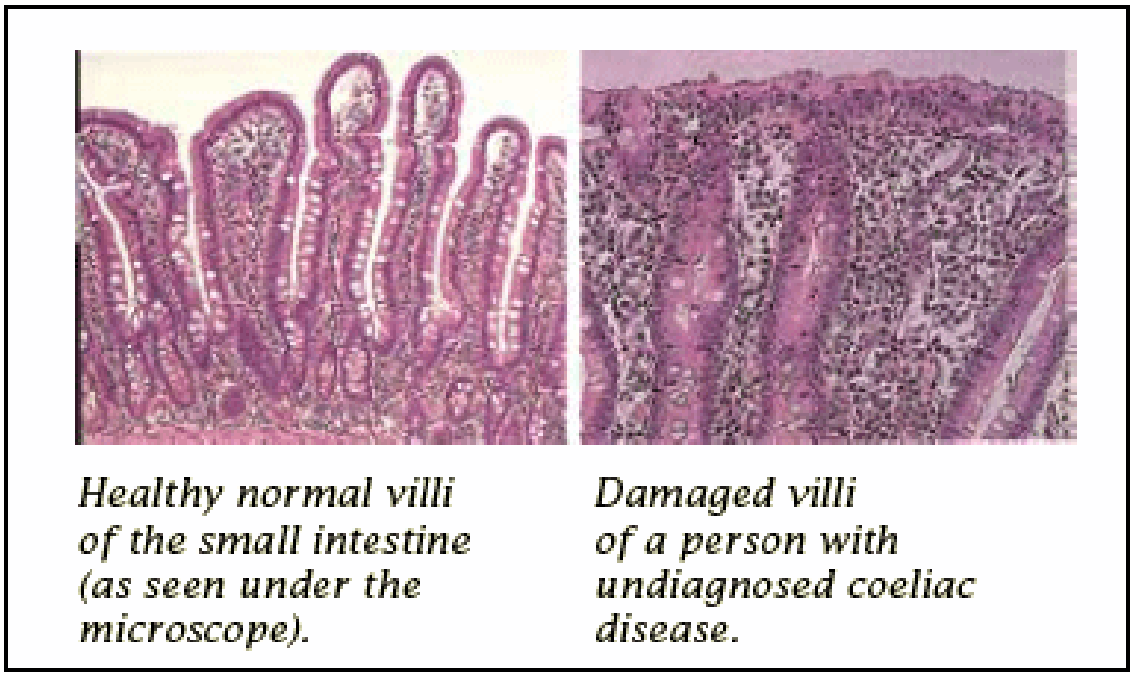

Figure 1. Villi Atrophy. Source: Rewers (2005).

Symptoms

Researchers have discovered a wide-array of symptoms leading to a positive identification of celiac disease. The most commonly recognized indicators of the disease relate to the malabsorption of food in the gastrointestinal system. The patient will have chronic diarrhea with fatty, greasy, and unusually foul-smelling stools. The patient may additionally complain of excessive gas, bloating, abdomen distention, weight loss, and fatigue (Fasano and Catassi 2001).

While not all patients exhibit outward signs of digestive problems, undiagnosed celiac disease can lead to a mixture of other health conditions. The inability to process and convert food adequately can lead to deficiencies in iron, vitamin K, and vitamin D resulting in anemia, easy bruising, and osteoporosis (Fasano and Catassi 2001). Infants and children afflicted with the disorder also exhibit signs of failure to thrive resulting in

lack of proper growth and development. Additionally, common indicators can be psychophysical and behavioral disturbances such as depression, irritability, and impaired concentration (Fasano and Catassi 2001).

Celiac disease can also manifest itself as a chronic skin disease known as dermatitis herpetiformis. This abnormality is described as symmetrical blistering skin lesions characterized by pathognomonic granular immunoglobulin IgA deposits on the uninvolved skin. These rashes are typically found on the elbows, knees, and buttocks. This skin condition affects about 10-20% of celiac patients while 90% of people with dermatitis herpetiformis have the gluten intolerance disease (Alaedini and Green 2005).

Several research reviews have suggested links between celiac disease and the following other medical disorders (Alaedini and Green 2005, Fasano and Catassi 2001).

- Endocrine Disorders – type 1 diabetes, autoimmune thyroid disorders, Addison disease, reproductive disorders, alopecia areata
- Neurological Disorders – Cerebellar ataxia, neuropathy, migraine, autism, epilepsy with intracranial calcifications
- Cardiac Disorders – Idiopathic dilated cardiomyopathy, autoimmune myocarditis, congenital heart defects
- Hepatic Disorders – Primary biliary cirrhosis, autoimmune hepatitis, autoimmune cholangitis
- Other Disorders – anemia, osteoporosis, selective IgA deficiency, Sjögren syndrome, juvenile chronic arthritis, Turner syndrome, Down syndrome, dental enamel defects

Diagnosis

Despite the use of small bowel biopsy as a gold standard for diagnosis, sporadically false-negatives have arisen due to patchy mucosal changes. Additionally, endoscopic biopsy does not typically reach the proximal jejunum where villous atrophy is frequently most severe. These issues have shifted towards a new definition of celiac disease using new serological markers to diagnosis including the presence of serum IgA autoantibodies to tissue transglutaminase (IgA TG) and HLA-DQB1*0201 or *0302 alleles (Rewers 2005).

Patients afflicted by this disorder can be diagnosed at any age, yet typically the disease is not detected until adolescence. This gluten intolerance mainly affects people of European descent being most prevalent in the Europe Union, North America, South America, and Australia. Frequency of celiac disorders among Caucasians is now thought to be in the range of 1 in 100 people (Wieser and Koehler 2008). However, equivalent ailment rates have been reported in North Africa, Middle East, and India (Rewers 2005). Additionally, celiac disease is passed down genetically. The probability of contracting the allergy increases to a 1 in 22 chance if a person has a first-degree relative with celiac disease and a 1 in 39 chance if they have a second-degree relative (Fasano 1996).

Treatment

Presently, the essential treatment in remedying the negative effects of celiac disease is a strict adherence to a life-long gluten-free diet. This diet implies no consumption of wheat, rye, barley, and related cereals such as spelt, kamut, and

triticale. These restricted grains are found in the tribe, *Triticeae*, within the grass family of *Poaceae* (Sollid and Lundin 2009). Nontoxic grains classified as safe include corn, sorghum, millet, rice, buckwheat, teff, quinoa, and amaranth. The inclusion of oats in a gluten-free diet is still regarded as suspect due to likelihood of cross-contamination during processing (Alaedini and Green 2005).

A gluten-free diet is challenging to celiac patients due to the wide-spread use of wheat and other gluten containing grains in staple foods like bread, cakes, and pasta. These grains are also extensively used as additives, thickeners, binders, and preservatives in processed foods like broth, processed meats, marinades, canned goods, candy, pudding, and medications (Cureton and Fasano 2009). Because of food label confusion, the average family shopping for gluten-free foods takes between 10 to 20 hours longer per month. Due to new medical knowledge and awareness of celiac disease, manufacturers have recognized the need and potential profit in clearly labeling and producing gluten-free foods.

In 2004, the Food Allergen Labeling and Consumer Protection Act (FALCPA) was signed into law. This directive mandates all FDA regulated food products, labeled after the 1st of January 2006, clearly state on the package whether the food contains any "major" food allergen (Cureton and Fasano 2009). The top eight allergens categorized as major include milk, eggs, fish, shellfish, tree nuts, peanuts, soybeans, and wheat. FALCPA makes label reading more straightforward for celiac patients, yet constant vigilance is still needed since rye and barley are not included in the major allergen list.

According to Wieser and Koehler (2008), gluten-free foods for celiac patients are produced under the regulations of the Codex Alimentarius Standard for Gluten-Free Foods adopted by the Codex Alimentarius Committee on Nutrition and Food for Special Dietary Uses. The "Draft Revised Codex Standard" edited in March 2006 proposes a maximum level of 20 mg of gluten per kg for naturally gluten-free foods (e.g. based on rice or corn flour) and 200 mg/kg for foods rendered gluten-free (e.g. wheat starch).

GLUTEN-FREE MARKET

Gluten-free foods are experiencing rapid growth in the marketplace due to increased availability and awareness of celiac disease. The 2007 Mintel Executive Summary on Food Allergies and Intolerance showed that the gluten-free market has seen 300% sales growth since 2000. Currently, estimated sales figures of gluten-free foods in the United States topped $696 million in 2006. This figure is expected to increase by 25% of the next four years reaching $1.7 billion by the end of 2010 (Cureton and Fasano 2009).

With this increase in demand for gluten-free products, research and development departments are striving to replace gluten containing grains in everyday food staples. In 2007, new food and beverage products claiming to be gluten-free reached 636 compared to just 202 new products in 2004 (Cureton and Fasano 2009). Most of these new products are in the snack and bakery sector (Table 1).

Table 1. Top Categories for Gluten-Free Foods in 2007

Category	Number of New Food and Beverage Products
Snacks	174
Bakery	94
Dairy	62
Confectionery	56
Sauces and seasoning	51
Processed fish, meat, and egg products	45
Beverages	43
Meals and meal centers	28
Side dishes	27
Desserts and ice cream	24

Source: Data from Cureton and Fasano (2009).

Even though there has been significant growth of gluten-free foods, there are still major concerns and challenges in improving the quality of life of celiac patients. One chief concern is the high cost of this diet. A large celiac support group identified taste and cost as the most important factors when purchasing gluten-free products (Sollid and Lundin 2009). On average, the cost of a gluten-free food is five times greater than its gluten containing counterpart (Table 2).

Table 2. Cost Comparison Between Wheat and Gluten-Free Products

Cost of Wheat Products		Cost of Gluten-Free Products	
Wheat Flour	$0.34/lb	Brown Rice Flour	$1.89/lb
Wheat Bread	$1.09/loaf	Gluten-Free Bread	$6.00/loaf
Wheat Pasta	$0.87/lb	Gluten-Free Pasta	$3.69/lb
Chocolate Chip Cookie	$2.69/lb	Gluten-Free Chocolate Chip	$12.83/lb
Wheat Crackers	$1.63/lb	Rice Crackers	$9.12/lb

Source: Data from Cureton and Fasano (2009).

GLUTEN-FREE BREAD

Introduction

Gluten is an essential part of the overall structure and quality of baked goods. The gluten fraction of wheat is primarily composed of two main protein groups. Gliadins are prolamins primarily responsible for the cohesiveness and extensibility of dough while glutenins are glutelins responsible for elasticity (Pyler 1988b). Combining these two proteins provides the viscoelastic properties necessary for producing a cohesive gluten network for structure and gas retention in wheat bread. Because of this, cereal technologists have a difficult task of replacing and replicating the gluten complex in developing gluten-free cereal products.

Gluten-free bread formulations produce doughs lacking the cohesive and elastic nature of traditional wheat breads. The absence of gluten makes these doughs more fluid and more similar to cake batter in terms of viscosity and rheological properties (Lazaridou and Biliaderis 2009). Consequently, researchers use the term batter based breads when describing gluten-free breadmaking. Due to the fluidity of these doughs, they require minimal mechanical mixing with a kitchen mixer and do not require hand-kneading (Schober et al 2005).

Flour

A variety of flours have been employed in gluten-free baked goods either alone or in combination with other flours and starches. These cereals include corn, amaranth, buckwheat, teff, arrow root, quinoa, rice, and sorghum (Schober 2009). Factors affecting the functionality of these flours depend on their genetics, growth conditions,

particle size, milling, and processing conditions. As previously mentioned, these flours lack gluten and depend on other ingredients to develop a gas holding network to provide structure and volume in bread.

Starch

Starches are commonly added to gluten-free formulations to improve texture and appearance. Not only do starches provide physiological health benefits, but they also can provide the following functions: gelling, thickening, adhesion, moisture retention, and anti-staling (Abdel-Aal 2009). Commercially available starches are derived from a variety of sources including corn, wheat, potatoes, rice, and cassava. All these starches have differing pasting, gelling, thermal, and texture properties based on their chemical structure and composition. Differences in starch functionality depend mostly on the glucose polymers of linear amylose and branched amylopectin (Abdel-Aal 2009).

In thermal processing of starchy foods, starch is directly involved in the gelatinization process. When heated or cooked enough, water absorption causes the starch granules to swell (Hoseney 1994). After these granules become disrupted, amylose seeps out creating a viscous slurry or paste depending on the concentration. Typically, the pasting properties are based on starch type, amylose content, amylose/amylopectin ratio, molecular weight, starch damage percentage, moisture content, shear rate, temperature, time, and the inclusion of other ingredients like sugar (Abdel-Aal 2009).

Several aspects of starch properties help improve gluten-free products. Starches which materialize rigid gels can be used to enhance the consistency of gluten-free

batters. These gel forming starches have also been recommended to improve the gas holding capacity and stabilize air cells (Schober 2009). Other researchers have proposed that starch dilutes the endosperm and bran particles of non-wheat whole flours (Schober et al 2005). These particles hinder the formation of a homogeneous starch gel and obstruct even gas cell formation. The addition of pre-gelatinized starch can also improve gluten-free breads by lowering the gelatinization temperature. By speeding up the gelatinization process, there is an increase in batter viscosity and ability to trap air cells while ultimately improving overall crumb structure and volume (Schober et al 2005).

Hydrocolloids

Hydrocolloids are a diverse group of biopolymers that bind and form gels with water. This group of polysaccharides and proteins stem from plants, animals, seaweed, and microbial sources (Abdel-Aal 2009). In the food industry, they have an assortment of uses by improving texture, appearance, and product stability. Hydrocolloids or gums can be separated into three distinct groups: gelling agents, thickeners, and emulsifiers (Abdel-Aal 2009).

In gluten-free bread formulations, hydrocolloids are added to enhance viscoelastic and gas retaining properties. The reaction between these gums with other starches results in improved rheological properties along with better texture and stability in the final baked product. Specifically, hydroxypropyl methylcellulose (HPMC) has proven to be a functional aid for gluten-free bread. HPMC is a surface active substance which helps stabilize foams. This gum aids in aeration and allows for the

development of small bubbles while preventing gas cell coalescence (Schober 2009). The visible outcome of this reaction is larger loaf volumes with softer crumb structures.

Proteins

Dairy-based proteins can be used to improve a variety of foods. The two main proteins of milk are casein and whey proteins which both have an emulsifying effect based on their physicochemical composition (Ardent et al 2009). In bakery products, these proteins are used for flavor enhancement, texture improvement, and shelf-life expansion. Specifically, caseinates have an emulsifying and stabilizing effect; whey proteins have gelling characteristics; high-heat non-fat dry milk increases water absorption and imparts browning during baking (Gallagher et al 2003).

Including milk proteins in gluten-free applications has potentially both positive and negative impacts on the bread. Gallagher and others (2003) found that adding whey protein isolate with additional water content improve volume and crumb softness. Other quality improvements include a more desirable crust color and higher acceptability scores during sensory analysis. However, without additional water, different dairy powders decreased volume and crumb softness. Another drawback for adding dairy proteins is the allergic potential for celiac patients with secondary lactose intolerance (Schober 2009).

Water

Water is an essential component of any type of bread production to hydrate ingredients and activate yeast while acting as a dilutor and solvent. In gluten-free bread, soft batters with increased water content (100-150% added water on a percent

flour basis) result in lower viscosity batter systems with enhanced bread volume (Schober 2005). This additional water helps dilute suspended bran and endosperm particles to produce a higher quality end product. Conversely, thicker batters tend to be more brittle, lack flexibility, and have reduced oven-spring (Schober 2005).

GLUTEN-FREE CAKE

Introduction

Unlike bread, gluten development is neither required nor desired in high-ratio (i.e. more sugar than flour) cake formulation. Cake batter is an oil-in-water emulsion which relies on mixing to entrap and disperse air bubbles throughout. As more air bubbles are introduced, the batter becomes more aerated, batter density (g/ml) decreases, and viscous resistance to flow (G") increases (Hoseney 1994). During baking, these entrapped bubbles form the nuclei for the accumulation of generated leavening CO_2 gas and water vapor. With heat, the pressure inside these air nuclei increases causing expansion. Because of this, large numbers of small air bubbles are needed to ensure uniform gas distribution and fine crumb grain (Pyler 1988c).

