

**DEVELOPMENT OF SPECIALTY BREADS AS
NUTRACEUTICAL PRODUCTS**

A Thesis

by

LINDSEY RENÉE HINES

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

MASTER OF SCIENCE

May 2007

Major Subject: Food Science and Technology

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ABSTRACT

Development of Specialty Breads as Nutraceutical Products. (May 2007)

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Bread is widely consumed and is an ideal vehicle for nutraceutical delivery. Sorghum bran, flax, and inulin are nutraceutical ingredients that may be incorporated into bread to provide health benefits. Because celiacs can not consume bread containing wheat flour, a need exists for gluten-free bread containing nutraceutical ingredients.

In preliminary research, bread containing 12% brown sorghum bran and 5% flaxseed was developed. In an effort to reduce rapid staling, soy flour was substituted for 0, 2, 4, and 6% of the wheat flour. Bread was evaluated based on specific volume, crumb firmness, color, moisture, nutrition content, and sensory evaluation. Sorghum bran was high in dietary fiber (45%) and antioxidants (oxygen radical absorbance capacity, ORAC). Flaxseed also contained dietary fiber (28%) and omega-3 fatty acids. Soy flour was high in protein.

High levels of soy flour increased crumb firmness and decreased specific volume. The bread containing 2% soy flour was preferred, however, by panelists in sensory evaluation. Per 56 g serving, this bread provided ~3 g dietary fiber, ~396 mg omega-3 fatty acids, and ~3417 μmol TE antioxidant activity. The use of low levels of soy flour in bread containing sorghum bran and flaxseed may help improve palatability

and increase consumption of dietary fiber, antioxidants, and omega-3 fatty acids in bread.

A gluten-free bread containing sorghum bran, flax, and inulin was also developed for consumers with celiac disease. Breads were evaluated based on the same parameters as described above. Inulin was high in soluble fiber (90%). The optimum formula was 10% inulin, 5% sorghum bran, and 5% flax. This formula had improved specific volume, reduced crumb firmness, and an attractive dark colored crumb. One 56 g serving of the bread provided ~2 g dietary fiber, ~1882 μmol TE antioxidant value, and ~287 mg omega-3 fatty acids. When compared to commercially available gluten-free bread mixes, the optimum formula was significantly improved with regards to crumb firmness and provided acceptable specific volume. Because of the improved bread qualities and high levels of health-promoting nutraceutical ingredients, the optimum formula could likely compete in the gluten-free bread market.

DEDICATION

This thesis is dedicated to my family, especially to my husband, Heath Hines. You have encouraged me in every way possible and inspired me to believe in myself. Thank you for never allowing me to give up on my dreams and being there to support me each step of the way.

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CHAPTER I

INTRODUCTION

The nutraceuticals market is a fairly new and growing segment of the food industry. Growth has been fueled by several factors. Consumers are increasingly interested in maintaining better health, and information is becoming more readily available linking diet to health. In addition, consumers want to avoid spending money on healthcare and prescription drugs, especially the aging Baby-Boomer generation. Finally, new FDA regulations permitting qualified health claims allow food manufacturers to make more claims based on emerging science. Today's health issues drive the functional food market, with cardiovascular disease and cancer topping the list as the number one and two causes of death in the United States. Therefore, products that contain functional ingredients to prevent these conditions will be in high demand. The functional foods market was worth \$20.2 billion in 2002. The fortified cereals, breads, and grains segment made up \$5 billion of that figure, and is expected to grow an average of 5.4% per year through 2007 (Business Communications Co. 2003).

Because bread is so widely consumed, it is an ideal vehicle for nutraceutical delivery (Awika et al. 2003a). Additionally, the popularity of home bread machines allows consumers to bake fresh bread easily and quickly. Research has been done to develop bread mixes with nutraceutical ingredients including sorghum bran, flaxseed, and barley

This thesis follows the style and format of Cereal Chemistry.

flour (Rudiger 2003, Gordon 2001). Sorghum bran is high in antioxidants, which scavenge cancer-causing free radicals. In addition, sorghum bran is high in dietary fiber, which promotes intestinal health. Flaxseed is rich in omega-3 fatty acids which have been shown to reduce the risk for cardiovascular disease (CVD), and barley flour contains soluble fiber that also reduces risk for CVD. Designed to be baked in a bread machine, these mixes are simple to prepare and may provide health benefits to the consumer. In preliminary trials, the combination of sorghum bran and flaxseed was optimized to provide maximum dietary fiber and antioxidant value without sacrificing bread quality.

One drawback of bread baked in bread machines is that it stales quickly. The use of small amounts of soy flour in bread increases water absorption and bread moisture, resulting in increased yield, decreased cost, and increased shelf life. In preliminary trials, the addition of a small amount of soy flour seemed to decrease staling and improve the texture of day-old bread. The first objective of this study was to improve texture and shelf life of the optimized formula by substituting soy flour at low levels.

While whole grain products offer many nutritional advantages, approximately 1% of the U.S. population is unable to consume wheat-containing products due to celiac disease, wheat intolerance, or wheat allergy. Celiac disease is an immune mediated condition triggered by the consumption of wheat and other cereals such as barley, triticale, rye, spelt, and kamut, and affects 3 million people in the United States (Fenster 2004). The only treatment is the lifelong adherence to a gluten-free diet. Because awareness and diagnosis of celiac disease is increasing, more patients are looking for

better quality gluten-free products. Although gluten-free products are a niche market, demand is likely to increase because celiac patients must remain on a gluten-free diet for life. Gluten-free breads and bread mixes are available, but often have poor quality. In addition, few, if any gluten-free breads exist that also provide nutraceutical benefits. Therefore, the second objective of this research was to develop a gluten-free bread that contains nutraceutical ingredients and competes favorably with commercially available products.