Late in the baking process, starch granules gelatinize to 'set' the cake structure and support the aerated system. After the internal layer temperature becomes high enough, gelatinized particles absorb the surrounding water and swell in size (Pyler 1988c). Because of this absorption, the once hydrated protein network becomes glassy, brittle, and resists any further volume expansion. Upon cooling, the swollen starch molecules occupy more space in the system which means the final cake will not collapse as the pressure in the gas cells diminish (Hoseney 1994).

Flour

Flour proteins act as the skeletal framework for a cake. This support provides viscosity to prevent gas cells from having substantial mobility and coalescing. However, flour starch granules are the core of the cake framework since they gelatinize to 'set' the structure and support the final aerated texture. To combat the delay of starch gelatinization due to high levels of sucrose added to high ratio cake systems, chlorination is applied to the cake flour. After treating, the modified starch polymers have highly hydrophilic regions which swell more rapidly when the starch gelatinization onset temperature is reached (Hoseney 1994).

Trouble-Shooting

The use of sorghum flour results in cakes with inferior volume, mouthfeel, and overall quality. Functionality problems linked with sorghum flour include large particle size, deficient polar lipids, and high starch gelatinization temperatures (Schober 2009). Glover and others (1986) investigated these deficiencies of incorporating sorghum into cake formulations. They concluded finer milling by way of pin milling resulted in smaller particle size and higher starch damage. This milling technique also seemed to improve water binding and batter viscosity resulting in improved overall cake quality.

Sorghum also lacks other functional properties due to the absence of glycol- and phospholipids (Taylor et al 2006). This lack of natural emulsifiers (i.e. polar lipids) results in lower volumes and inferior crumb structure in comparison to cakes baked with wheat lipids. Due to this insufficiency, emulsifiers should be added to the gluten-free cake batter. These surface active agents have both lipophilic and hydrophilic ends which

reduce the interfacial tensions between the oil and water phases in the batter (Pyler 1988a). The improvement in batter stability ultimately results in decreasing bubble coalescence and increasing gas cell retention (Hoseney 1994).

Glover and others (1986) also found a high percentage of ungelatinized starch in the center of sorghum-composite cakes after baking. The primary factor for this is presumed to be the high gelatinization temperature of sorghum starch. This hypothesis was confirmed by replacing sucrose with glucose. The use of glucose/dextrose instead of sucrose/saccharose resulted in more complete starch gelatinization as well as improved cake volume and crumb properties (Glover et al 1986). Saccharose, a longer disaccharide molecule, delays starch gelatinization by lowering the water activity and binding to starch chains (Hoseney 1994). The use of glucose, a monosaccharide, in cake results in earlier starch gelatinization.

SORGHUM

Introduction

With an increasing sector of the population desiring gluten-free foods, there are many opportunities to utilize sorghum as a gluten-free grain. *Sorghum bicolor* (L.) Moench is a cereal in the grasses (Poaceae) family. The grain is native to the tropical areas of Africa, and was first domesticated around 3,000 to 5,000 years ago (U.S. Grains Council 2004). The genus *Sorghum* was established in 1794, and was then divided into three species: *S. halepense*, *S. propinquum*, and *S. bicolor* (Waniska and Rooney 2000a). The cultivated sorghum species *S. bicolor* can be loosely classified into

four categories based on intended use: grain, sweet, broom, and grass (U.S. Grain Council 2004). Cane type sorghum, commonly referred to as sorgos, has sweet and heavy stalks used to manufacture sweetener syrup. Silage and animal feed can also be made from the leaves and stalks of sweet sorghum. Broom corn sorghum has branches which are lengthened and rigid when reaching maturity. As a result, this fibrous substance is selected for whisk brooms, basketry, and house construction materials (Kimber 2000). Grass sorghums include both sudan and tunis grass. These grass type sorghums make excellent forage, silage, and feed stuffs. Finally, grain sorghum is commonly known as kafir, durra, milo, and millet. With nearly 95% of the nutritive value of corn, grain sorghum is principally used as a nutritionally valuable food source for both animals and humans (U.S. Grains Council 2004).

Production

The United States precedes India, Nigeria, and Mexico as the world's largest producer of sorghum grain (U.S. Grain Council 2004). In 2007, total sorghum production in the United States reached 505 million bushels up 82 percent from the previous year (NASS 2008). According the U.S. Grain Council (2004), the top five states in production in ranking order are Kansas, Texas, Nebraska, Louisiana, and Oklahoma. Due to climate and soil conditions, the sorghum belt in the U.S. runs from South Dakota to Southern Texas mainly on dry land acres. Typically in the United States, planting season occurs during May to mid-June while harvest is completed in September to November depending on crop readiness (Schober et al 2006).

The following factors are essential for the growth of the sorghum plant (Kimber 2000):

1) *Length of day*. Sorghum is a short day plant. The plants are day-length sensitive which means it initiates reproduction when day length reaches 12 hours.
2) *Rainfall amount*. Even though sorghum can prosper in drought conditions, this versatile crop will also grow in rainy weather.
3) *Altitude*. Sorghum grows at elevations from sea level to 3,000 meters.
4) *Temperature*. Seeds germinate satisfactorily at 10 to 35ºC. Ideal growing temperature is 30ºC. Frost conditions kill the sorghum plant.
5) *Soil type*. Sorghum can be effectively grown in a wide-array of soils ranging from light and sandy to heavy clay.

Structure and Appearance

Varying in proportion due to cultivar and environmental conditions, the sorghum caryopsis is composed of three distinctive anatomical parts (Waniska and Rooney 2000b):

1) *Pericarp* – This outer layer is separated into three histological tissues: epicarp, mesocarp, and endocarp. The epicarp is the outer most layer which is coated with a thin waxy film. The mesocarp is unique when compared to other cereal grains since it contains starch granules. The endocarp is the inner pericarp tissue compiled of cross and tube cells.

2) *Endosperm* – This storage organ is an assembly of the aleurone layer, peripheral, floury, and corneous regions. The aleurone layer contains protein bodies, enzymes, oil in the form of spherosomes, and ash in the form of phytin bodies. Both the peripheral and corneous sections appear transparent and also affect the functionality and digestibility of sorghum. The corneous and floury segment of the endosperm is made up of starch granules, protein bodies, and cellulose rich cell walls.

3) *Germ* – This embryo is a diploid combining one male and one female gamete. It is mainly comprised of the embryonic axis and scutellum. The embryonic axis houses the new plant material: the radicle which forms the primary roots and the plumulae which forms the leaves and stems. Not only does the scutellum serve as a bridge between the endosperm and germ, it also is a cache for reserve nutrients such as protein, enzymes, oils, and minerals.

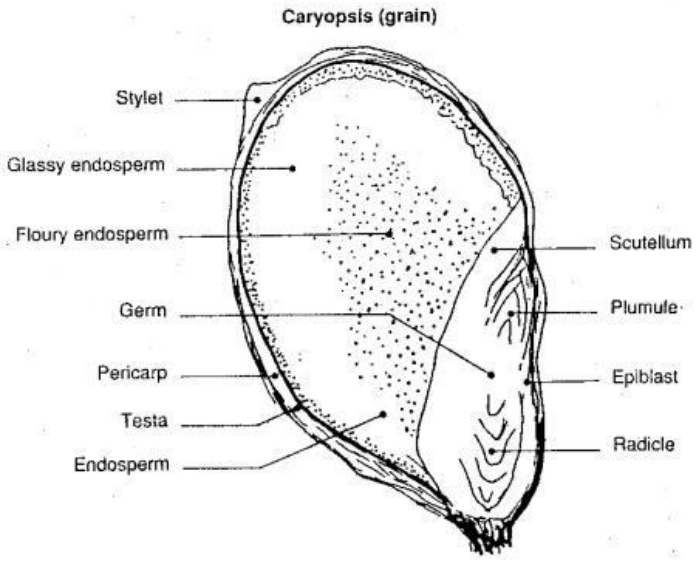

Figure 2. Cross-Section of Sorghum Seed.
Source: Waniska and Rooney (2000b).

Classification and Genetics

Sorghums can be loosely characterized by appearance, color, and total extractable phenolic content. White food grade sorghum has a white pericarp with no pigmented testa (Waniska and Rooney 2000b). White sorghums contain very small amounts of extractable phenol quantities with no detectable tannins or anthocyanins. While no tannins are present, red sorghums are comprised of a red pericarp and considerable extractable phenols. Black sorghums consisting of a black pericarp have a very high quantity of anthocyanins. Tannin varieties have varying degrees of pericarp pigmentation with substantial amounts of condensed tannins (Awika and Rooney 2004).

Sorghum genetics and kernel structure drastically influence the total phenol content. According to Dykes and Rooney (2006), pericarp color of the sorghum kernel is regulated by the R and Y genes. Possible combinations include:

	Pericarp Color	*Genotype*
homozygous recessive Y	White	rryy or RRyy
recessive R and dominant Y	Yellow	rrYY
dominant R and Y	Red	RRYY

Additionally, an intensifier gene (I) can also exaggerate the pericarp color especially in red cultivars.

While most research bases phenolic content on color, Boren and Waniska (1992) showed pericarp color and intensity are not good indicators of tannin content. Depending on the pigmented testa, sorghums with a white, yellow, red, or brown color pericarp may or may not have tannins. The presence of a pigmented testa is generated

by having both a dominant B_1 and B_2 gene. Brown pigments and perhaps tannins are regulated by the spreader gene, S (Dykes and Rooney 2006).

According to Waniska and Rooney (2000b), revelations about its genetic makeup and other chemical analyses have led the separation of sorghum into three distinct categories. Type I sorghum ($b_1b_1B_2\_$, $B_1\_b_2b_2$, $b_1b_1b_2b_2$) has no pigment testa, no tannins, and low degrees of phenols. Type II sorghum ($B_1\_B_2\_ss$) has tannins deposited in vesicles within the testa layer which can be extracted with acidified methanol. Type III sorghum ($B_1\_B_2\_S\_$) has tannins deposited along the cell walls of the testa with some present in the pericarp. Tannins from Type III can be extracted by either methanol or acidified methanol when using a vanillin/HCl assay.

Phenolic Acids, Flavonoids, and Tannins

All sorghums (Sorghum bicolor (L.) Moench) contain phenolic acids while some varieties possess flavonoids and condensed tannins (Dykes and Rooney 2006). The main sources of these phenolic compounds are situated in the pericarp, testa, aleurone layer, and endosperm (Hahn et al 1984). The category and amount of phenols present in sorghum grain varies due to plant genetics, environment factors, and cultivar type (Dicko et al 2006).

Phenols are of particular interest in food products due to their effects on astringency, bitterness, browning reactions, color, antioxidant activities, and protein components (Singleton et al 1999). Estimating these compounds can serve as a quality-grade marker while being informative and beneficial when developing new food technologies and applications.

Phenols are the most widely distributed secondary metabolite primarily responsible for the oxygen capacity in most plant-derived products (Dicko et al 2006). Phenols are defined as a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group (Vermerris and Nicholson 2008). Among cereals, sorghum ranks highest in total phenolic content reaching upwards of 6% (w/w) in some cultivars (Awika and Rooney 2004).

Phenolic compounds are a culprit for imparting bitterness and astringency in many foods and beverages. Varying from simple phenolic molecules to polymers with high molecular weight, there are more than 15 different classes of dietary phenolic compounds. The flavonoid group can be subdivided into 13 classes and included flavanones, flavanols, isoflavones, flavans, and anthocyanins (Drewnowski and Gomez-Carneros 2000). Plant tannins are high-molecular weight (greater than 500) polyphenols that tend to impart a more astringency (Drewnowski and Gomez-Carneros 2000). Conversely, lower-molecular weight phenols impart a more bitter taste. Astringency is defined as a drying or puckering mouth feel detectable throughout the oral cavity. This reaction may occur due a complicated response between polyphenols and proteins of the mouth and saliva (Drewnowski and Gomez-Carneros 2000).

Phenolic compounds impart bitterness and astringency since they serve as natural pesticides for plants against pathogens, predators, and parasites. Levels of these types of off-flavors are variable and alterable due to a variety of factors including plant genetics, type of cultivar, ripeness, and environmental surroundings as well as processing and storing techniques (Waniska and Rooney 2000b). To illustrate the belief

phenolics serve as a buffer against predation, immature sprouts and seedlings contain a greater amount of total overall phenol content when compared to mature plants (Drewnowski and Gomez-Carneros 2000).

Utilization

Depending on the world region, sorghum is made into a diverse assortment of food products. While the U.S. typically uses sorghum for animal feed, approximately 40% of the global crop is utilized for human consumption (Waniska and Rooney 2000a). In developing parts of the world, sorghum is employed in porridges, couscous, malted beverages, and unfermented/fermented flat breads. These traditional products are typically made with whole grain corneous flour. The whole grain may be achieved by grinding or decorticated then grinding the sorghum to produce either a fine particle flour or finished product (Waniska and Rooney 2000a).

In the U.S., sorghum has recently gained interest due to its gluten-free status along with the creation of more hybrids suitable for human consumption. Being an attractive alternative for people with wheat intolerances, sorghum is increasingly being incorporated into snack foods and bakery products. This growing demand from celiacs has lead to sorghum being commercially available in gluten-free bread, pasta, cookies, cereal, beer, and bakery mixes for brownies, cakes, and pancakes (U.S. Grains Council 2004).

While the health benefits and nutritional aspects are appealing, incorporating sorghum into traditionally wheat-based formulations has several complications. The total phenolic acid content of sorghum imparts a bitter taste to the finished product.

Secondly, sorghum lacks the wheat gluten proteins which provide structure and gas retention properties to baked goods. These shortcomings lead to poor cell structure, low volume, bitter flavor attributes, and other irregularities when developing new formulations in breads, cakes, and cookies (Schober et al 2006).

Despite its newly gained fame in gluten-free food products, roughly 90 percent of sorghum in the U.S. is consumed as animal feed (Stroade and Boland 2008). The starch and protein in sorghum are more problematic in animal digestibility when compared to corn. Sorghum can be further processed to improve its feed intake and efficiency through techniques such as grinding, crushing, steaming, steam flaking, popping and extruding. These processing steps of breaking the seed coat, reducing the particle size, and increasing surface area improve the end-use value of sorghum and yield a nutritional equivalent to corn (Stroade and Boland 2008).

In addition to feed and food applications, sorghum can be manufactured into numerous other products. Since it has poor conductivity, sorghum is prevalently used in biodegradable packaging materials (Stroade and Boland 2008). Additionally, Archer Daniels Midland is employing sorghum in housing wallboard. Lastly, sorghum is the second most utilized crop for ethanol production in the United States. Approximately 10 percent of the U.S. sorghum crop is consumed by ethanol production (NASS 2008). Five of the eight manufacturing plants using sorghum as a renewable fuel resource are located in Kansas since this state is continuously a top producer and reliable source of sorghum (Stroade and Boland 2008).

Quality Issues of Sorghum in Gluten-Free Applications

While sorghum offers a gluten-free alternative to wheat, complications arise when incorporating this grain into baked goods. Quality issues arise when milling, processing, and implementing sorghum grain in food products. Comparing the structure and chemistry differences with wheat illustrate the difficulties of integrating sorghum flour into formulations.

Even on a kernel basis, sorghum differs dramatically with wheat. Sorghum has a higher proportion of germ relative to the size of the endosperm. This equates to higher oil content in the kernel, approximately 3.4% in sorghum compared to 2.2% in wheat (Taylor and Dewar 2001). Depending on milling techniques, this high oil content could lead to high oil content flour which is more susceptible to rancidity during storage (Hoseney 1994).

The kernel structure of sorghum also affects other aspects of the milling process. Dissimilar to wheat, sorghum grain does not have a furrowing crease in the kernel (Taylor and Dewar 2001). In theory, this phenomenon should make the milling of sorghum more straightforward. However, the outer bran layer (pericarp) of sorghum is more friable than other cereals. To further complicate the milling process, the starchy endosperm of sorghum, unlike wheat, contains both a hard or corneous outer part and a soft or floury inner part. Sorghum varieties with a higher proportion of corneous endosperm are considered more desirable to mill since they offer higher yields of endosperm flour (Taylor and Dewar 2001).

All varieties of sorghum contain varying amounts of polyphenolic compounds including anthocyanins, anthocyanidins, tannins, and other flavonoids. These pigments are concentrated in the pericarp and glumes, yet may extend into the endosperm (Waniska and Rooney 2000b). These compounds impart fluctuating amounts of bitterness and color defects in certain food products.

The gelatinization temperature range of sorghum starch lies between 68-78ºC (Hoseney 1994). This range is drastically higher than wheat starch gelatinization temperature span of 58-64ºC. Higher gelatinization temperatures along with water-insoluble glucuronoarabinoxylans impart difficult challenges when incorporating sorghum into bread making applications (Taylor and Dewar 2001).

Despite the previous mentioned structural and chemistry differences, sorghum proteins are the main reason for quality issues in baked goods. These proteins known as kafirins are incapable of forming dough with sufficient gas-holding and visco-elastic properties (Taylor and Dewar 2001). This hindrance makes dough strengtheners, improvers, and oxidizing agents essential when incorporating sorghum flour into leavened baked products. Additionally, the protein of sorghum is deficient in the essential amino acid lysine. This deficiency results in negative consequences on digestibility and nutritional value of sorghum protein (Taylor and Dewar 2001).

Extensive research has been conducted in producing acceptable non-wheat substitutions in baked goods. Early work by Jongh (1961) indicated emulsifiers with just starch generated yeast-leavened bread-like products. The addition of glycerol monostearate caused starch granules to aggregate and sustain gas bubbles. Following

this breakthrough, scientists have experimented with various food additives to improve gas holding capacity of sorghum flour. Hart and others (1970) ascertained methyl cellulose (4000 cP viscosity) at an addition rate of 4% increased gas retention, loaf volume, and prevented collapsing.

FLOUR TREATMENTS

Introduction

Bleaching is an all-encompassing term used in flour production to convey both color removal by oxidizing yellow flour pigments and chemical maturation by oxidizing thiol groups (Pyler 1988a). Bleaching agents can be classified into three categories: bleaching agents only which have no influence on baking quality, maturing agents only which have no influence on color removal, or dual effects on both maturing and bleaching.

Treatment Levels

To determine the type of oxidant used as flour maturing/bleaching agents, the following factors must be evaluated (Stauffer 1990):

- *Safety*. Is the compound or its residue harmful to consumers or production employees?
- *Legality*. Is the compound allowed by the respective government in baked products?
- *Technological effectiveness*. Does the compound improve color and/or dough performance?

- *Cost effectiveness*. Is the compound the cheapest way to produce the desired effect?
- *Ease of application*. Can the compound be applied in a convenient and timely manner?

The level of oxidant treatment is determined by a variety of factors including flour type, extraction level, wheat variety, growth environment, storage length, milling process, other additives applied, and intended use (Pyler 1988a). For example, lower grade flour contains a greater amount of thiol groups. This means more of the oxidant must be applied since its effectiveness decreases as the level of extraction increases.

Types of Treatments

Select bleaching agents oxidize yellow pigment in flour to yield whiter and brighter product. These yellow pigments are expressed as carotenoid which consists of xanthophyll, carotene, and flavones (Pyler 1988a). Oxidizing agents used in the bleaching process have long, unsaturated carbon chains. These chains readily add oxygen to the double bonds in the carotene pigments to yield colorless compounds (Pyler 1988a).

An example of chemical oxidants permitted in flour to act as color bleaching agents are gaseous nitrogen peroxide and solid benzoyl peroxide. While both agents are unable to create a maturing effect on flour, benzoyl peroxide, a lipid, is more readily used since it is more effective in removing color (Pyler 1988a). This fine white powder is added to flour at 25-100 ppm (Stauffer 1990). For handling purposes, the powdered

benzoyl peroxide is typically mixed in by the miller with inert fillers like calcium carbonate or starch.

Maturing agents such as potassium bromate and azodicarbonamide (ADA) act as dough improvers while having more perceptible bleaching action (Pyler 1988a). While potassium bromate is extremely effective as a dough strengthener, it has been labeled a category 2B possible carcinogen (IARC 1999). This has lead to most commercial bakeries suspending use of this flour aid since it is banned in the European Union, Canada, China, and Brazil. ADA has the ability to improve machinability, increase loaf volume, and improve overall end quality. However, this flour aid also has been banned from use in Europe and Australia due to links as a possible cause of asthma and increasing allergic reactions of other food ingredients (WHO 1999). A widely approved and accepted method for maturing flour and oxidizing thiol groups utilizes L-ascorbic acid. Even though it is only two-thirds as effective of potassium bromate, ascorbic acid can have positive effects on dough while causing no nutritional or safety concerns (Pyler 1988a).

Chlorination

Chlorination is typically applied to cake flour as well as certain cookie flours to improve baking performance. Besides bleaching, this treatment also changes the functional properties of the flour. At the flour mill, chlorine gas is administered to the flour in metal cylinders. The process entails air, gas, and flour being mixed together in an agitator. Generally, chlorine gas is added from 1,100-2,300 ppm (1.8-3.7 oz/cwt) to improve cake color, symmetry, volume, grain, and texture (Hoseney 1994). Since

chlorination produces hydrochloric acid as a byproduct, the pH of the treated flour is lowered. As a result, pH is used to determine the degree of chlorination. Best flour performance is achieved by properly bleaching the flour to a 5.8 to 6.1 range (Pyler 1988c).

While the exact mechanisms are still uncertain during this complex process, the chlorine gas reacts with the following flour components: lipids, pentosans, starch, proteins, and water-soluble substances. The chlorine increases the hydrophilic qualities of the flour and ultimately increases batter viscosity (Hoseney 1994). Additionally, the gas imparts chemical changes to encourage starch swelling and gelatinization. Research conducted by Huang and others (1982) indicated that depolymerization and oxidation of starch occurred during chlorination. At 90ºC, the chlorinated flour produced cake batters with greater swelling capacity and solubility of starch granules. Moreover, chlorination interrupts intra- and intermolecular hydrogen bonds in flour protein molecules while cleaving peptide bond reactions which ultimately increase protein dispersibility and gluten solubilization (Pyler 1988b).

Apprehension has arisen in recent years concerning the safety and toxicity of chlorination. Health authorities in many countries have banned the use of chlorinated flour. The European Union, for instance, banned the use of chlorine as a flour improver in November 2000 (Catterall 2000). These bans along with mill safety concerns and public opinion on chemicals in food processing have left researchers scrambling to develop a safe replacement for chlorination.

Heat Treatment

With emphasis on finding viable alternatives to chlorination, an increased interest has focused on heat treating flour as a substitution. Several studies have suggested heating flour with temperatures ranging from 49-140ºC and times fluctuating from 15 minutes to 4-5 days (Russo and Doe 1970, Thomasson et al 1995, Fustier and Gélinas 1998, Catterall 2000). Lower temperatures are generally linked to longer treatment times while high temperatures have lower exposure intervals. This exposure to heat denatures the protein and enzymes in the flour while lowering minimum starch gelatinization temperature and increasing batter expansion between 85-94ºC (Russo and Doe 1970). This difference in viscosity is connected to a cake's ability to transform from foam to sponge form and reduce shrinkage during baking (Thomasson et al 1995).

Russo and Doe (1970) showed that the optimum heat treatment temperature of flour is 120ºC to improve baking performance in high ratio layer cakes. While holding time was not determined to be a critical factor, this study illustrated that too high treatment temperatures had deleterious effects on baking texture and flavor. The research performed by Thomasson and others (1995) also focused on replacing chlorine treatment with heat exposure. This study concluded soft wheat flour heat at 125ºC for 30 minutes supplemented with 0.12% (fwb) xanthan gum produced higher volume cakes with similar crumb structure when compared to chlorinated control flour. Further work by Fustier and Gélinas (1998) confirmed heat treating flour increased batter viscosity. This report also revealed that heat treatment increased cohesiveness and springiness while reducing gumminess in final cake texture.

Heat treatment has also been suggested to be a viable method of improving bread quality particularly in weak, substandard flour. While incorporating it reduces dough extensibility, heat-treated flour has been shown to increase resistance, viscosity, and stiffness (Gélinas et al 2001). These factors lead to an increase dough elasticity and produce positive effects on oven spring and loaf volume (Pyler 1988). These effects mimic oxidizing agents traditionally used in making bread such as ADA and ascorbic acid. In research performed by Gélinas and others (2001), heat-treating flour at 80ºC for 15 minutes had positive effects on bread specific volume, texture, number of crumb cells, and overall appearance.

OZONE

Introduction

Since ozone possesses the ability to decompose free radicals without leaving chemical residues, the application for this strong oxidizing agent has broad appeal for use in the food industry. In the U.S., ratification of new legislation by means of the Food Quality Protection Act has created renewed interest in innovative food processing and sanitizing systems (Kim et al 2003). Additionally, continued environmental concerns over toxic chemicals have increased the demand and focus on new agents for sanitizers, bleaching agents, pesticides, and other chemicals in the food industry. In June 1997, ozone received a generally recognized as safe (GRAS) status as a disinfectant for foods (Kim et al 1999). This allowed ozone to be used in treating bottle water and sanitizing bottle water plants. In 2001, the Food and Drug Administration (FDA) allowed the use of ozone as a direct-contact food-sanitizing agent (Federal

Register 2001). This ruling exonerated any obstacles for using ozone in the $430 billion food production business. This approval from the FDA has kick-started a revival for using ozone as an antimicrobial agent in the treatment, storage, and processing of various food products.

Ozone Properties

Even though ozone is a naturally occurring substance found in the atmosphere of the earth, it can also be produced synthetically. Freshly generated ozone in nature is characterized by a fresh, clean smell of air following a thunderstorm (Muthukumarappan et al 2009). Ozone is an allotropic modification of oxygen that contains three atoms (O_3) compared to the two (O_2) in a standard oxygen molecule. The structure of ozone consists of three atoms of oxygen in the form of an isosceles triangle with an angle of 116.8 degree between the two O-O bonds (Figure 1). The distance between the two bonded oxygen atoms is 1.27 Å.

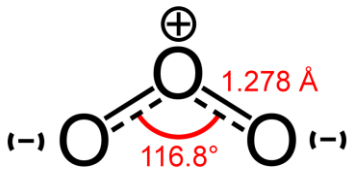

Figure 3. Ozone Molecule Formula.
Source: Taken from Muthukumarappan et al (2009).

As a gas, ozone is blue; both liquid (-111.9°C at 1 atm) and solid ozone (-192.7ºC) are an opaque blue-black color (Hunter 1995). Additionally, ozone is somewhat unstable as a gas at normal temperatures and pressures, is partially soluble in water, and is the strongest disinfectant currently available for contact with foods

(Muthukumarappan et al 2009). Ozone has an oxidation-reduction potential of 2.075 V (Brady and Humiston 1978). This high electrochemical potential (E^0, V) indicates ozone is a very favorable oxidizing agent for food applications (Equation 1). The physical properties of ozone are listed in Table 1.

Equation 1: Electrochemical Potential for Ozone.

$$O_3\ (g) + 2H^+ + 2e^- \leftrightarrow O_2\ (g) + H_2O\ \{E^0 = 2.075\ V\}$$

Table 3. Physical Properties of Ozone.

Physical Properties	**Value**
Boiling point, ºC	-111.9
Density, kg/m^3	2.14
Heat of formation, kJ/mole	144.7
Melting point, ºC	-192.7
Molecular weight, g/mole	47.9982
Oxidation strength, V	2.075
Solubility in water, ppm (at 20ºC)	3
Specific gravity	1.658

Source: Data from Muthukumarappan et al (2009).

Ozone Production

To generate ozone, air or another gas containing normal oxygen is exposed to a high-energy source. The introduction of high-energy converts molecules of oxygen to molecules of ozone. Since it is unstable and quickly decomposes to normal oxygen, ozone must be manufactured on site for immediate use. Ozone production is predominately achieved by one of three methods: electrical discharge methods, electrochemical methods, and ultraviolet (UV) radiation methods (Muthukumarappan et al 2009).

Electrical (corona) discharge methods are the most widely used in commercial settings even though it consumes a large amount of electricity. Substantial electrical energy (5000 V) is required for the ozone producing electrical discharge field to be formed (Muthukumarappan et al 2009). During this process, adequately dried air or O_2 passes between two high-voltage electrodes divided by a dielectric material, which is typically glass. The ozone/gas mixture released from the ozonator normally includes 1 to 3% ozone when using dry air and 3 to 6% when using high purity oxygen (Muthukumarappan et al 2000).

The electrodes used in this technique are usually either concentric metallic tubes or flat, plate-like electrodes. When voltage reaches these electrodes, a corona discharge forms between the two electrodes, and the O_2 in the discharge gap is transformed into ozone (Figure 2). This corona discharge is a physical occurrence characterized by a low-current electrical discharge across a gas-containing gap at a voltage gradient surpassing a certain critical value (Taylor et al 1996). Initially, oxygen molecules (O_2) are split into oxygen atoms (O), and then the individual oxygen atoms merge with the remaining oxygen molecules to form ozone (O_3).

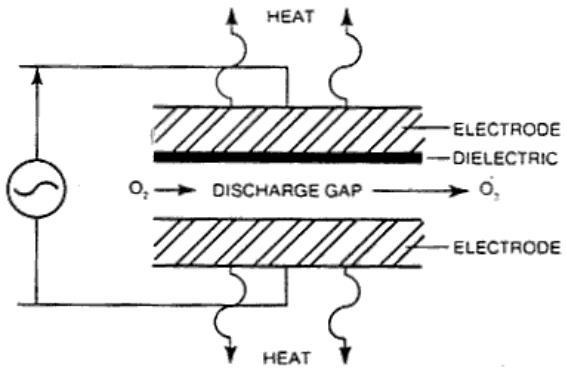

Figure 4. Ozone Generation by Corona Discharge Method
Taken from Muthukumarappan et al (2009).

Ozone Applications

In both gas and aqueous phases, ozone can be used for a variety of purposes in the food industry. Since the oxidizing power of ozone is 1.5 times greater than chlorine, this sanitizer and antimicrobial agent can efficiently inhibit various types of bacteria, molds, yeast, and viruses (Xu 1999). Another benefit, the by-product of ozone treatment is less harmful when compared to chlorine's harmful halogenated compounds and brominated disinfection by-products (EPA 1999). Gaseous ozone can be applied to fruits and vegetable during storage and transportation to enhance shelf life (Kim et al 2003). Aqueous ozone can be applied as an antimicrobial agent to food surfaces, packaging materials, and food processing equipment as well as decreasing microbial spoilage in chilled water and meat carcasses (Xu 1999, Kim et al 1999).

Limited research has been performed on using ozone on cereal and cereal products. Most of the published research focuses on using ozone washing and tempering wheat, controlling insects and fungus in stored grain, and improving flour quality in grain (Ibanoglu 2002, Dubois et al 2006, Mendez et al 2003, Chittrakorn 2008). Ibanoglu (2002) researched the effect of washing soft and hard wheat kernels with water and ozonated water. While no significant differences were found in lightness or Farinograph data, soft wheat flour washed with ozonated flour had lower dough extensibility and more resistance to extension. Mendez and others (2003) treated a variety of grains with 50 ppm of gaseous ozone to control pests during storage. While this treatment did destroy 92-100% of insects, no effects on nutrition or kernel properties were found without any deleterious effects of bread making functionality.