CHAPTER II

LITERATURE REVIEW

Sorghum and Its Composition

Sorghum (*Sorghum bicolor*, L. Moench) is a cereal grain commonly grown in hot, dry regions of the world. While grown for human consumption in Africa and Asia, it is used primarily as animal feed in the United States (Rooney et al. 1992). Typical food applications for sorghum grain, flour, and meal include porridges, couscous, tortillas, bread, and fermented beverages (Gordon 2001).

All sorghums contain phenolic compounds that can affect the color and nutritional value of the grain. The type and amount of phenolic compounds present are controlled by genetics. The R, Y, I, B₁, B₂, and S genes control the color of the pericarp and testa. Various combinations of dominant and recessive R and Y genes result in a white, yellow, or red pericarp, while the intensifier (I) gene affects the intensity of the pericarp color. The presence of dominant B₁ and B₂ genes results in a pigmented testa that contains condensed tannins. The dominant S gene, when present with dominant B₁ and B₂ genes, produces a brown pericarp (Hahn et al. 1984).

Sorghums are classified by the USDA Federal Grain Inspection Service (FGIS) according to the presence of a pigmented testa. Type I sorghums do not have a pigmented testa and contain no condensed tannins. Type II sorghums have a pigmented testa with a recessive spreader gene (B₁-B₂-ss), while type III sorghums possess a pigmented testa with a dominant spreader gene (B₁-B₂-S-). All sorghums with a pigmented testa are classified as brown sorghums, regardless of pericarp color. Brown

sorghums can be detected by the Clorox bleach test, in which kernels with a pigmented testa turn black, while those without remain light (Hahn et al. 1984).

Phenolic Compounds in Sorghum. The major phenolic compounds present in sorghum are phenolic acids, flavonoids, and tannins. All sorghums contain phenolic acids and most contain flavanoids. However, only sorghums with a pigmented testa contain condensed tannins.

The phenolic acids (Fig. 1) are derivatives of benzoic or cinnamic acid and are substituted with hydroxyl (OH) or methoxy (OCH₃) groups. In sorghum, phenolic acids exist as free acids, soluble and insoluble esters, and are concentrated in the outer layers of the grain. Many phenolic acids inhibit microbial growth and may impart grain mold resistance (Hahn et al. 1984).

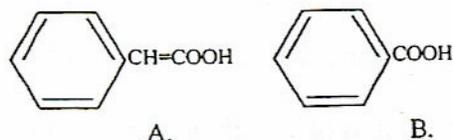


Fig. 1. Basic phenolic acid structures (A. cinnamic acid; B. benzoic acid).

The major class of flavanoids (Fig. 2) found in sorghum is flavans. The most common flavans are flavan-3-en-3-ols, also called anthocyanidins. In plants, anthocyanidins exist as glucosides at the 3 or 7 position and are called anthocyanins. The combination of anthocyanins and anthocyanidins are primarily responsible for the pericarp color of sorghum.

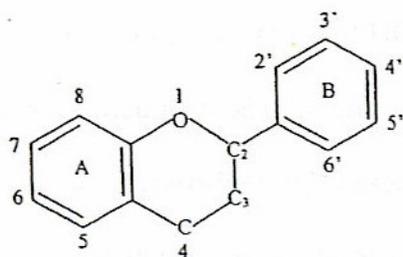


Fig. 2. Basic flavonoid ring structure.

Tannins (Fig. 3) are defined as water soluble phenolic compounds with molecular weights between 500 and 3000 that are able to precipitate alkaloids, gelatin, and other proteins (Santos-Buelga and Scalbert 2000). In fact, the protein-precipitating characteristic of tannins has long been used to turn animal skin into leather. These properties are responsible for the astringent taste that tannin-rich foods produce in the mouth and also impart tannin-containing sorghums with bird and mold resistance. Tannins can be divided into two classes: hydrolysable and condensed tannins. Hydrolyzable tannins (eg. tannic acid) break down into sugars and a phenolic acid (gallic or ellagic acid) when treated with acid, alkali, or some enzymes (Hahn et al. 1984). Hydrolysable tannins have not been found in sorghum. Condensed tannins are found in sorghum kernels with a pigmented testa. Polymers of flavan-3-ol units, they are referred to as proanthocyanidins because anthocyanidins are released when they are treated with mineral acids. Only condensed tannins, or proanthocyanidins, have been found in sorghum.

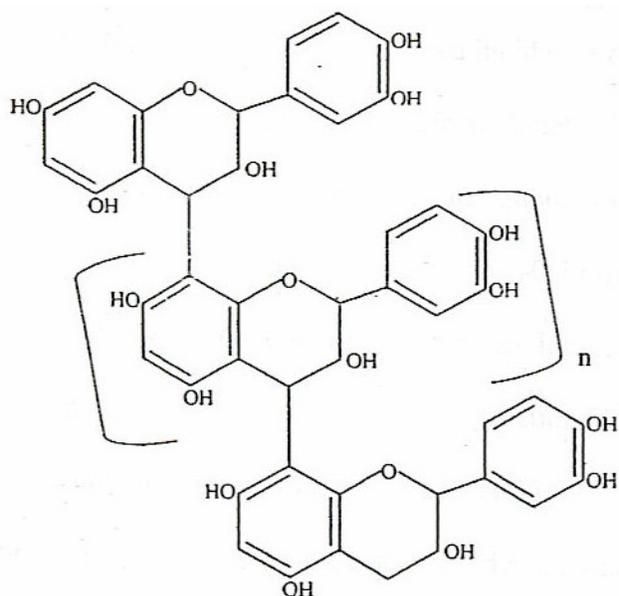


Fig. 3. Proanthocyanidin (tannin) polymer.

Two methods are commonly used to measure the levels of phenolic compounds in sorghum. The Folin-Ciocalteu method measures total phenols, including phenolic acids, flavonoids, and tannins, and is based on the reducing power of phenolic hydroxyl groups. This method uses gallic acid as a standard. The vanillin-HCl procedure is a colorimetric method that measures proanthocyanidins (tannins). It is based on the reaction of tannins with vanillin in the presence of HCl to give a bright red color (Hahn et al. 1984).