The Oxygreen® process was created to improve flour quality as well as decrease insects and mycotoxins (Dubois et al 2006). This ozonation procedure was aimed to modify flour properties in high ratio cakes, sponge cakes, and bread without the addition of ascorbic acid or amylase. These modifications improved baking performance by acting as an oxidative agent during kneading and baking. Dubois and others (2006) studied the safety of the process on grain. These researches concluded the Oxygreen® process did not alter the content of vitamins, proteins, carbohydrate, or lipid contents while acting as a powerful oxidant controlling insects and aflatoxins.

Recent research by Chittrakorn (2008) focused on treating soft wheat flour with ozone for 10, 20, 30, 36, and 40 minutes with an application rate of 0.06 L/min. Ozone treated flour had a lower pH with a slight increase in lightness (L) values. Additionally, these treated flours produced cakes with improved cake volume, brightness, and softness. When compared to chlorinated flour, ozone treated flour produced similar cake structure with increased volumes.

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Chapter 2:

EFFECT OF SORGHUM FLOUR TREATED WITH OZONE AND HEAT ON THE QUALITY OF GLUTEN-FREE BREAD AND CAKE

ABSTRACT

In order to improve the quality of products available for consumers who require a gluten-free diet, this study examined the effects of heat and ozone treatments on sorghum flour functionality in gluten-free bread and cake. In the ozone treatment experiment, commercially milled food-grade sorghum flour was subjected to ozone at the rate of 0.06 L/min for 15, 30, and 45 minutes. In the heat treatment experiment, commercially milled food-grade sorghum flour was subjected to dry-heat at two temperatures (95ºC and 125º) for 15, 30, and 45 minutes. Characterization of flour from each treatment included measurements of flour pH, color, and pasting properties. Evaluation of bread quality from each treatment included measurements of specific volume, color, crumb properties, and crumb firmness. Evaluation of cake quality from each treatment included measurements of specific gravity, volume, symmetry, uniformity, color, crumb structure, and crumb firmness.

Bake testing using ozonated sorghum flour in a high-ratio white layer cake formulation showed that volume significantly increased ($p<0.05$) as ozonation time increased. Additionally, longer ozonation exposure times increased cells per slice area, lightness, and slice brightness values in gluten-free cakes while reducing crumb firmness. Despite improving lightness and slice brightness values, ozonation did not significantly increase ($p>0.05$) the specific volume of gluten-free batter based bread.

In the heat treatment experiment, the optimum time and temperature relationship for improving sorghum flour was 125ºC for 30 minutes. This treatment level produced bread with the highest specific volume (3.08 mL/g) and the most cells

per slice area (50.38 cells/cm^2). This treatment level also produced cakes with the highest volume (72.17 cc) and most cells per slice area (79.18 cells/cm^2). Additionally, cake and bread made from this heat treatment was deemed more acceptable in comparison to the control during consumer testing. The control sorghum flour in both studies produced breads and cakes with low volume, poor crumb properties, and dense textures. These results can assist in the product development process in advancing the quality of sorghum-based gluten-free foods for the consumers who require a gluten-free diet.

INTRODUCTION

Celiac disease, an autoimmune disorder affecting the gastrointestinal system, afflicts 1% of the population in regions comprised mainly of Caucasian decent (Sollid and Lundin 2009). The basis of the disorder is an inflammation of the upper small intestine villi after ingesting gluten proteins from wheat, rye, barley, and possibly oats (Alaedini and Green 2005). Currently, the only effective and existing treatment for the disease is a life-long elimination of gluten-containing foods from the diet. With increased awareness and diagnosis of the disease, gluten-free foods are experiencing rapid growth in the marketplace.

Comprised of two protein fractions, gliadin and glutenin, gluten is an essential part of the overall structure and quality of baked goods. Combining these two proteins provides the viscoelastic properties necessary for producing a cohesive gluten network for structure and gas retention in wheat bread (Hoseney 2004). Subsequently, cereal technologists have an arduous task of duplicating the gluten complex in developing gluten-free cereal products. While there are a few gluten-free baked goods on the market, these products have a rigid texture, open crumb structure, bland taste, grey/off-color, and brief shelf-life.

In the U.S., sorghum has recently gained interest due to its gluten-free status along with the creation of more hybrids suitable for human consumption. Being an attractive alternative for wheat allergy sufferers, sorghum is increasingly being incorporated into snack foods and bakery products. This growing demand from celiacs has lead to sorghum being commercially available in gluten-free bread, pasta, cookies,

cereal, beer, and bakery mixes for brownies, cakes, and pancakes (U.S. Grains Council 2004).

Heat treatment has been suggested to be a viable method of improving cake and bread quality particularly in weak, substandard flour. Exposure to heat denatures the protein and enzymes in the flour while increasing batter expansion (Russo and Doe 1970). This difference in viscosity is connected to a cake's ability to transform from foam to sponge form and reduce shrinkage during baking. In bread applications, heat-treated flour has been shown to increases resistance, viscosity, and stiffness (Gélinas et al 2001). These factors lead to an increase in dough elasticity and produce positive effects on oven spring and loaf volume (Pyler 1988). These effects mimic oxidizing agents like ADA and ascorbic acid traditionally used in making bread.

Since ozone possesses the ability to decompose free radicals without leaving chemical residues, the application for this strong oxidizing agent has broad appeal for use in the food industry. The Oxygreen® process was created to improve flour quality as well as decrease insects and mycotoxins (Dubois et al 2006). This ozonation procedure was aimed to modify flour properties in high ratio cakes, sponge cakes, and bread without the addition of ascorbic acid or amylase. These modifications improved baking performance by acting as an oxidative agent during kneading and baking. Neither heat treatment nor ozonation have been previously researched for their effects on sorghum flour.

Therefore, the objectives of the present study were:

1. To investigate the effect of ozone on the properties of sorghum flour and the potential use of ozone treated flour for gluten-free cake and bread production
2. To investigate the effect of heat on the properties of sorghum flour and the potential use of heat treated flour for gluten-free cake and bread production

The ultimate objective of the study was that findings from this research can assist in the product development process and in advancing the quality of sorghum-based gluten-free foods for the consumers who require a gluten-free diet.

MATERIALS AND METHODS

MATERIALS

Cake Materials

Whole grain sorghum flour with the same lot number was purchased from Twin Valley Mills (Ruskin, NE). Other ingredients used were: dextrose (Archer Daniels Midland, Decatur, IL), emulsified shortening (Archer Daniels Midland, Decatur, IL), non-fat dried milk (Great Value, Wal-Mart Stores, Inc., Bentonville, AR), dried egg whites (Century Foods International, Sparta, WI), iodized salt (Kroger, Cincinnati, OH), and double-acting baking powder (Clabber Girl, Terre Haute, IN),

Bread Materials

Whole grain sorghum flour with the same lot number was purchased from Twin Valley Mills (Ruskin, NE). Other ingredients used were: unmodified potato starch (Bob's Red Mill, Milwaukie, OR), iodized salt (Kroger, Cincinnati, OH), granulated sugar (Kroger, Cincinnati, OH), hydroxypropyl methylcellulose (Methocel K4M, E 464, Dow Chemical Co., Midland, MI), non-fat dried milk (Great Value, Wal-Mart Stores, Inc., Bentonville, AR), and active dry yeast (Red Star Yeast, Milwaukee, WI).

TREATMENT PROCEDURES

Ozonation Treatment

Ozone gas was generated by a pilot scale ozone generator (Clear Water Tech, Inc., San Luis Obispo, CA) using oxygen produced by an oxygen generator (Dwyer Instruments, Inc. San Luis Obispo, CA) (Figure 1). Ozone gas was tumbled in a motorized metal drum (Miag, Braunschweig, Germany) filled with 3 lbs of sorghum

flour. The ozone was administered at a rate of 0.06 L/min for 15, 30, and 45 minutes. Excess ozone was neutralized by bubbling the gas through a solution containing 250 ml distilled water and 4 g of potassium iodide with a starch indictor. Treated flour was placed in glass pans under a fume hood for 72 hours to help alleviate the strong ozone odor.

Figure 1. Pilot scale ozone generator and oxygen generator

Heat Treatment

For heat treating, 2 lbs of flour was evenly distributed on a 60 x 30 x 2.5-cm aluminum pan approximately 0.5 cm thick. Then, flour was placed in a convection oven (Whirlpool, St. Joseph, MI) and heated either at 90ºC or 125ºC for 15, 30, and 45 minutes. After heating, flour was cooled to room temperature and rehydrated to approximately 12% moisture content in a fermentation cabinet.

FLOUR PROPERTIES

pH measurement

The pH of flour samples was measured using AACC method 02-52. Ten grams of flour were added to 100 ml of distilled water. The flour mixture was continuously stirred on a stirring plate for 15 minutes. Flour samples were allowed to stand for 10 minutes, and then decanted to evaluate the pH of the liquid supernatant. A Fisher Scientific Accumet portable pH/mV/Ion meter (Model AP63, Thermo Fisher Scientific, Inc. Waltham, MA) with a glass pH electrode was used to attain the pH values. Calibration was performed before each use with pH 4.0 and pH 7.0 buffer solutions.

Flour Color

A Minolta CR-300 colorimeter (Konica Minolta, Osaka, Japan) was used to measure the color of flour samples. The instrument was calibrated against a standard white tile (No: 17033201, L=97.83, a=-0.41 and b=1.90). Each flour sample was placed in the granular materials attachment and compacted by tapping 20 times. The Minolta Chroma Meter was placed in the granular attachment, and measurements were subsequently taken and recorded. Flour color results were reported in terms of 3-dimensional color values: L*, a*, b*. Lightness is determined by L* values (0 = black and 100 = white). Red and green hues are attributed to a* values (+60 red color and -60 green color). Yellow and blue colors are indicated by b* values (+60 yellow color and -60 blue color). The instrument was calibrated against a standard white tile (No: 17033201, L=97.83, a=-0.41 and b=1.90).

Moisture Content

The moisture contents of the flours were measured using AACC method 44-15A. The procedure determines the moisture content as the loss in weight of a sample when heated under specified conditions. Approximately 2-3 grams of flour were placed in aluminum sample pans and heated by a mechanical-convection oven set at 130^{o}C for 1 hour. After heating, samples were placed into a desiccator to cool for 60 minutes. The following formula was used to calculate percent moisture (AACC method 44-01):

$$\% \text{ Moisture} = 100\% - \frac{(\text{wt of sample after oven drying})\ 100}{\text{original wt of sample}}$$

Protein Content

The protein content of the sorghum flour was measured using AOAC 990.03 approved method, nitrogen determination by combustion using a LECO FP-528 instrument (Leco Corporation, St. Joseph, MI). In the sample, nitrogen freed by combustion at high temperatures in pure oxygen is measured by thermal conductivity detection. This value was converted to the equivalent protein by using a 6.25 conversion factor.

Fat Content

The fat content of the sorghum flour was measured using AOAC 920.39 approved method. This method determines crude fat in the samples by ether extraction with a subsequent solvent evaporation. The fat content was reported as a percentage of the original sample weight.

Ash Content

The ash content of the sorghum flour was measured using AOAC 942.05 approved method. Two grams of the sample was weighed into a porcelain crucible and placed in a temperature controlled furnace preheated to 600^{o}C. After a two hour period, the crucible was then transferred directly to a desiccator, cooled, and weighed. Ash content was reported as a percentage of the whole sample.

Fiber Content

The crude fiber content of the sorghum flour was measured using the Ankom Method, based on the AOAC 962.09 approved method. The Ankom Crude Fiber solvent solubilizes non-fiber components of the flour. The sample is subsequently filtered, rinsed, and dried to determine the crude fiber content. Crude fiber was reported as a percentage of the original sample weight.

Starch Pasting Properties

The pasting properties of sorghum starch from each flour sample were determined using a Rapid Visco Analyser (RVA Model 4, Newport Scientific, Australia) according to AACC method 76-21. Prior to analysis, the flour samples were analyzed for moisture content. The quantity of starch and water were adjusted on each sample to ensure a 14% moisture content. The following correction formula for 14% moisture content was employed:

$$M_2 = M_1 \times (100\text{-}14) / (100\text{- Moisture Content of Sample})$$

$$W_2 = 25.0 \text{ mL} + (M_1\text{-}M_2)$$

Where M_1 = sample mass for the material (4 g)

M_2 = corrected sample mass

W_2 = corrected water volume

The corrected volume of distilled water for each respective sorghum flour sample was poured into the aluminum RVA canister. The designated corrected flour sample was gently mixed into the water using the RVA mixing paddle. The mixture was blended and hydrated in a circular motion to avoid any flour clumping. The parameters assessed during the RVA test include pasting temperature (temperature at which starch granules begin to swell and gelatinize due to water uptake and defined as an increase of 25 cP over a period of 20 sec), peak time (time at which peak viscosity was recorded), peak viscosity (maximum paste viscosity achieved in stage 2, the heating stage of the profile), breakdown (difference between peak viscosity and trough), set back (difference between final viscosity and trough), and final viscosity (viscosity at the end of run). The viscosity measurements were recorded in centipoise cP units (1 cP = 1 mPa sec^{-1}).

CAKE BAKING PROCEDURE

Baking tests of the treated flour samples were conducted to study cake baking potential. All cakes were baked according to AACC high ratio white layer cake, Method 10-90 (AACC 2000). Water and double-acting baking powder were calculated to optimum levels. Dextrose was substituted for sucrose to lower the starch gelatinization temperature. The final formula is shown in Table 1. All dry ingredients were sifted and placed into the mixing bowl. Subsequently, emulsified shortening and 60% of the distilled water were added. These ingredients were mixed at stir speed using a 300 watt

KitchenAid mixer (Ultra Power, St. Joseph, MI) for 30 seconds, then scraped down and mixed on speed #2 for 4 minutes. One half of the remaining water was added to the batter, mixed at stir speed for 30 seconds, scraped, and mixed again on speed 2 for 2 minutes. The remaining 20% of the water was added, mixed on stir speed for 30 seconds, scraped, and mixed for an additional 2 minutes on second speed. Two lightly greased 8 inch pans were filled with 425 grams of batter and baked at 190ºC (375ºF) for 22 minutes. After baking, each cake was de-panned, placed on wire racks, and cooled at room temperature for 2 hours.

Table 1. Formula for high ratio sorghum white layer cakes

Ingredients	% Flour Basis	Amount (g)
Flour	100.0	200.0
Dextrose	140.0	280.0
Shortening	50.0	100.0
Non-fat dry milk	12.0	24.0
Dried egg white	9.0	18.0
Salt	3.0	6.0
Baking powder	5.5	11.0
Distilled water	135.0	270.0

BAKING QUALITY OF CAKES

Specific Gravity of Batter

Specific gravity was determined by dividing the weight of the cake batter by the weight of an equal volume of distilled water.

Volume, Symmetry, and Uniformity

A plastic measuring template was used to calculate volume, contour, and symmetry indices according to AACC method 10-91 (AACC 2000). Cakes were sliced in half, and the interior of the cake was placed against the template. Volume index was calculated by adding the center height of the cake with the points halfway between the center and outer edges.