Health Benefits of Phenolic Compounds. Diets high in fruits and vegetables are protective against a variety of diseases, especially cardiovascular disease and certain types of cancer. Antioxidants and dietary fiber are thought to be the primary source of these protective effects (Ross and Kasum 2002). Phenolic compounds possess important

antioxidant activity and are found in fruits, vegetables, nuts, and cereal grains (Halstead 2003).

Wine has been recognized for its phenolic content and associated health benefits. The so-called “French Paradox” refers to the epidemiological observation that the incidence of coronary heart disease is much lower in wine-consuming areas of France compared to areas where wine is not as frequently consumed. The phenolic compounds in wine are reported to possess several cardioprotective effects, including preventing oxidation of LDL cholesterol, inhibiting platelet aggregation, and promoting vascular relaxation due to the production of nitric oxide (Halsted 2003). Phenolic compounds in wine include proanthocyanidins (condensed tannins), quercetin and epicatechin, as well as the non-phenolic antioxidant resveratrol (Halsted 2003).

The tannins from sorghum are also powerful antioxidants (Riedl and Hagerman 2001, Chung et al. 1998a, Hagerman et al. 1998). For phenolic compounds, the ability to act as antioxidants is dependent on conjugation, number and arrangement of phenolic substituents, and molecular weight. Because condensed tannins are highly polymerized and contain many phenolic hydroxyl groups, they are especially effective antioxidants (Hagerman et al. 1998). Even when complexed with proteins, sorghum tannins appear to retain at least half of their antioxidant capacity (Reidl and Hagerman 2001).

In addition to antioxidant activity, tannins have several other demonstrated health benefits. Sorghum tannins have been shown to reduce susceptibility to skin cancer and inhibit the growth of human melanoma colony cells (Gómez-Cordovés et al. 2001). Tannins also possess antimutagenic and antimicrobial attributes (Chung et al. 1998a,

1998b).

However, tannins have been called a “double-edged sword” (Chung et al. 1998a) because they have both positive and negative health effects. Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes, and affect the utilization of vitamins and minerals. Many researchers have reported reduced feed efficiency of animals fed on a high tannin diet. Tannins are reported to interfere with the digestion and/or absorption of carbohydrates from sorghum and in vitro amylolysis of sorghum starch (Chung et al. 1998b). The major effect of dietary tannins is not from reduced food consumption or digestion, but from reduced efficiency in converting the digested and absorbed nutrients into new body substances (Chung et al. 1998a). However, the so-called anti-nutritional effect of tannins does not pose a problem for populations where animal proteins and cereals such as rice, wheat, corn or barley are included. In fact, Awika and colleagues suggest that the reduced caloric value or slower digestibility of high-tannin foods could be a positive attribute, considering the prevalence of obesity in the U.S. (2003a).

Tannins and tannic acid are listed as Category I carcinogens by the Occupational Safety and Health Administration (OSHA). Topical application of tannic acid at 5-5000 μM has been reported to induce skin cancer in rats. Betel nuts contain 11-26% tannins, and are suspected of causing the high incidence of cheek and esophageal cancer in the Far East. However, the correlation between consumption of betel nuts and cancer may not be related to tannins themselves, but to other molecules attached to the tannins (Chung et al. 1998a). The dose of tannins required to induce cancers in a targeted area

such as the cheek is likely greater than the dose obtained through food intake.

Therefore, the dose and type of tannins required to elicit anticarcinogenic effects must be determined (Chung et al. 1998a).

Recent evidence suggests that tannins and other flavanoids from foods are much more bioavailable than previously thought (Ross and Kasum 2002). Spencer and colleagues showed that procyanidins are unstable in a simulated gastric juice (pH 2) and are degraded to monomers and dimers (2000). Proanthocyanidins up to trimers can be absorbed by the intestinal cell monolayer, and the remaining polymers are fermented by colonic microflora into other flavanoid monomers which may have additional health benefits (Déprez et al. 2001, Déprez et al. 2000). Processing conditions also affect the content and distribution of proanthocyanidins. Awika and colleagues demonstrated that sorghum and its tannin-rich fractions can be processed into food products while retaining significant levels of these compounds (2003a).

ORAC Assay for Antioxidant Capacity. The oxygen radical absorbance capacity (ORAC) assay is a method commonly used to measure the antioxidant activity of fruits and vegetables. The ORAC method measures the ability of antioxidants to protect a fluorescent protein from damage by free radicals (Awika et al. 2003b). A peroxy radical is generated by AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride), which then attacks the fluorescent probe. The loss of fluorescence is an indication of the extent of damage to the protein from its reaction with the peroxy radical (Ou et al. 2001). The results are compared to trolox (6-hydroxy-2,5,7,8-tetra methyl-2-carboxylic acid) as a standard, a water soluble analogue of vitamin E. In the method described by Cao et al.

(1993), β -phycoerythrin (β -PE) was used as the probe. More recently, Ou et al. described a modification that replaced β -PE with fluorescein (3',6'-dihydroxyspiro[isobenzofuran-1[3H],9'[9H]-xanthen]-3-one). This modification reduces variability in the procedure, and generally gives values that are 2-3 times higher than with β -PE (Awika et al. 2003b).

The ORAC assay offers several advantages over other procedures. First, it is automated and standardized, so results can be compared across laboratories. Second, the ORAC method is reported to mimic antioxidant activity of phenols in biological systems better than other methods because it uses biologically relevant radicals and considers both time and degree of activity of antioxidants (Awika et al. 2003b). ABTS is another method used to measure antioxidant activity and is highly correlated with ORAC (Awika 2003).

Phenol and Dietary Fiber Content of Sorghum Bran. Most of the phenolic compounds in sorghum are located in the testa and pericarp layers, which can be separated as bran during abrasive milling. The bran fraction contains 2-7 times the level of phenols in the whole grain (Hahn and Rooney 1986). Awika (2000) removed fractions of bran by abrasive milling, and demonstrated that the fraction corresponding to 10% removal of bran contained the highest level of tannins (17.5 mg CE/100 mg). Table I compares the ORAC antioxidant activity and phenolic content of the grain and bran fractions of several cereals. Brown, type III sorghums contain significantly higher levels of phenols and antioxidant activity than other cereals (Awika 2003).

TABLE I
ORAC and Phenol Content of Selected Sorghums and Cereals^a

Sample	Antioxidant Activity ($\mu\text{mol TE/g}$)		Phenol (mg/g)	
	Grain	Bran	Grain	Bran
High Tannin Sorghum	453	2400	12.4	53.4
Black Sorghum	219	1008	7.3	35.6
White Sorghum	22	64	0.8	4.8
Wheat Bran	ND ^b	31	ND	0.3

^aDM basis. Data from Awika (2003).

^bNot determined.

In addition to containing high levels of phenols, sorghum bran is also a rich source of dietary fiber. The bran fraction is composed of 45% dietary fiber, which can be classified as 97% insoluble and 3% soluble fiber (Awika 2000, Hahn and Rooney 1986). Because sorghum bran is high in both dietary fiber and phenols, it is an ideal ingredient for use in functional foods.

Soy Flour

With the increasing evidence about soy's cardiovascular health benefits, the FDA approval of the soy health claim, and positive press coverage, the consumer's perception of soy has changed. Once viewed negatively, the presence of soy products in food is now considered desirable by many consumers. In response to the health benefits and improved consumer perception, processors have sought to increase the utilization of soy products in food (Limpert 2004).

In a commonly used process for the production of soy flour, soy beans are cleaned, de-hulled, and flaked. The flakes are then solvent extracted to remove the oil and flash-desolventized. Finally, the flakes are toasted and milled to produce soy flour (Porter and Skaarra 1999). Soy flour is toasted to different extents to inactivate lipoygenase enzymes. Untoasted soy flour with 90% protein dispersibility index (PDI) has a grassy flavor and is used mostly as a crumb whitener at levels below 1%. Soy flour with 70 PDI has a cereal taste that generally does not affect flavor, while fully toasted 20 PDI soy flour has a slightly nutty flavor that may be compatible with variety breads (Milo Ohr 2000). Soy flour is composed of approximately 50% protein, 2.5-3.5% fat, 18% total dietary fiber, and 4-8% moisture (Limpert 2004). It is also high in isoflavones, particularly daidzein and genistein.

Soy flour is added to foods for several reasons. One use of soy flour is in fortification of cereal-based foods such as baked goods. Because the amino acid profile of soy protein is complimentary to that of wheat protein, the nutritional value of soy-fortified wheat products can be increased (Riaz 1999). The use of soy flour as a nutraceutical ingredient is also increasingly common. The FDA allows products containing 6.25 grams of soy protein per serving to carry the claim “reduces risk for heart disease.” However, to meet the health claim, a high amount of soy flour must be used. For example, assuming that soy flour contains 50% protein, almost 35% soy flour (baker’s percent) would have to be substituted for wheat flour to provide 6.25 grams per serving. A third use for soy flour is as a bread improver. Untoasted, enzyme-active soy flour is used as a crumb whitener and to improve texture and volume in white bread,

rolls, and buns. Because the flour contains enzymes that cause off-flavor volatiles, it is only used at up to 0.5% of wheat flour (Riaz 1999).

Because soy flour can retain large amounts of water during baking, each 1% addition of soy flour increases final bread moisture by 0.3 – 0.5%. This results in increased yield, decreased cost, and increased shelf life. The increase in bread moisture may also reduce the rate of staling during storage (Riaz 1999). Research by Cargill found that inclusion of 3% 70 PDI soy flour in a 7-grain variety bread had no sensory effect and reduced costs by \$0.44/100 lbs of dough due to increased water retention (Limpert 2004). Therefore, soy flour has the potential for use in bread to decrease costs while improving texture and staling rate.

Flaxseed – A Source of α -linolenic Acid, Dietary Fiber, and Lignans

The flax plant (*Linum usitatissimum*) produces tiny, flat seeds that are oval with a pointed tip (Gordon 2001). The seeds are composed of oil (42%), dietary fiber (28%), and protein (20%). The U.S. Food and Drug Administration allows the inclusion of up to 12% (by weight) of flaxseed in foods. Flaxseed has generated interest as a functional food ingredient for preventing CVD because it contains three key compounds: α -linolenic acid (an omega-3 fatty acid), soluble fiber, and lignans (Bloedon and Szapary 2004). The combined findings from nine clinical trials suggest that whole or ground flaxseed (15-50 g/day) can modestly reduce total and LDL cholesterol by 1.6 to 18% in both hypercholesterolemic and normocholesterolemic patients without significant changes in HDL or triglyceride levels (Bloedon and Szapary 2004).

may be able to directly reduce serum cholesterol by modulating enzymes involved in cholesterol metabolism (Bloedon and Szapary 2004). Lignans also act as antioxidants, and may reduce free radical induced damage that results in bone loss in postmenopausal women (Arjmandi 2001). Because lignan from flaxseed is stable during baking (Muir and Westcott 2000), it has potential for use as a functional ingredient in bread.

The dietary fiber present in flaxseed is approximately $\frac{1}{3}$ soluble and $\frac{2}{3}$ insoluble. The efficacy of soluble fiber in reducing serum cholesterol levels is well documented (ADA 2002). In a randomized, crossover trial, 50 g/day of partially defatted flaxseed reduced total cholesterol (4.6%) and LDL cholesterol (7.6%) without affecting HDL cholesterol levels (Jenkins et al. 1999). The cholesterol lowering properties of flaxseed are generally attributed to dietary fiber, lignans, or a combination of these two components (Bloedon and Szapary 2004). In addition, the soluble fiber from flaxseed behaves as a typical viscous fiber, acting to reduce blood glucose response (Oomah 2001).