Calculations: Volume index = B + C + D Contour = 2C – B –D Symmetry = | B – D |

These letter values designated for calculations are illustrated in figure 2 below:

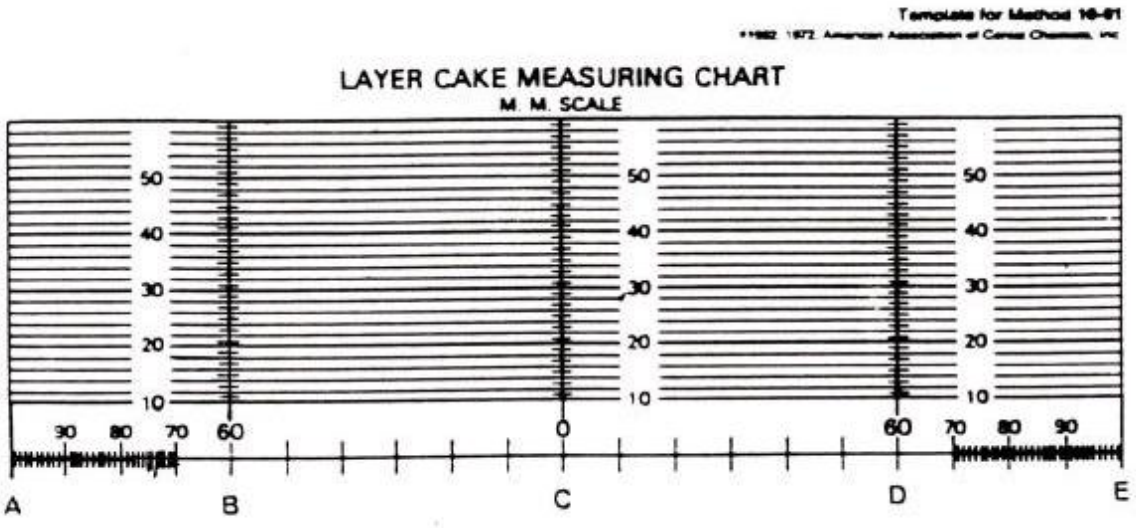

Figure 2. AACC Layer Cake Measuring Chart
Source: AACC (2000).

Textural properties

A texture profile analysis (TPA) was performed on each cake to measure firmness using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, United Kingdom). The TPA was performed using a 1 inch diameter cylinder probe. The test

setting was carried out at a constant speed of 2.0 mm/sec for pre-test, test, and post-test. A distance of 10 mm was used to compress the sample with 3 seconds between each stroke and trigger force of 5 grams. Each cake had 3 representative samples cut with the dimensions of 2 inch wide, 2 inch deep, and 1 inch tall. Slices were analyzed 2 hours post baking.

Internal cake color

The color of cake crumb was measured with a Minolta colorimeter (CR-300). L*, a*, b* values were recorded.

Internal crumb structure

Crumb and gas cell structure for each cake were evaluated using a C-Cell imaging system (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). Cakes were sliced in the center with an electric knife at a thickness of 15 mm. Image analysis parameters measured include slice brightness, average cell diameter and volume, average cell wall thickness, and average crumb fineness (number of cells/cm^2).

BREAD BAKING PROCEDURE

Baking tests of the treated flour samples were conducted to study bread baking potential. The batter bread formula was made in accordance with previous sorghum research described by Schober and others (2005, 2007). The base formulation is listed in Table 2. The dried yeast was allowed to reactivate and hydrate in 30ºC water for 5 minutes prior to mixing. The remaining ingredients were blended together to break up any clumps and then added to the hydrated yeast mixture. The batter was mixed with a

Hobart mixer model N-50 (The Hobart Mfg. Co., Troy, OH) using the flat paddle attachment for 30 seconds on low speed. After scraping, the batter was mixed for an additional 90 seconds on medium speed. After mixing, 250g of batter was placed into greased baking tins (9 cm x 15 cm x 5.5 cm) and proofed in a proofing cabinet (National Manufacturing Co., Lincoln, NE) set at 30ºC with 87% relative humidity. The batter was proofed to a height of 4.5 cm. After proofing, the batter was sprayed with water and placed in an electric reel oven (National Manufacturing Co., Lincoln, NE) for 30 minutes at 232ºC (450ºF). After baking, bread was de-panned, placed on wire racks, and cooled at room temperature for 2 hours.

Table 2. Formula for sorghum batter based bread

Ingredient	% Flour Basis	Amount (g)
Sorghum flour	70.0	140.0
Potato starch	30.0	60.0
HPMC	2.0	4.0
Active dry yeast	2.0	4.0
NaCl	1.75	3.5
Non-fat dry milk	1.0	2.0
Sucrose	1.0	2.0
Distilled Water	105.0	210.0

Baking Qualities of Bread

Specific Volume

After cooling for 2 hours, loaf weights were taken along with loaf volumes measured by rape seed displacement (AACC Method 10-05). Loaf specific volume was calculated by dividing loaf volume (mL) by loaf weight (g).

Internal Bread Color

The color of bread crumb was measured with a Minolta colorimeter (CR-300). L*, a*, b* values were recorded.

Internal Crumb Structure

Crumb and gas cell structure for each bread loaf was evaluated using a C-Cell imaging system (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). The bread was cut into 2.5 cm slices using an electric knife with cutting jig to ensure uniformity of slice surface and thickness. To avoid irregularities between slices, only the four slices from the center of the bread were used for analysis. Image analysis parameters measured include average cell diameter and volume, average cell wall thickness, average crumb fineness (number of cells/mm^2), and slice brightness.

Textural Properties

A texture profile analysis (TPA) was performed on each bread to measure firmness using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, United Kingdom). The TPA was performed using a 25 mm diameter cylinder plastic probe attached to a 30 kg load cell. The test setting was carried out at a constant speed of 2.0 mm/sec for pre-test, test, and post-test with a trigger force of 5.0 g to compress the center of the crumb at distance of 40% of the slice thickness (2.5 cm). A distance of 10 mm was used to compress the sample with 5 seconds between each stroke. Slices were analyzed 2 hours post baking.

CONSUMER STUDY

A consumer study was held in Call Hall at Kansas State University to evaluate the acceptance of gluten-free bread and cake. In the bread study, the control sorghum flour and sorghum flour heat treated at 125ºC for 30 min were implemented into the gluten-free bread formulation used in the previous bread baking experiments. In the cake study, the control sorghum flour and sorghum flour heat treated at 125ºC for 30 were implemented into the gluten-free cake formulation used in the previous cake baking experiments. This heat treated flour was selected since it produced cakes and bread with the highest volume with superior crumb structure in the previous bake testing experiments. Since ozone imparted a strong off-flavor and odor, ozonated flour was not used in the sensory test.

A total of 100 untrained panelists volunteered to participate in the consumer study. Each panelist was given a pre-screening form to obtain information about age, gender, education completed, frequency of cake and bread consumption, buying habits of cake and bread, and potential food allergies (Appendix 3). If a panelist claimed to have a food allergy, they were asked not to participate in the study. Panelists also signed an informed consent form to notify them about the purpose and guidelines of the study (Appendix 2).

In both the cake and bread study, the two respective samples labeled with random three-digit codes were placed on white paper plates. Both samples were given to the panelists at the same time along with ballots having corresponding three-digit codes. The panelists were asked to test each sample in the specified order to eliminate

bias. Unsalted saltine crackers and distilled water were provided for cleansing their palate between samples.

Each ballot contained a 9-point hedonic scale for each attribute. The 9-point scale displayed the degree of liking with 9 being like extremely, 5 being neither like nor dislike, and 1 being dislike extremely. The attributes evaluated were overall acceptability, appearance, flavor, color, and texture. Consumers were also given the opportunity to write additional comments on the bottom of the ballot (Appendix 4-5).

STUDY DESIGN

Preliminary Work

Preliminary experimental work was performed to evaluate the effectiveness of various treatment times and exposure levels of ozonation and heat treatments on sorghum flour. In the ozonation trials, sorghum flour was treated with ozone gas for 5, 10, 15, 20, 25, 30, 35, 40, 45, and 60 minutes. Treatment levels for the main experiment were selected after baking trials to achieve an adequate representation of the effects of sorghum flour treated with ozone on the quality of gluten-free bread and cake. In the heat trials, sorghum flour was treated with heat for 5, 10, 15, 20, 25, 30, 35, 40, 45, and 60 minutes at two different temperatures, 90ºC and 125ºC. Treatment levels for the main experiment were selected after baking trials to achieve an adequate representation of the effects of sorghum flour treated with heat on the quality of gluten-free bread and cake. Additional research was performed to evaluate the possible synergist effects of combining heat and ozone treatments. No supplementary effects

were seen on the volume, color, or crumb properties of bread or cake made from dually treated sorghum flour.

Statistical Design

In the ozone experiment, three time treatment levels were evaluated for all tests. In the heat experiment, three treatment times at two different treatment temperatures were evaluated for all tests. In both experiments, three replications of each treatment were treated as blocks in a randomized block design. Triplicate readings of each physical, chemical, and textural test were performed. Sensory analysis was performed only once for the consumer study.

All data from the physical, chemical, textural, and sensory tests were analyzed using SAS, Software Release 9.1.3 (SAS, Institute Inc., Cary, NC, 2003). When treatment effects were found significantly different, the least square means with Tukey-Kramer groupings were used to differentiate treatment means. A level of significance was observed at $\alpha < 0.05$.

RESULTS AND DISCUSSION

PART 1: OZONE TREATMENT

Properties of Ozonated Flour

The pH of the control sorghum flour was 6.14 while the pH of ozone treated flour decreased linearly as exposure time increased. The 45 minute treatment produced flour with the lowest pH of 5.91 (Table 3). This may occur from the oxidation of flour components by ozonation promoting the formation of acid products. Langlais and others (1991) reported ozone has the ability to oxidize carbohydrate, amino acid, and unsaturated fatty acid components leading to the development of acid products.

The lightness (L*) values for the control and ozonated flour (Table 3) were significantly different ($p<0.05$). Indicating yellowness of the flour, the b* values significantly decreased ($p<0.05$) as ozone exposure time increased. This finding indicates ozone has an ability to decolorize some food components by oxidizing pigments such as carotenoids in the sorghum flour. Weiwei and Xueling (2008) investigated the effect of ozone treatment on the color of wheat flour. They showed flour treated with ozone improved flour color by increasing L* values and decreasing yellowness values. Their findings mimic the results in this experiment by suggesting ozonated sorghum flour appears to be brighter or whiter than non-treated sorghum flour. Pyler (1988) states the color of flour has significant influence on the ultimate crumb color of baked goods. This would lead to the hypothesis that sorghum flour with higher L* values would improve brightness values in the final product. This

improvement in color may lead to an increase in the overall consumer acceptance of gluten-free baked goods.

Gelatinization, pasting, and set back profiles of the control sorghum flour and ozone treated sorghum flour are listed in Table 4. Peak viscosity, defined as the highest viscosity during the heating cycle, increased as time of ozonation increased. Oxidative treatments like chlorination has been reported to change the starch surface properties by increasing surface hydrophobicity. Varriano-Marston (1985) reported oxidative polymerization of starch ruptured chains connecting crystallites and the amorphous region. This disruption increases the surface porosity of starch and allows this open starch structure to bind tightly with water and bind more oil. Hoseney (1994) stated chemical modification through oxidation results in greater swelling capacity of starch and increases batter viscosity. This increase in the starch swelling properties helps prevent cakes from collapsing during cooling by occupying the void space in the structure as the cake temperature lower and gas cell pressure decreases. The increase in peak viscosity in ozonated sorghum flour may be due to the oxidation of starch by ozone leading to an increase in starch granule swelling during heating.

The breakdown value denotes the stability of the paste during heating. This measurement is the difference between the peak viscosity and the viscosity after stirring the hot paste at 95°C for a specific time. This decrease in viscosity is caused by the alignment of polymer molecules with the shear field. Flour with a sharp drop in viscosity indicates weakening by mechanical disruption. In this experiment, break down decreased as exposure to ozonation increased (Table 4).

Table 3. Comparison of pH and L*a*b* values of untreated and ozonated sorghum flour*

Flour Name	pH	Color of Flour		
		L*	a*	b*
Control	6.14 ± 0.007[a]	80.11 ± 0.042[a]	0.22 ± 0.014[a]	13.72 ± 0.325[a]
15 min	6.09 ± 0.006[b]	81.95 ± 0.720[b]	0.22 ± 0.121[a]	10.93 ± 0.121[b]
30 min	5.99 ± 0.006[c]	82.94 ± 0.822[bc]	0.33 ± 0.015[a]	9.95 ± 0.686[b]
45 min	5.91 ± 0.010[d]	83.74 ± 0.437[c]	0.52 ± 0.047[a]	9.22 ± 0.384[b]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Table 4. Comparison of gelatinization and pasting properties of untreated and ozonated sorghum flour*

Flour Name	Peak Viscosity (x 1000 cP)	Breakdown (x 1000 cP)	Setback (x 1000 cP)	Final Viscosity (x 1000 cP)	Peak time (min)	Pasting Temp (Cº)
Control	4.354 ± 0.08[a]	2.469 ± 0.03[a]	3.749 ± 0.03[a]	6.034 ± 0.07[a]	5.05 ± 0.00[a]	71.33 ± 0.46[a]
15 min	4.30 ± 0.08[a]	2.428 ± 0.03[a]	4.001 ± 0.07[b]	6.312 ± 0.02[b]	4.95 ± 0.04[a]	70.55 ± 0.71[a]
30 min	4.584 ± 0.04[b]	2.247 ± 0.01[b]	4.002 ± 0.01[b]	6.420 ± 0.04[b]	4.92 ± 0.05[a]	70.18 ± 0.25[a]
45 min	5.099 ± 0.04[c]	2.018 ± 0.00[c]	4.005 ± 0.07[b]	6.606 ± 0.02[c]	4.92 ± 0.00[a]	71.13 ± 0.04[a]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Ozone Bread Baking Test

Bread Specific Volume

No significant differences were noted ($p>0.05$) for the specific volume of breads produced from non-ozonated and ozonated flours (Table 5). Specific volumes fluctuated only slightly between 2.77 mL/g (control sorghum flour) to 2.81 mL/g (15 min ozonated sorghum flour). Despite increasing gas cell size and diameter, ozonation produced over-elastic dough with a poor ability to hold the gas released during the proofing process. Inability to hold these gas cells resulted in a constrained loaf volume. This result of low loaf volumes and open crumb structure as a result of over-oxidation was in agreement

with other research investigating oxidation treatment levels (Berglund et al 1991; Bonet et al 2006).

Bread Crumb Structure

Results from the C-Cell Digital Imaging software are also listed in Table 5. Values for cell volume ranged from 12.10 mm^3 (control sorghum flour) to 18.15 mm^3 (45 min ozonated flour). Values for cell diameter ranged from 2.97 mm (control sorghum flour) to 4.06 mm (30 min ozonated flour). As seen in figure 3, breads produced from ozonated flour had higher cell volume and diameters which translated into an open and ragged crumb structure. This coarse texture is a principal symptom of an over-matured dough which has become excessively elastic. Kulp (1981) stated that an excessively high dose of an oxidant treatment produces an over-elastic dough which lacks sufficient extensibility. This allows for easy expansion of gas cells late in proofing and during the oven spring stage of baking. This seems to be the explanation for the ozonated flour having large gas cells and open crumb structure.