Dietary Fiber

The American Dietetic Association recommends that the public should consume an adequate amount of dietary fiber from a variety of plant foods. The adequate intake (AI) level for dietary fiber was established by the Institute of Medicine in 2005 as 25 grams per day for adult women and 38 grams per day for adult men. However, only about half of the recommended amount is consumed, regardless of age or gender. The Food and Drug Administration allows products containing 2.5 grams of dietary fiber per

serving to be labeled as a “good source” of fiber; while those containing 5 grams per serving can be labeled as “high fiber.”

The American Association of Cereal Chemists (AACC 2001) defines dietary fiber as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation” (AACC 2001).

Dietary fiber can be divided into two categories based on physiological action in the body. Soluble fibers that form a viscous gel in the stomach delay gastric emptying. This results in decreased blood glucose response and a feeling of satiety. Viscous soluble fibers interfere with bile acid adsorption in the large intestine. By increasing excretion of bile acids, cholesterol must be removed from the blood and converted to bile by the liver to replenish the lost supply, thereby reducing cholesterol levels. In contrast, insoluble fiber decreases transit time through the intestine and promotes laxation. In addition, research shows that it reduces risk for colon cancer and diverticulitis (ADA 2002). Both soluble and insoluble fibers contribute bulk to foods without providing calories. Therefore, high-fiber diets are often larger in volume and lower in calories, and thereby reduce risk for obesity and assist in weight maintenance (Cho et al. 2004).

Baking With Fiber, Omega-3 Fatty Acids, and Antioxidants

The minimum formula for bread must include flour, yeast, salt, and water. Other ingredients may be added, including sugar, fat, enzymes, and surfactants. Flour is the major structural component of bread. Water acts as a plasticizer and solvent and is added to hydrate the gluten proteins in flour to produce a viscoelastic dough that can retain gas. Yeast converts fermentable carbohydrates into carbon dioxide and ethanol, thereby leavening the dough. Sugar serves as a source of fermentable carbohydrates for the yeast and also adds flavor. Salt contributes flavor and increases dough strength by shielding charges on the gluten proteins. Fat is often added to increase volume and prolong shelf life. Cysteine is a reducing agent that decreases dough development time by splitting disulfide bonds present in gluten. Surfactants such as sodium stearyl lactylate (SSL) are added to improve crumb structure, increase loaf volume, and delay staling (Hosney 1994). Xylanase is added to improve volume, crumb structure, dough strength, and extensibility.

Gluten, the water insoluble protein fraction of wheat flour, is responsible for the unique viscoelastic properties of dough. Gluten's two components, glutenin and gliadin, are responsible for its functionality. Glutenin is soluble in dilute acid or base and is made up of low-molecular weight (40,000-50,000 Da) and high-molecular weight (80,000-120,000 Da) subunits connected by disulfide linkages. Gliadin is soluble in 70% aqueous alcohol, and is composed of single-chain proteins with molecular weights ranging from 30,000 to 100,000 Da. Gliadin contributes plasticity to dough, while glutenin is responsible for the elastic nature of dough and contributes most to dough

strength (Stauffer 2001). During mixing, gluten proteins hydrate and unfold, forming thread-like polymers that interact via hydrogen bonds, hydrophobic interactions, and disulfide bonds. These polymers form a web-like gluten matrix that is able to entrap air bubbles, allowing the dough to rise (Damodaran 1996).

The effect of added fiber on bread quality is well documented. Whole grain breads tend to have higher density and lower volume than white pan bread. Added fiber dilutes the gluten network and at high levels interferes with the dough's gas retention abilities (Hosney 1994). Vital wheat gluten is often added to bran and multi-grain breads at 3-5% of flour to improve loaf volume (Stauffer 2001). Park et al. (1997) determined the effect of wheat fiber and psyllium husk fiber substitution for 5% and 10% of flour in white bread. Fiber substitutions increased water absorption, increased mixing time, reduced volume, decreased crumb grain quality, and slightly decreased caloric value. Fiber substitution also slowed the rate of crumb firming, which was likely caused by the increased moisture content of the loaves.

Flaxseed can confer both quality improvements and health benefits to bread. The Flax Council of Canada reports that soluble fiber, namely flaxseed gum, has been shown to improve loaf volume, oven spring, and keeping qualities. The water-binding properties of flaxseed can also help to increase shelf life (Payne 2000). Flaxseed gum has been shown to improve loaf volume, oven spring, and keeping quality, with optimum levels of flax substitution approaching 15% of flour weight.

Several researchers have successfully incorporated antioxidants into bread. Park and colleagues (1997) added fat-coated L-ascorbic acid (AsA), cold-water-dispersable

(CWD) β -carotene, and CWD all-rac- α -tocopheryl acetate (ToAc) to bread. The freshly baked loaves contained 76, 67, and 96% of added antioxidant, respectively. However, the AsA disappeared quickly during storage over 7 days, while CWD β -carotene and ToAc remained stable. Awika (2003) determined the effects of baking on the antioxidant activity of cookies and bread baked with brown sorghum bran. Brans in cookies retained significantly more ABTS activity (72-78%) than brans in bread (57-60%). ABTS is a measure of antioxidant activity highly correlated to the ORAC value discussed previously. Due to a lower moisture content and shorter mixing and baking times, the phenolic compounds in cookies were retained better than those in bread. However, even with baking losses, significant levels of antioxidants can be delivered in bread. A bread containing 6% brown sorghum bran had a measured ORAC value of 1,940 $\mu\text{mol TE}$ per 56 g serving (Rudiger 2003), more than half of the intake suggested by Prior and Cao (2000).

Nutraceutical Bread Summary

Brown sorghum bran is high in phenolic compounds and dietary fiber, while flaxseed provides high amounts of omega-3 fatty acids and lignans. When added at low levels, soy flour has the potential to improve bread texture and shelf life. The possibility exists for these three ingredients to be combined in a bread mix to provide high levels of nutraceutical compounds with improved texture and shelf life.

The objectives of this section of the research were to:

- 1) Optimize the levels of sorghum bran and flaxseed in a bread mix and evaluate the baked product's physical and nutritional qualities.

- 2) Determine the degree of soy flour substitution that will provide maximum textural improvements and increased yield with minimum sensory effects.

Celiac Disease

Celiac disease is an immune mediated condition affecting the small intestine that is triggered in genetically susceptible individuals by the consumption of the gliadin fraction of gluten (Fasano et al. 2003). The villi of the intestinal mucosa become flattened, reducing the production of disaccharidases and peptidases necessary for digestion. This deficiency in digestive enzymes and the reduced surface area of the small intestine results in malabsorption of virtually all nutrients. Depending on the extent of damage to the small intestine, patients may be relatively symptom-free or may have significant GI distress, malabsorption, and malnutrition. Some patients lack intestinal symptoms, and instead initially present with symptoms such as type I diabetes mellitus, anemia, osteoporosis, arthritis, infertility, or Down syndrome (Fasano et al. 2003).

Work has been done to determine the gliadin peptide sequence that results in the celiac immune response. All gliadins (α , β , γ , and ω) appear to be active in causing epithelial damage, and the most immunoreactive amino acid sequences in gliadins are not well characterized (Lähdeaho et al. 1995). Tučková et al. (2002) digested gliadin and tested the different fractions for the degree of immune response elicited, finding that the peptide sequence FQQPQQQYPSSQ produced the highest immune response. In examining ω -gliadins, Ensari et al. (1998) determined that the octapeptide sequence PQQPFPQQ is important in the immunopathology of celiac disease. Lähdeaho and

colleagues (1995) identified two peptides associated with celiac disease: peptide 9 (QPYPQPQFP) in α - and α/β -gliadins, and peptide 42 (LGQGSFRPSQ) found only in α -gliadin. While this work may one day create the possibility of genetically modified wheat that is safe for celiac patients, all sources of gluten must currently be avoided.

Gluten-Free Diet. The only treatment for celiac disease is the lifelong adherence to a gluten-free diet. Consumption of wheat, rye, and barley, as well as less common cereals such as spelt, kamut, einkorn, and triticale must be avoided. A growing body of research strongly suggests that oats can be safely consumed by celiacs, but there is concern about contamination by wheat during processing (Thompson 2001). Sorghum, flax, corn, rice, millet, buckwheat, amaranth, quinoa and teff are all grains that can be safely included in the gluten-free diet (Mechanic-Schlossmann et al. 2003). Tubers such as potatoes and cassava, beans, and oilseeds are also gluten-free. However, because wheat is ubiquitous in the food supply, its elimination from the diet presents a significant challenge to celiac patients and usually results in decreased quality of life (Lee and Newman 2003).

The Codex Alimentarius standard for gluten-free foods requires that the nitrogen content of foods derived from gluten-containing grain cannot exceed 0.05 g per 100 g grain on a dry matter basis. It is estimated that wheat starch meeting the Codex standard may contain 40 to 60 mg gluten per 100 g, which is equivalent to 200 to 300 ppm gliadin. (Thompson 2001). Currently, a draft revised standard for gluten-free foods is being developed by the Codex Committee on Nutrition and Foods for Special Dietary Uses in order to re-define the amount of gluten that is allowed in gluten-free foods. In

the new definition, foods made from naturally gluten-free ingredients may not contain more than 20 ppm gluten (10 ppm gliadin). Gluten-free foods made from ingredients that contain gluten (such as wheat, rye or barley) may not contain more than 200 ppm gluten (100 ppm gliadin). Currently, approval of the revised definition is pending until more information regarding tolerance levels to gluten can be determined (Joint FAO/WHO Food Standards Program 2004).

Standards for the gluten-free diet vary by country. In the United States and Canada, the diet contains no gluten, and is based on naturally gluten-free grains such as rice and corn. However, in the United Kingdom and Scandinavia, foods such as wheat starch that have been rendered gluten-free are included in the diet. Because the minimum dose of gliadin required to elicit an immune response in celiac patients is unknown, dietitians in the United States advise against the use of gluten-free wheat starch (Thompson 2001).

Prevalence of Celiac Disease. Until recently, celiac disease was thought to be rare in the United States. However, Fasano and colleagues (2003) recently conducted the largest multicenter epidemiologic study ever performed to establish the prevalence of celiac disease in the United States. This study indicated that the prevalence of celiac disease is 1:133 in patients who are considered not-at-risk, 1:22 among first-degree relatives of celiac patients, and 1:39 among second-degree relatives. Affecting 3 million Americans, celiac disease is the most common autoimmune disease in the United States. However, only 15% of the 3 million celiacs in the U.S. are currently diagnosed (Fenster 2004). Increased awareness of the prevalence of celiac disease by physicians will lead to

an increased diagnosis rate and an increase in demand for gluten-free products, particularly high quality gluten-free breads.

Gluten-Free Bread

Quality Problems. Because gluten is the major structure forming protein in bread, the formulation of gluten-free bread presents significant challenges. Many commercially available gluten-free breads are dry and crumbly with poor mouthfeel and flavor (Arendt et al. 2002). Specific volume is characteristically low due to the absence of a gluten network that is able to trap air in the dough, resulting in small, dense loaves. In addition, gluten-free breads stale more quickly than wheat breads (Kadan et al. 2001) and must be eaten soon after baking. In order to ensure acceptability of gluten-free bread, the product must have sensory characteristics similar to wheat flour yeast breads (Gallagher et al. 2003). However, it would be more realistic to consider gluten-free breads as a different product for a population with special dietary needs (Haque and Morris 1994). The following discussion summarizes some of the factors that influence the quality of gluten-free bread.