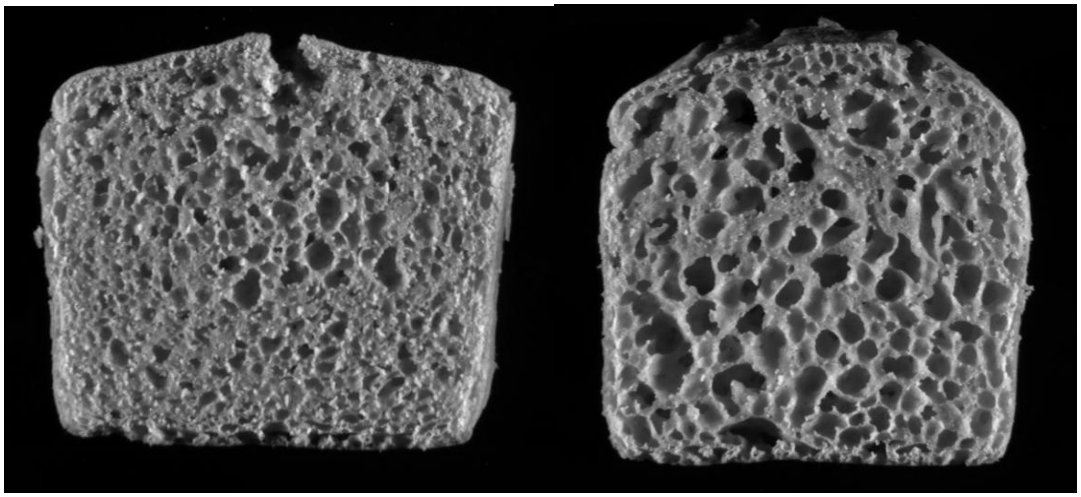

Figure 3. Comparison of bread crumb structure produced from untreated and ozonated sorghum flour. On left: control untreated sorghum flour; on right: 30 min ozonated flour

Table 5. Comparison of specific volume and C-Cell analysis of bread produced from untreated and ozonated sorghum flour*

Flour Name	Specific Volume (mL/g)	Cell Volume (mm^3)	Cell Diameter (mm)	Wall Thickness (mm)	Cells per Slice Area ($cells/cm^2$)
Control	2.77 ± 0.03[a]	12.10 ± 0.34[a]	2.97 ± 0.11[a]	0.55 ± 0.00[a]	44.74 ± 0.65[a]
15 min	2.81 ± 0.04[a]	14.11 ± 0.73[b]	3.28 ± 0.14[a]	0.57 ± 0.01[a]	44.05 ± 1.01[a]
30 min	2.78 ± 0.09[a]	18.10 ± 0.85[c]	4.06 ± 0.33[b]	0.57 ± 0.01[a]	43.26 ± 1.48[b]
45 min	2.80 ± 0.06[a]	18.15 ± 0.85[c]	4.04 ± 0.26[b]	0.56 ± 0.01[a]	43.98 ± 1.48[ab]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Bread Crumb Color

Color has traditionally been pivotal for the acceptance or rejection of food products. Waniska and Murty (1982) discussed the importance of color in sorghum product quality. In certain cases, white color is not required, but it is generally preferred. As predicted due to ozonated flours having higher L* values, slice brightness and lightness (L* value) increased as ozonation time increased. Pomeranz (1960) observed this correlation between flour color and crumb color with a coefficient of 0.987. The opportunity to increase brightness and lightness values would be recommended to increase the acceptability of sorghum gluten-free which is generally considered grey and dull in appearance. As shown in Table 6, slice brightness and L* values increased linearly as ozonation exposure time increased. Values for slice brightness ranged from 97.88 (control sorghum flour) to 106.23 (45 min ozonated flour). Values for lightness (L*) ranged from 87.00 (control sorghum flour) to 90.99 (45 min ozonated flour).

Table 6. Comparison of slice brightness and L*a*b* values of bread produced from untreated and ozonated sorghum flour*

Flour Name	Slice Brightness	Color of Bread Crumb		
		L*	a*	b*
Control	97.88 ± 0.30[a]	87.00 ± 0.27[a]	22.01 ± 0.14[a]	8.09 ± 0.95[a]
15 min	101.13 ± 0.97[b]	86.67 ± 0.88[a]	22.21 ± 0.36[a]	8.17 ± 0.62[a]
30 min	102.96 ± 1.68[b]	86.11 ± 0.71[a]	22.32 ± 0.26[a]	7.58 ± 0.80[a]
45 min	106.23 ± 1.84[c]	90.99 ± 1.07[b]	22.85 ± 1.06[a]	6.95 ± 0.78[a]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Bread Texture Profile Analysis

Significant differences ($p<0.05$) were found in bread slice firmness (Table 7). The treated ozonated flours produced softer crumb texture as treatment time increased. The control sorghum flour produced the firmest bread texture (855.00 g Force) while the 45 min ozonated flour produced the softest crumb structure (691.67 g Force). A possible explanation for the decrease in firmness is the open crumb structure of the ozonated bread. This open structure provides less resistance to the probe during TPA. The control bread had a finer crumb structure and provided more resistance during the deformation test. While the ozonated bread had lower firmness values, these large voids in the crumb structure would not be perceived as a desirable trait. Hoseney (1994) reported that the most desirable white pan bread would have a soft crumb along with a fine cell structure.

Table 7. Comparison of firmness of crumb in bread produced from untreated and ozonated sorghum flour*

Flour Name	Firmness (g)
Control	855.00 ± 7.07[a]
15 min	773.33 ± 5.16[b]
30 min	733.33 ± 8.16[c]
45 min	691.67 ± 7.53[d]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Ozone Cake Baking Test

Cake Batter Specific Gravity

The specific gravity measurement estimates the amount of air incorporated into a batter. A lower specific gravity is indicative of a batter with more air and viscosity (Pyler 1988). A viscous batter provides the structure to help retain air bubbles during mixing (Kim and Walker 1992). Since no new air cells are formed after mixing, air cells can dissipate due to coalescence or rising to the surface (Hoseney 1994). Typically, a specific gravity around 1.0 indicates a low number of air cells incorporated into the batter (Pyler 1988). It has been shown that batter specific gravity has an effect on volume, tenderness, and final crumb structure of cake. Pyler (1988) stated a specific gravity of 0.925 is optimum for a white layer cake with 140% sugar level with a mixing time of 10 minutes. Cake batters with high specific gravity produce cakes with low volume and dense crumb grain.

The specific gravity of the cake batters from the control and ozonated flours are listed in Table 8. The results show ozonation reduced specific gravity indicating a greater number of gas cells incorporated into the cake batter. Flour treated with ozone

for 30 and 45 min had significantly lower ($p<0.05$) specific gravities than the control. Additionally, these flours translated this advantage to higher cake volumes with more air cells per slice area (Table 9 and 10).

Table 8. Comparison of specific gravity of cake batter produced from untreated and ozonated sorghum flour*

Flour Name	Specific Gravity
Control	1.04 ± 0.01[a]
15 min	1.02 ± 0.01[a]
30 min	0.96 ± 0.01[b]
45 min	0.95 ± 0.01[b]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Volume, Symmetry, and Uniformity

Indices for cake volume, symmetry, and uniformity calculated using a plastic measurement template are listed in Table 9. Cakes made from 30 minute ozone treated flour had significantly higher volumes ($p<0.05$) when compared with cakes made from untreated control flour. Ozone treated flour had sufficient strength to support the overall cake structure without collapsing during cooling (Figure 4). This added strength may be due to ozone oxidizing and modifying the properties of flour components like starch, protein, and lipids. Oxidative treatments like chlorination have been shown to increase the hydrophobicity on the surface of wheat starch (Seguchi 1990). This helps improve the bubble stability while allowing the oxidized starch to swell to a greater extent in comparison to unoxidized starch. This increased viscosity of the batter helps prevent the cake from collapsing during baking as well as cooling (Hoseney 1994).

Additionally, the chlorinated flours are more acidic which will cause the structure of cakes to set faster since starch gelatinization occurs sooner in the oven (Amendola and Rees 2003).

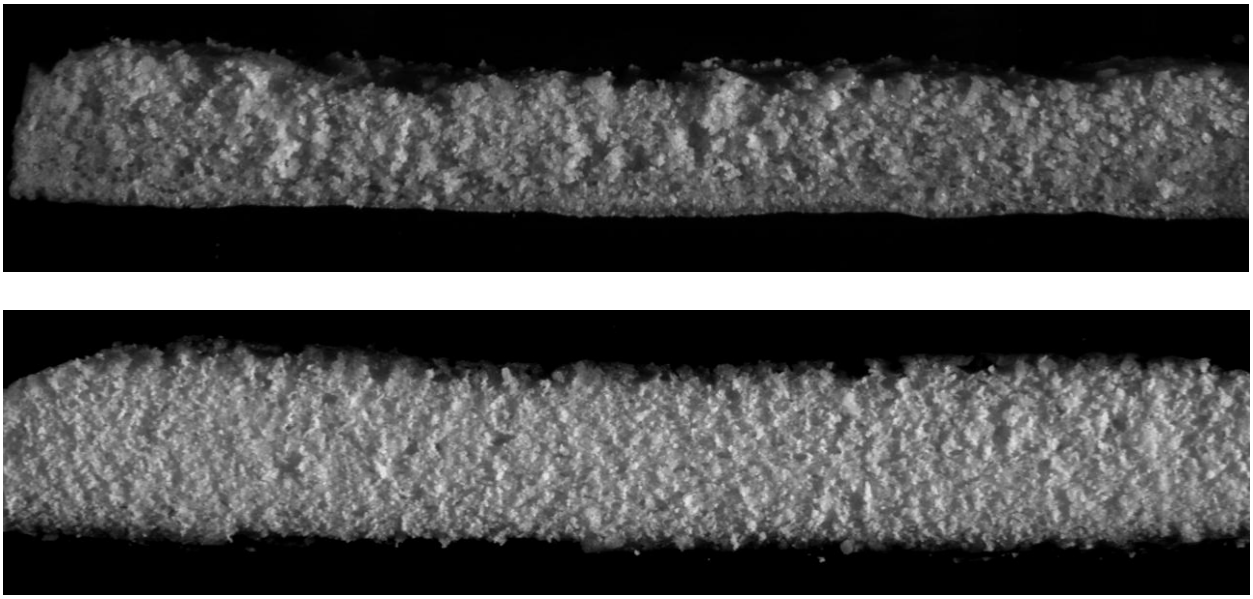

Figure 4. Comparison of volume of cakes produced from untreated and ozonated sorghum flour. On top: untreated control flour; on bottom: 30 min ozonated flour

Table 9. Comparison of volume, symmetry, and uniformity indexes of cake produced from untreated and ozonated sorghum flour*

Flour Name	Volume Index	Symmetry Index	Uniformity Index
Control	58.50 ± 0.71[a]	3.00 ± 2.83[a]	1.00 ± 0.00[a]
15 min	64.83 ± 1.33[b]	2.17 ± 1.17[a]	0.50 ± 0.55[a]
30 min	76.83 ± 1.72[d]	2.17 ± 1.17[a]	0.50 ± 0.55[a]
45 min	72.00 ± 1.26[c]	3.50 ± 0.84[a]	1.17 ± 0.41[a]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Crumb Structure

As ozonation time increased from 15 min to 45 min, cell diameter decreased and number of cells per slice area increased (Table 10). The size and number of air bubbles present in the cake batter are indicative of the final crumb structure and volume. In the untreated treated sorghum flour, cakes had the highest cell volume and diameter, yet the number of cells per slice area indicated that less air bubbles were incorporated into the cake batter. This could imply that the cake batters made from the control flour can not hold gas cells leading to larger gas cells through coalescence. As seen in Table 4 and 8, the 30 and 45 minute ozonation treatments had the largest increase in viscosity and decrease in specific gravity. This indicated large numbers of gas cells were incorporated into the batter, and this advantage translated to significantly higher ($p<0.05$) cells per slice area. This increase in small air cells leads to an increase in perceived quality of the final cake since it possesses a finer and more uniform crumb structure.

Table 10. Comparison of C-Cell analysis of cakes produced from untreated and ozonated sorghum flour*

Flour Name	Cell Volume (mm^3)	Cell Diameter (mm)	Wall Thickness (mm)	Cells per Slice Area ($cells/cm^2$)
Control	7.22 ± 0.45[a]	2.13 ± 0.09[a]	0.45 ± 0.01[a]	69.96 ± 2.24[a]
15 min	7.15 ± 0.29[a]	2.07 ± 0.04[a]	0.46 ± 0.01[a]	70.41 ± 1.77[a]
30 min	6.63 ± 0.30[b]	1.92 ± 0.06[b]	0.44 ± 0.01[a]	72.98 ± 2.19[b]
45 min	6.34 ± 0.09[b]	1.90 ± 0.03[b]	0.44 ± 0.01[a]	74.49 ± 2.08[c]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Crumb Color

Values for slice brightness values ranged from 74.70 (control sorghum flour) to 81.82 (45 min ozonated sorghum flour) (Table 11). Lightness values also significantly increased ($p<0.05$) as ozonation time increased. Similar bleaching effects were found in research by Chittrakorn (2008) when ozoning soft wheat flours for white layer cake production. This bleaching action through oxidative treatments is believed to destroy the carotenoid and flavonoid pigments found in the endosperm. This decolorization step subsequently produces a whiter flour (Hoseney 1994).

Table 11. Comparison of slice brightness and L*a*b* values of cake produced from untreated and ozonated sorghum flour*

Flour Name	Slice Brightness	Color of Cake Crumb		
		L*	a*	b*
Control	74.70 ± 0.57^{a}	88.77 ± 0.71^{a}	15.93 ± 2.06^{a}	19.08 ± 1.41^{a}
15 min	77.82 ± 1.44^{bc}	90.70 ± 0.88^{ab}	15.81 ± 1.44^{a}	18.98 ± 1.50^{a}
30 min	79.30 ± 1.35^{bc}	91.15 ± 0.71^{bc}	15.84 ± 1.14^{a}	17.54 ± 1.14^{b}
45 min	81.82 ± 0.94^{d}	92.86 ± 1.07^{c}	16.10 ± 1.25^{a}	17.60 ± 1.69^{b}

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Texture Profile Analysis

Significant differences ($p<0.05$) were found in cake firmness between treatments (Table 12). Firmness is a textural parameter which measures the peak force during the first compression cycle (Bourne 1978). The treated ozonated flours produced softer crumb texture as treatment time increased. The control sorghum flour produced the firmest cake (635 g Force) while the 45 minute ozonation treatment produced the softest cake (518.33 g Force).

Table 12. Comparison of firmness of crumb in cake produced from untreated and ozonated sorghum flour*

Flour Name	Firmness (g)
Control	635.00 ± 7.07[a]
15 min	598.33 ± 11.69[b]
30 min	563.33 ± 12.11[c]
45 min	518.33 ± 11.69[d]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

PART 2: HEAT TREATMENT

Properties of Heat Treated Flour

As indicated in Table 13, heat treatment had no significant ($p>0.05$) impact on the color indices of flour. As Catterall (2000) reported, one of the main weaknesses of heat treating flour is the lack of any bleaching effect. Their study concluded heat treated flour will give a slightly darker crumb color, yet this negative color defect poses the most concern in making products like angel food cakes. Additionally, no significant differences ($p>0.05$) were found between the pH of untreated and heat treated sorghum flour.

Gelatinization, pasting, and set back profiles of the control sorghum flour and heat treated sorghum flour are listed in Table 14. No significant differences ($p>0.05$) were found in the breakdown, setback, or peak time between all samples. A significant increase ($p<0.05$) in peak and final viscosity were found in flour samples heated for 30 and 45 minutes at both 95ºC and 125ºC. This increase in the starch swelling properties helps prevent cakes from collapsing during cooling by occupying the void space in the structure as the cake temperature lower and gas cell pressure decreases.