Continuous Phase Formation. The presence of a continuous phase is essential in the creation of good-quality gluten-free bread. In order to mimic the viscoelastic properties that gluten imparts, polymeric substances are often added to gluten-free breads to improve quality. Theoretically, any ingredient capable of improving the coherence between starch granules without impairing the dough's ability to rise can function as a gluten substitute (Özboy 2002). Gums and hydrocolloids or proteins are

often used in research and industry to serve as gluten substitutes, thereby creating a continuous phase in the dough.

Ács et al. (1996) investigated several gums (xanthan, guar, locust bean, and traganth) as gluten substitutes in a corn starch based gluten-free bread. They found that the gums increased volume and reduced crumb firmness, with 1-3% xanthan gum having the greatest effect. Haque and Morris (1994) found that the combination of 1% psyllium and 2% HPMC in gluten-free rice bread stabilized the dough over the range of proofing and baking temperatures, resulting in improved specific volume and sensory scores. Cato et al. (2004) found that the combination of 3.3% HPMC and 0.8% CMC in rice bread provided the necessary dough viscosity to trap air bubbles. The resulting product had a rigid but porous cell structure and good loaf volume. Özboy (2002) found that 2% of a commercial hydrocolloid containing xanthan gum and carageenans produced a good quality corn starch based bread. The addition of a surfactant (DATEM) and the proper amount of water (105%) were also important for quality.

Using a slightly different approach, Moore and colleagues (2004) found that the formation of a continuous protein phase through the addition of eggs is critical for improved keeping quality of gluten-free bread. Eggleston et al. (1992) studied the use of egg whites, xanthan gum, and margarine on the quality of cassava bread. Margarine increased the batter's ability to rise, while the egg whites stabilized the structure during baking. This study also concluded that margarine, and more significantly egg white, reduced the extent of starch gelatinization and solubilization in the cassava bread, which is important in preventing an excessively rubbery product.

Dispersed Phase Characteristics. The type of starch used to replace wheat flour has an effect on gluten-free bread quality due to differences in gelatinization temperature and starch granule size. Starch gelatinization temperature plays a role in the expansion of dough during baking. Kusunose and colleagues (1999) found that if the starch in bread dough gelatinizes and sets the dough structure before expansion is complete (as in potato starch), a smaller loaf will result. Starch granule size is also a factor in determining quality, particularly in the crumb grain of gluten-free bread (Table II). Flours with a high proportion of large starch granules produce bread with a more open grain with larger cells and thicker cell walls, while flours with smaller starch granules produce a finer grained crumb with smaller cells and thinner cell walls (Hayman et al. 1998).

TABLE II
Characteristics of Starch Granules from Various Cereal Grains and Tubers

Starch	Gelatinization Temperature Range (°C)	Granule Shape	Granule Size (µm)
Wheat	58-64	Round or lenticular	20-35
Corn	62-72	Round or polyhedral	15
Sorghum	68-78	Round	25
Rice	68-78	Polygonal	3-8
Tapioca	59-65	Round or polyhedral	20
Potato	57-65	Oval	100

Rice flour and corn starch-based products make up a large portion of the experimental gluten-free breads in the literature (Özboy 2002, Kadan et al. 2001,

Toufeili et al. 1994, Haque et al. 1994). Sanchez et al. (2002) found that a flour mixture consisting of 74.2% cornstarch, 17.2% rice flour, and 8.6% cassava flour optimized loaf volume, crumb-grain score, and bread score. This study also found that the addition of 0.5% soy flour improved bread texture by preventing the coalescence of air bubbles, resulting in a finer crumb structure. Kusunose and colleagues (1999) found that bread containing tapioca starch had the largest volume and oven spring. However, tapioca starch granules fused together to form a gas discontinuous system. This resulted in large initial loaf volume but caused shrinkage upon cooling due to negative pressure inside the loaf. In contrast, wheat starch granules remain intact after gelatinization, creating cracks in the air bubble cell membranes that prevent shrinkage during cooling.

White sorghum flour is also used in gluten-free breads. It has a nutritional profile similar to wheat, and does not adversely affect the flavor or color of the final product (Lovis 2003). Schober and colleagues (2005) demonstrated that differences in gluten-free bread making quality exist among sorghum hybrids. Sorghum flours from different hybrids exhibited significant differences in crumb grain, number of air cells, and crumb firmness, although loaf volume was unaffected.

Fiber is another component that affects the quality of gluten-free breads, and may be present in the dispersed phase of the dough. Because most gluten-free products are made from refined grains, celiacs may not consume an adequate amount of dietary fiber. Grehn et al. (2001) reported that adult celiac patients following a gluten-free diet consumed less dietary fiber than the control group consuming a normal diet. In addition,

Mariani et al. (1998) found that following a gluten-free diet worsens the already unbalanced diet of adolescents, providing low levels of dietary fiber.

The addition of dietary fiber to gluten-free breads generally results in poorer quality due to decreased gas retention capacity. Moore and colleagues (2004) found that the use of whole grain brown rice and buckwheat flour increased dietary fiber content but decreased the specific volume of gluten-free breads. Kadan and co-workers (2001) added 10% rice bran (flour basis) to a rice based gluten-free bread. Although the loaf volume was significantly decreased and firmness increased, the authors considered it a better product because it had improved flavor and appearance and reduced crumbliness.

Dietary fiber generally increases water absorption of dough due to its high water binding capacity (Rudiger 2003). Gluten-free bread dough typically resembles cake batter and is sticky and difficult to handle. However, incorporating ingredients that increase water absorption improve the dough's handling properties (Gallagher et al. 2003). Therefore, dietary fiber could improve handling properties of gluten-free bread dough.

Inulin as a Functional Soluble Fiber. Shown in Fig. 5, inulin is a linear $\beta(2-1)$ fructan. It is indigestible in the human small intestine, but is completely fermented by colonic bacteria in the large intestine (Roberfroid 2002). It is also highly hygroscopic. When used in wheat bread, inulin increases loaf volume and dough stability, improves slice-ability, and creates a uniform and finely grained crumb (Anon 1999). Gallagher et al. (2002) used 8% inulin in gluten-free bread, which increased fiber from 1.4% to 7.5% compared to the control and enhanced the crust color.