Table 13. Comparison of pH and L*a*b* values of untreated and heat treated sorghum flour*

Flour Name	pH	Color of Flour		
		L*	a*	b*
Control	6.14 ± 0.006[a]	80.11 ± 0.042[a]	0.22 ± 0.014[a]	13.72 ± 0.325[a]
90ºC/15 min	6.14 ± 0.006[a]	80.60 ± 0.491[a]	0.20 ± 0.032[a]	13.44 ± 0.273[a]
90ºC/30 min	6.12 ± 0.006[a]	80.30 ± 0.655[a]	0.19 ± 0.080[a]	13.76 ± 0.249[a]
90ºC/45 min	6.15 ± 0.006[a]	80.30 ± 0.348[a]	0.17 ± 0.055[a]	13.10 ± 0.197[a]
125ºC/15 min	6.14 ± 0.006[a]	79.72 ± 0.617[a]	0.20 ± 0.056[a]	13.13 ± 0.170[a]
125ºC/30 min	6.12 ± 0.006[a]	80.09 ± 0.749[a]	0.19 ± 0.015[a]	13.50 ± 0.234[a]
125ºC/45 min	6.15 ± 0.006[a]	78.93 ± 0.488[a]	0.18 ± 0.058[a]	13.82 ± 0.083[a]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Table 14. Comparison of gelatinization and pasting properties of untreated and heat treated sorghum flour*

Flour Name	Peak Viscosity (x 1000 cP)	Breakdown (x 1000 cP)	Setback (x 1000 cP)	Final Viscosity (x 1000 cP)	Peak time (min)	Pasting Temp (Cº)
Control	4.354 ± 0.08[a]	2.069 ± 0.03[a]	3.749 ± 0.03[a]	6.034 ± 0.07[a]	5.05 ± 0.00[a]	71.33 ± 0.46[a]
90ºC/15 min	4.351 ± 0.03[a]	2.037 ± 0.03[a]	3.770 ± 0.02[a]	5.984 ± 0.08[a]	5.05 ± 0.00[a]	71.03 ± 0.06[a]
90ºC/30 min	4.457 ± 0.03[b]	2.098 ± 0.02[a]	3.792 ± 0.04[a]	7.051 ± 0.03[b]	5.00 ± 0.00[a]	71.45 ± 0.42[a]
90ºC/45 min	4.455 ± 0.04[b]	2.081 ± 0.01[a]	3.656 ± 0.03[a]	6.800 ± 0.00[b]	4.95 ± 0.04[a]	70.18 ± 0.04[b]
125ºC/15 min	4.374 ± 0.01[a]	2.075 ± 0.05[a]	3.621 ± 0.02[a]	5.920 ± 0.07[a]	4.95 ± 0.04[a]	71.15 ± 0.00[a]
125ºC/30 min	4.380 ± 0.03[a]	2.037 ± 0.02[a]	3.602 ± 0.02[a]	6.955 ± 0.01[b]	5.05 ± 0.00[a]	71.13 ± 0.04[a]
125ºC/45 min	4.405 ± 0.09[b]	2.095 ± 0.14[a]	3.733 ± 0.03[a]	7.092 ± 0.02[b]	4.95 ± 0.00[a]	70.23 ± 0.04[b]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Bread Baking Test

Bread Volume

Values for the specific volume of breads ranged from 2.62 mL/g (control sorghum flour) to 3.08 mL/g (heated treated sorghum at 125ºC for 30 min) (Table 15). This significant difference in specific volume is illustrated in figure 3. This increase in specific volume may due be to the modification of proteins in sorghum flour by oxidizing the free sulfhydryl groups. Gujral and Rosell (2004) investigated the effects on oxidation on breadmaking quality of rice flour. They found that the oxidation of the

sulfhydryl units resulted in an increase of disulfide cross-linkages. As a consequence, a stronger dough was obtained with a greater resistance to mechanical shock, improved oven spring, and ultimately a larger loaf volume.

Figure 5. Comparison of specific volume of bread produced from untreated and heat-treated sorghum flour. On left: untreated control flour; on right: heated treated flour at 125ºC for 30 min

Bread Crumb Structure

Cell volume and diameter remained small while more cells per slice area were produced (Table 15). Gallagher and others (2003) expressed a greater number of smaller gas is desirable when improving volume and overall crumb structure of gluten-free breads. Gélinas and others (2001) studied the effects of heat on substandard flour to improve bread making potential. They found the number of crumb cells within a fixed area increased after heat treatment. They also found heat-treated flour greatly improved the fineness of crumb grain. Their findings are emulated in this study since

the internal grain structure was perceived to improve with heat treatment. Illustrated in figure 6, heat treated flour produced a finer and more uniformly-sized cell structure while the control flour produced an irregular crumb structure with large gaps between the crust and crumb. The control flour clearly had a weaker crumb structure which collapsed after initial oven spring.

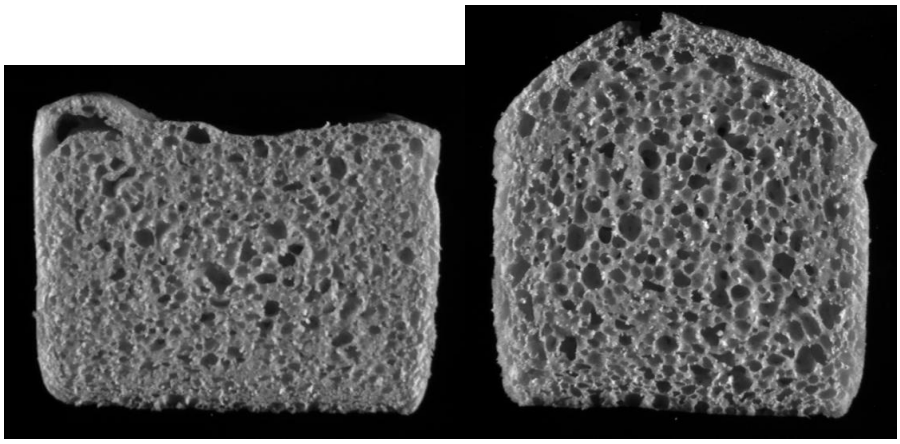

Figure 6. Comparison of bread crumb structure produced from untreated and heat-treated sorghum flour. On left: untreated control flour; on right: heated treated flour at 125ºC for 30 min

Table 15. Comparison of specific volume and C-Cell analysis of bread produced from untreated and heat treated sorghum flour*

Flour Name	Specific Volume (mL/g)	Cell Volume (mm^3)	Cell Diameter (mm)	Wall Thickness (mm)	Cells per Slice Area ($cells/cm^2$)
Control	2.62 ± 0.02^a	10.93 ± 0.15^a	3.08 ± 0.08^a	0.56 ± 0.01^a	44.87 ± 0.55^a
90ºC/15 min	2.65 ± 0.04^a	11.76 ± 0.73^a	3.03 ± 0.14^a	0.55 ± 0.01^a	46.06 ± 1.52^a
90ºC/30 min	2.84 ± 0.03^b	11.64 ± 1.00^a	3.05 ± 0.13^a	0.52 ± 0.02^a	50.18 ± 2.37^b
90ºC/45 min	3.04 ± 0.02^c	10.64 ± 0.83^a	3.08 ± 0.15^a	0.54 ± 0.02^a	49.04 ± 1.99^b
125ºC/15 min	2.51 ± 0.07^a	9.65 ± 0.93^a	3.07 ± 0.09^a	0.55 ± 0.03^a	49.08 ± 2.05^b
125ºC/30 min	3.08 ± 0.07^c	10.37 ± 1.33^a	3.07 ± 0.16^a	0.56 ± 0.01^a	48.95 ± 2.24^b
125ºC/45 min	2.90 ± 0.09^b	10.53 ± 0.11^a	3.06 ± 0.11^a	0.55 ± 0.01^a	48.12 ± 2.20^b

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Bread Crumb Color

The values for slice brightness and L*a*b* values are listed in Table 16. Brightness and lightness (L*) decreased as treatment times and temperatures increased. This highlights one of the drawbacks of heat treatment over other forms of oxidative processes. Fesler (2003) discussed the lack of bleaching effect of treating flour with heat. He reported that heat treated flour will always give a slightly darker crumb color since the original color of the flour is slightly darker in appearance.

Table 16. Comparison of slice brightness and L*a*b* values of bread produced from untreated and heat treated sorghum flour*

Flour Name	Slice Brightness	Color of Bread Crumb		
		L*	a*	b*
Control	99.60 ± 0.94^{a}	87.50 ± 0.44^{a}	22.26 ± 0.13^{a}	9.04 ± 0.19^{a}
90ºC/15 min	99.23 ± 0.70^{a}	88.36 ± 0.90^{a}	22.61 ± 0.29^{a}	12.50 ± 0.66^{b}
90ºC/30 min	97.90 ± 1.52^{a}	88.68 ± 1.02^{a}	22.29 ± 1.01^{a}	12.45 ± 0.71^{b}
90ºC/45 min	94.53 ± 2.84^{b}	87.93 ± 1.52^{a}	22.19 ± 0.41^{a}	14.13 ± 0.41^{c}
125ºC/15 min	98.59 ± 0.72^{a}	87.32 ± 0.81^{a}	21.54 ± 1.28^{a}	11.15 ± 0.38^{b}
125ºC/30 min	97.47 ± 1.61^{a}	87.49 ± 1.53^{a}	22.48 ± 1.06^{a}	11.43 ± 0.70^{b}
125ºC/45 min	93.76 ± 1.91^{b}	85.73 ± 1.59^{b}	22.87 ± 0.41^{a}	11.49 ± 0.34^{b}

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Bread Texture Profile Analysis

Firmness as it relates to baked goods is defined as the resistance of the crumb to deformation (He and Hoseney 1990). Significant differences ($p<0.05$) were found in firmness values between breads made with non-heated and heat treated sorghum flour (Table 17). Values ranged from 885 g Force (control sorghum flour) and 820 g Force (heat treated sorghum flour at 125ºC for 45 min). A possible explanation for the reduced firmness in breads made with heat treated flour relates to loaf volume. Sabanis

and others (2009) noted a negative correlation between crumb firmness and loaf volume of -0.89 ($p<0.05$). In their study, bread with lower specific volumes had denser and more tightly-packed crumb structures leading to higher crumb firmness values. In this study, texture also seemed to be affected by the cell structure. Heat-treated flour produced breads with higher specific volumes along with finer, uniformly sized cells ultimately leading to a softer and more elastic texture. Pyler (1988) stated consumers of white pan bread prefer a soft, resilient crumb and relate these attributes to product freshness.

Table 17. Comparison of firmness of crumb in bread produced from untreated and heat treated sorghum flour*

Flour Name	Firmness (g)
Control	885.00 ± 7.07[a]
90ºC/15 min	873.33 ± 10.33[a]
90ºC/30 min	830.00 ± 17.89[b]
90ºC/45 min	825.00 ± 15.17[b]
125ºC/15 min	865.00 ± 10.49[a]
125ºC/30 min	836.67 ± 8.16[b]
125ºC/45 min	820.00 ± 14.14[b]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Baking Test

Cake Batter Specific Gravity

The specific gravity of a batter has a direct influence over the final cake volume (Kim and Walker 1992). Lower weight per unit volume along with lower specific gravity translates into greater total cake volume. Specific gravity is a gauge of the amount of air is incorporated into the batter (Pyler 1988). A more viscous batter with lower

specific gravity prevents large air bubbles from coalescing and leaving the batter from the surface.

Table 18 shows the mean specific gravity values of the untreated control sorghum flour compared to the heat treated flour. Significant differences ($p<0.05$) were found among the treatments. The values ranged from 0.93 (heat treated sorghum flour for 30 min at 125ºC) to 1.04 (control untreated sorghum flour). This decrease in specific gravity may mean more air was incorporated into the batter system. The following sections will compare the volume and crumb structure of cakes.

Table 18. Comparison of specific gravity of cake batter produced from untreated and heat-treated sorghum flour*

Flour Name	Specific Gravity
Control	1.04 ± 0.01[a]
90ºC/15 min	1.02 ± 0.03[ab]
90ºC/30 min	0.95 ± 0.02[c]
90ºC/45 min	0.94 ± 0.01[c]
125ºC/15 min	0.99 ± 0.01[b]
125ºC/30 min	0.93 ± 0.01[c]
125ºC/45 min	0.94 ± 0.04[c]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Volume, Symmetry, and Uniformity

The volume, symmetry, and uniformity indices for cakes made with heat treated and non-heat treated flour are listed in Table 19. Significant differences ($p<0.05$) were found in volume indexes between the control and heated flours. Values ranged from 58.50 (control sorghum flour) and 72.17 (heat treated sorghum flour at 125ºC for 30 min). Studies have shown that heat treatment can increase batter viscosity and improve the overall caking baking properties of flour (Russo and Doe 1970; Guy and Pithiwala

1981; Thomasson et al 1995; Catterall 2000; Cook 2002). It has been suggested that the improvements originate from heat denaturing the proteins on the starch granule surface exposing more of the hydrophobic side-chains of amino acids which are mainly buried in native proteins (Catterall 2000). This activation of the starch surface may allow for the formation of starch-lipid or starch-protein complexes which stabilize the cake batter during baking (Kulp, 1981). Guy and Pithawala (1981) suggest untreated flour forms a weaker gel system than heat treated flour due to the slower and less extensive swelling of the starch granule. These positive effects of heat treating flour seem to resonate in this experiment. Batter viscosity increased while specific gravity decreased meaning more air bubbles were trapped in the batter system. Additionally, this stronger batter system helped prevent against gas cell coalescence and collapsing during cooling. This improvement in structure strength and volume is illustrated in figure 7.

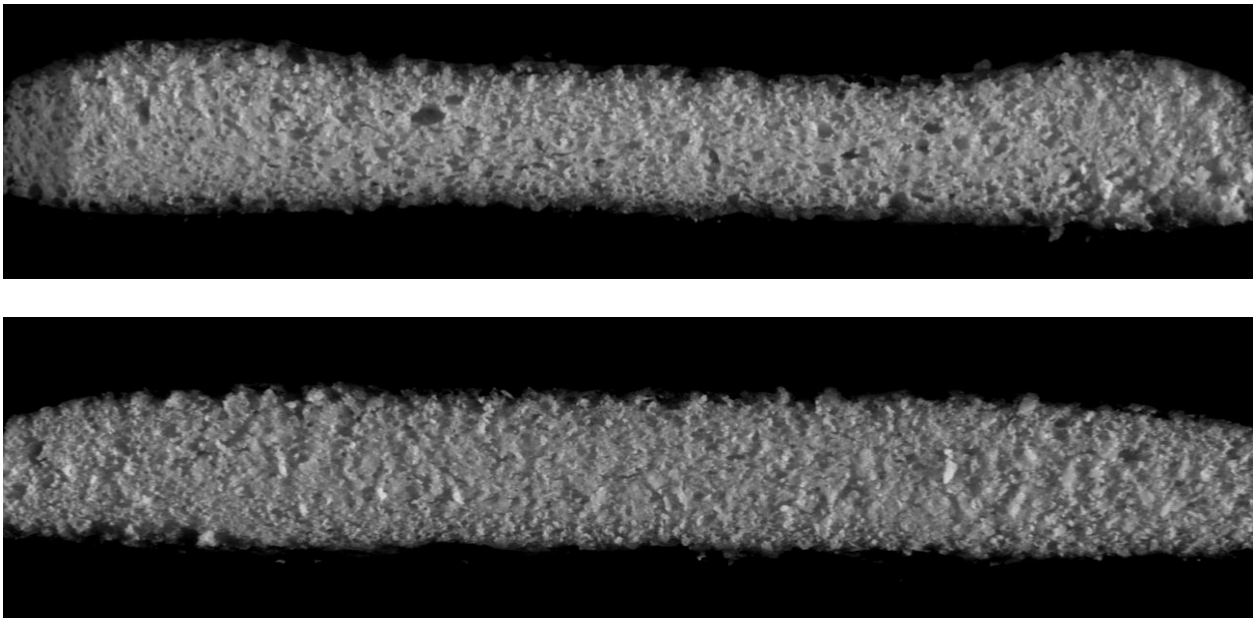

Figure 7. Comparison of cake volumes produced from untreated and heat-treated sorghum flour. On top: untreated control flour; on bottom: heated treated flour at 125ºC for 30 min

Table 19. Comparison of volume, symmetry, and uniformity indexes of cake produced from untreated and heat treated sorghum flour*

Flour Name	Volume Index	Symmetry Index	Uniformity Index
Control	58.50 ± 0.71[a]	3.00 ± 2.83[b]	1.00 ± 0.00[a]
90ºC/15 min	60.50 ± 2.17[a]	1.00 ± 0.89[a]	1.33 ± 0.82[a]
90ºC/30 min	71.50 ± 1.52[c]	1.50 ± 1.05[a]	0.50 ± 0.55[a]
90ºC/45 min	67.17 ± 1.83[b]	1.33 ± 1.03[a]	0.33 ± 0.52[a]
125ºC/15 min	67.67 ± 1.83[b]	2.00 ± 0.82[ab]	0.67 ± 0.52[a]
125ºC/30 min	72.17 ± 1.94[c]	0.83 ± 0.75[a]	0.50 ± 0.55[a]
125ºC/45 min	65.33 ± 2.07[b]	1.67 ± 1.51[a]	1.00 ± 0.00[a]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Crumb Structure

One of the largest differences in perceived gluten-free cake quality relates to the visual appearance of the crumb (Gambús et al 2009). Digital imaging data for cakes made from heat treated sorghum are listed in Table 20. Significant differences ($p<0.05$) were found for cells per slice area. Values for cell per slice area ranged from 69.96 cells/cm^2 (control sorghum flour) to 79.18 cells/cm^2 (heat treated sorghum flour at 125ºC for 30 min). Cook (2002) reported the quality of cake crumb is linked to the number and size of air bubbles incorporated into the batter during mixing. Since heat-treating the sorghum flour increased viscosity and decreased specific gravity, it can be hypothesized that more air bubbles were entrapped during mixing. Nakamura and others (2008) concluded dry-heating flour stabilized the foam of the cake batter, and this stability of the foam was maintained during baking. This stability translated into reducing gas cell coalescence and increasing cake volume. In the present experiment, the increase in overall volume can be correlated with increase in gas cells per slice.

Table 20. Comparison of C-Cell analysis of cakes produced from untreated and heat treated sorghum flour*

Flour Name	Cell Volume (mm^3)	Cell Diameter (mm)	Wall Thickness (mm)	Cells per Slice Area ($cells/cm^2$)
Control	6.22 ± 0.12^{a}	1.96 ± 0.12^{a}	0.45 ± 0.01^{a}	69.96 ± 2.24^{a}
90ºC/15 min	7.00 ± 0.14^{b}	2.11 ± 0.14^{b}	0.47 ± 0.02^{a}	70.37 ± 2.68^{a}
90ºC/30 min	7.73 ± 0.38^{c}	2.26 ± 0.09^{c}	0.46 ± 0.02^{a}	76.21 ± 2.38^{b}
90ºC/45 min	7.73 ± 0.42^{c}	2.29 ± 0.04^{c}	0.47 ± 0.02^{a}	75.45 ± 2.41^{b}
125ºC/15 min	7.45 ± 0.30^{bc}	1.94 ± 0.05^{a}	0.45 ± 0.01^{a}	71.20 ± 1.85^{a}
125ºC/30 min	7.45 ± 0.51^{bc}	2.26 ± 0.13^{c}	0.46 ± 0.01^{a}	79.18 ± 2.73^{c}
125ºC/45 min	7.48 ± 0.28^{bc}	2.26 ± 0.09^{c}	0.45 ± 0.01^{a}	76.89 ± 2.65^{b}

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Crumb Color

Cake crumb brightness and L*a*b* values are listed in Table 21. Results mimic the previous color results for bread made with heat-treated flours. As temperature and time increase, the brightness and lightness values decrease. However, only the highest level of treatment (heat treated sorghum flour at 125ºC for 45 min) produced a cake with a significantly lower ($p<0.05$) lightness value when compared with the control. Despite the slightly darker crumb color, this defect is negligible in comparison to the positive effects of heat treatment on overall volume, texture, and crumb structure.

Table 21. Comparison of slice brightness and L*a*b* values of cake produced from untreated and heat treated sorghum flour*

Flour Name	Slice Brightness	Color of Cake Crumb		
		L*	a*	b*
Control	81.20 ± 1.56^{a}	88.77 ± 0.71^{a}	16.93 ± 0.65^{a}	19.08 ± 1.41^{a}
90ºC/15 min	81.02 ± 0.74^{a}	87.26 ± 1.01^{a}	22.37 ± 0.37^{b}	14.68 ± 0.98^{b}
90ºC/30 min	80.03 ± 1.43^{a}	86.81 ± 2.02^{b}	22.23 ± 0.26^{b}	14.38 ± 0.82^{b}
90ºC/45 min	78.72 ± 1.20^{b}	86.52 ± 1.22^{b}	22.54 ± 0.27^{b}	14.80 ± 0.47^{b}
125ºC/15 min	80.87 ± 1.20^{a}	88.90 ± 1.38^{a}	22.53 ± 0.98^{b}	18.96 ± 0.79^{a}
125ºC/30 min	81.97 ± 1.19^{a}	87.46 ± 1.51^{a}	22.29 ± 1.04^{b}	14.91 ± 2.15^{b}
125ºC/45 min	78.53 ± 1.07^{b}	85.66 ± 0.56^{b}	23.27 ± 0.85^{b}	14.84 ± 1.19^{b}

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Cake Texture Profile Analysis

The TPA results are listed in Table 22. Firmness values ranged from 635.0 g Force (control sorghum flour) to 555.0 g Force (heat treated sorghum flour at 95ºC for 45 min). Heat treatment of flours has been shown to improve texture, grain, volume, and eating quality of cake (Russo and Doe 1970; Hanamoto and Bean 1978). This decrease in firmness may be related to the amount of air incorporated into the cake batter during mixing. As previously mentioned, heat treatment reduced the specific gravity of the cake batter. This increase in amount of air bubbles in the batter system seems to translate into a tender baked product.

Table 22. Comparison of firmness of crumb in cake produced from untreated and heat treated sorghum flour*

Flour Name	Firmness (g)
Control	635.00 ± 7.07[a]
90ºC/15 min	618.33 ± 7.53[a]
90ºC/30 min	586.67 ± 12.11[b]
90ºC/45 min	555.00 ± 10.49[c]
125ºC/15 min	625.00 ± 10.49[a]
125ºC/30 min	601.67 ± 7.53[b]
125ºC/45 min	561.67 ± 11.69[c]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

CONSUMER STUDY

Out of 100 panelists, 58 were female while 42 were male. The age of panelists ranged from 18 to 80 years with 59% of panelists in the 18-25 age group. For bread consumption, 45% panelists claimed to eat whole grain bread everyday while 46% of panelists claimed to consume whole grain bread at least once a week. 34% of panelists typically bought white bread while 66% purchased wheat or 100% whole wheat bread.

For cake consumption, 67% panelists claimed to eat cake at least once a month while 33% consumed cake at least once every two weeks. 71% of panelists typically bought cake mixes while 11% purchased prepared cakes and 8% purchased other forms of cake such as angel food or pound cake. Unexpectedly, 70% of respondents claimed they may purchase gluten-free products while 14% claimed they would buy and 16% they would not buy gluten-free products.

Significant differences ($p<0.05$) were found for overall acceptability, flavor, and texture (Table 23). More acceptable gluten-free bread was made with the heat treated sorghum at 125ºC for 30 minutes in comparison with the untreated control flour. The overall acceptability score was 5.05 for the heat treated flour and 4.76 for the control flour. This higher score along improved flavor and texture values indicates heat treating sorghum flour has the potential to improve baked goods in the gluten-free market.

Significant differences ($p<0.05$) were found for overall acceptability, appearance, and texture (Table 24). More acceptable gluten-free cake was made with the heat treated sorghum at 125ºC for 30 minutes in comparison with the untreated control flour. The overall acceptability score was 6.65 for the heat treated flour and 5.98 for the control flour. This higher score along with improved appearance and texture values indicates heat treating sorghum flour has the potential to improve baked goods in the gluten-free market.

Lawless and Heymann (1999) stated food choices made by consumers are influenced by a variety of factors including income, culture, religion, and health concerns. However, the most driving factor in purchasing habits for most people is

taste. Palatability of foods is perceived by several sensory attributes such as appearance, flavor, aroma, and mouthfeel.

Table 23. Comparison of scores from consumer study of bread produced from untreated and heat treated sorghum flour*

Flour Name	Overall acceptability	Appearance	Flavor	Color	Texture
Control	4.76 ± 1.64[a]	5.89 ± 1.61[a]	4.34 ± 1.42[a]	5.92 ± 1.79[a]	4.65 ± 1.93[a]
125ºC/30 min	5.05 ± 1.53[b]	5.70 ± 1.65[a]	4.88 ± 1.50[b]	5.94 ± 1.48[a]	5.16 ± 1.35[b]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Table 24. Comparison of scores from consumer study of cake produced from untreated and heat treated sorghum flour*

Flour Name	Overall acceptability	Appearance	Flavor	Color	Texture
Control	5.98 ± 1.46[a]	6.02 ± 1.52[a]	6.24 ± 1.77[a]	6.17 ± 1.54[a]	5.85 ± 1.74[a]
125ºC/30 min	6.65 ± 1.19[b]	6.83 ± 1.26[b]	6.25 ± 1.42[a]	6.19 ± 1.07[a]	6.63 ± 1.51[b]

*Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

CONCLUSIONS

Overall, this research demonstrates that treating sorghum flour with ozone and heat affect the quality of gluten-free bread. In the ozone experiment, flour viscosity and lightness (L value) improved as ozone exposure time increased. While ozonation improved the volume, slice brightness, and texture in cakes, it did not have the same positive effects on gluten-free bread. Bread made from ozonated sorghum flour had an open ragged structure with equivalent volume to the control flour. In both applications, ozone also imparted a strong off-flavor and odor to the end products.

On the other hand, heat treatment had positive effects on the quality of both gluten-free bread and cake. Improvements in overall volume, crumb structure, texture, and overall consumer acceptance were found in both cakes and breads made with sorghum flour heat treated at 125ºC for 30 minutes. While no improvements were seen in color, heat treatment seems to be a viable option to improving sorghum flour without imparting pungent off notes. These results can assist in the product development process in advancing the quality of sorghum-based gluten-free foods for the consumers who require a gluten-free diet.

RECOMMENDED FUTURE WORK

- Performing more analytical tests on the chemical and physical properties of the sorghum flour after treatment could illustrate how ozone and heat alter flour and dough functionality. The protein, carbohydrate, and lipid fractions of the sorghum flour need to be investigated individually to see modifications due to treatment level. Other proposed tests may include solvent retention capacity, water absorption index, protein characterization through SE-HPLC, and thermal properties using a differential scanning calorimeter.
- Since ozonated flour imparts a strong odor and flavor to both cake and bread, research needs to focus on how to decrease these pungent aromas by using alternate processing techniques and methods. Additionally, the tumbling mechanism of the motorized drum needs to be investigated to ensure the ozone is being evenly dispersed during the ozonation treatment.
- More sensory testing is needed to more accurately capture the effect ozone and heat has on bitterness, astringency, and other organoleptic attributes of sorghum products. Descriptive analysis could be useful in obtaining complete sensory descriptions and variations of the gluten-free products made from sorghum flour with varying levels of heat and ozone applications.
- More research on staling is needed to help determine the effects of ozonation and heat treatment on the shelf life of sorghum-based breads and cakes. Reducing staling and extending shelf life is essential in order to commercially produce gluten-free baked goods as opposed to daily home baking.

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APPENDICES

Appendix 1:

Proximate analysis of Twin Valley Mill (control) sorghum flour

Flour Name	Moisture Content	% Crude Protein	% Crude Fat	% Crude Fiber	% Ash
Control	10.72	5.96	3.05	0.24	1.385

Appendix 2:

INFORMED CONSENT STATEMENT FOR

CONSUMER SENSORY ANALYSIS OF GLUTEN-FREE CAKE AND BREAD

The purpose of this project is to determine consumer preference of gluten-free cake and bread. . Testing is expected to take less than 5 minutes. All ingredients in these products are food grade and approved by FDA. If you have no food allergies, there are no known risks or discomforts associated with consumption of these products. Your data will be treated as research data and will in no way be associated with you other than for identification purposes, thereby assuring confidentiality of your performance and responses.

1. I (print name)_____, agree to participate as a panelist in a sensory consumer testing conducted by Dr. Fadi Aramouni.

2. I understand that this study is part of a research project.

3. I understand that there will be a free ice cream certificate upon completion of the testing session.

4. I understand that I do not have to participate in this research and there will be no penalty if I choose not to participate.

5. I understand that I may withdraw from the research at any time.

6. If I have any questions concerning this study, I understand that I can contact Dr. Fadi Aramouni at 216 Call Hall (785-532-1668).

7. If I have any questions about my rights as a panelist or about the manner in which the study is conducted, I may contact the Committee on Research Involving Human Subjects, 103 Fairchild Hall, Kansas State University, Manhattan, KS 66506 (785-532-6195).

SIGNATURE:_____ DATE:_____

Appendix 3:

CONSUMER PRE-SCREENING FORM FOR GLUTEN-FREE CAKE AND BREAD PRODUCTS

Please complete the information below:

Age:

□ 18-25 □ 26-30 □ 31-35 □ 36-40 □ 41-45 □ 46-50
□ 51-55 □ 56-60 □ 61-70 □ 71-80 □ 81-90 □ Over 90

Gender:

□ Male □ Female

Education Completed:

□ High School □ Some College □ B.S. □ M.S. □ Ph.D.
□ MD □ Other

What type of bread do you typically buy?

□ White □ Wheat □ 100% whole wheat
□ Artisan □ Other: _____

How often do you eat whole grain bread?

□ Every day □ 3-4 times a week □ At least once a week
□ Once every 2 weeks □ Once a month □ Never

What type of cake do you typically buy?

□ Prepared cake □ Pound cake □ Angel food
□ Cake mix □ Other: _____

How often do you eat cake?

□ Every day □ 3-4 times a week □ At least once a week
□ Once every 2 weeks □ Once a month □ Never

Would you purchase gluten-free products?

□ YES • NO • MAY BE

Do you suffer from any food allergies?

• Yes • No

If you have any food allergies, you cannot participate in this study.

Thank you for your willingness to help.

Appendix 4:
CONSUMER BALLOT FOR GLUTEN-FREE BREAD STUDY

Panelist #_____

Instructions:
You will be testing two samples of gluten-free bread. Samples are presented in the order to be tasted. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Take a drink of water and a bite of cracker before you start and as needed throughout testing.

SAMPLE: 294

Please check only one box that represents your response (X)

1. Please rate your overall acceptability of this sample

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

2. How much do you like or dislike the appearance of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

3. How much do you like or dislike the flavor of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

4. How much do you like or dislike the color of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

5. How much do you like or dislike the texture of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

Additional Comments:_____

CONSUMER BALLOT FOR GLUTEN-FREE BREAD STUDY

Panelist #_____

Instructions:
You will be testing two samples of gluten-free bread. Samples are presented in the order to be tasted. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Take a drink of water and a bite of cracker before you start and as needed throughout testing.

SAMPLE: 316

Please check only one box that represents your response (X)

1. Please rate your overall acceptability of this sample

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

2. How much do you like or dislike the appearance of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

3. How much do you like or dislike the flavor of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

4. How much do you like or dislike the color of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

5. How much do you like or dislike the texture of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

Additional Comments:_____

Appendix 5:
CONSUMER BALLOT FOR GLUTEN-FREE CAKE STUDY

Panelist #_____

Instructions:
You will be testing two samples of gluten-free cake. Samples are presented in the order to be tasted. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Take a drink of water and a bite of cracker before you start and as needed throughout testing.

SAMPLE: 416

Please check only one box that represents your response (X)

1. Please rate your overall acceptability of this sample

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

2. How much do you like or dislike the appearance of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

3. How much do you like or dislike the flavor of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

4. How much do you like or dislike the color of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

5. How much do you like or dislike the texture of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

Additional Comments:_____

CONSUMER BALLOT FOR GLUTEN-FREE CAKE STUDY

Panelist #_____

Instructions:
You will be testing two samples of gluten-free cake. Samples are presented in the order to be tasted. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Take a drink of water and a bite of cracker before you start and as needed throughout testing.

SAMPLE: 509

Please check only one box that represents your response (X)

1. Please rate your overall acceptability of this sample

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

2. How much do you like or dislike the appearance of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

3. How much do you like or dislike the flavor of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

4. How much do you like or dislike the color of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

5. How much do you like or dislike the texture of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
●	●	●	●	●	●	●	●	●
1	2	3	4	5	6	7	8	9

Additional Comments:_____