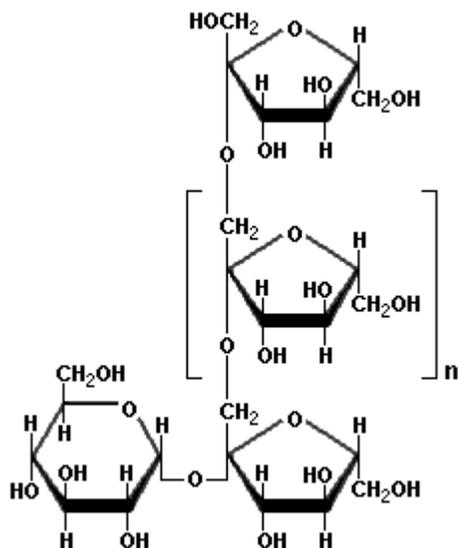


Fig. 5. Inulin.

In addition to potential functional properties in bread, inulin also possesses health benefits. Inulin acts as a probiotic, promoting the growth of health-promoting *Bifidobacteria* which are associated with a protective effect against colon cancer (Carabin and Flamm 1999). In addition, non-digestible oligosaccharides such as inulin have been shown to increase calcium absorption in adolescents and adults. This is significant because lactose intolerance and bone disease are commonly observed in celiac patients (Murray 1999). Cashman (2002) reviewed the use of inulin and its effect on calcium absorption in several studies. The effect appears to be dose dependent, with intakes of inulin ranging from 8 to 40 grams per day resulting in an 18-58% increase in

calcium absorption. Therefore, the use of inulin in gluten-free breads could provide beneficial health effects for celiacs.

The average daily consumption of inulin in the US is 1 to 4 g. Because inulin is fermented in the colon, excessive doses can cause GI symptoms including flatulence, bloating, and abdominal distention. Signs of intolerance can be seen with doses of 20-30 g per day. However, given labeling requirements for dietary fiber, consumers can make choices related to intake based on their individual tolerances (Carabin and Flamm 1999). Because of its functionality and potential health benefits, inulin has potential for use in gluten-free breads.

Use of Enzymes in Gluten-Free Bread. Enzymes have also been used to improve the quality of gluten-free breads by modifying the starches that compose the dispersed phase. Gujral and colleagues (2003) studied the effects of cyclodextrin glycoyl transferase (CGTase) (0.066 and 0.122 U/100 g flour) and α -amylase (15 and 30 U/100 g flour) on gluten-free rice bread. CGTase was most effective in increasing specific volume, decreasing crumb firmness, and slowing staling. The enzyme cleaved starch to produce cyclic molecules that formed inclusion complexes with the hydrophobic rice proteins, thereby acting as an emulsifier by reducing interfacial tension.

Use of Sorghum Bran, Flax, and Inulin in Gluten-Free Bread. The use of inulin in wheat based breads has shown promise. Besides improving bread loaf volume and crumb texture, it also increases dietary fiber, functions as a probiotic, and facilitates increased calcium absorption. Flax has also been shown to improve loaf volume of wheat flour breads. While sorghum bran has been used in wheat flour breads to provide

high antioxidant value, it tends to reduce loaf volume (Gordon 2001, Rudiger 2003). Therefore, it is hypothesized that the loaf-enhancing properties of inulin and flax could make it possible to incorporate sorghum bran in gluten-free bread without significantly affecting loaf volume.

Gluten-Free Bread Summary

A need exists for better-quality gluten-free breads for celiac patients. Additionally, ingredients with nutraceutical properties may be incorporated into gluten-free breads to provide health benefits tailored to the specific health needs of people with celiac disease. The objectives for this section of research were to:

- 1) Develop an acceptable gluten-free base formula that utilizes sorghum flour.
- 2) Determine the effects of sorghum bran, flax, and inulin substitution on gluten-free bread quality.
- 3) Determine optimum levels of flax, inulin, and sorghum bran to provide maximum levels of dietary fiber, antioxidants, and omega-3 fatty acids in one or two slices of bread.
- 4) Create a gluten-free bread formula that meets or surpasses the sensory and nutritional qualities of commercially available products.

CHAPTER III

PRODUCTION OF NUTRACEUTICAL BREAD CONTAINING SORGHUM BRAN, FLAXSEED, AND SOY FLOUR

Materials

Raw Materials. High protein bread flour (Superlative, 13.7% crude protein) was used for bread baking. Bran was milled from high tannin sorghum (CSC3 x R28) grown in College Station, TX in 2001. Select Grād™ milled golden flaxseed was obtained from Pizzey's Milling (Angusville, Manitoba, Canada), and 20 PDI soy flour was obtained from Cargill (Minneapolis, MN). Minor ingredients included granulated sugar, iodized salt, instant yeast (Fleischmann's), corn oil, vital wheat gluten, sodium stearyl lactylate (SSL), glycerol monostearate (GMS), Grindamyl™ POWERBake xylanase (Danisco, Copenhagen, Denmark), and L-cysteine.

Milling of Sorghum Bran. Bran was obtained by decorticating sorghum using a PRL mini-dehuller (Reichert et al. 1981) to remove 12% of the total grain weight as bran. The bran was then milled with an Alpine pin mill (Model A 250 CW) to pass through a US #100 mesh screen.

Methods

Analysis of Raw Materials. Sorghum bran, flax, and soy flour were evaluated for moisture, crude protein, crude fat, and ash according to standard AOAC procedures.

Sorghum bran and flax were also analyzed for total dietary fiber using the Prosky method (1985). Protein, fat, ash, and moisture content of the bread flour and soy flour were determined by near-infrared reflectance (NIR). Particle size distribution of raw materials was determined by sieving a 100 g sample through a series of appropriately sized screens using a Ro-Tap Testing Sieve Shaker (W.S. Tyler Co., Cleveland, OH).

Oxygen radical absorbance capacity (ORAC) of sorghum bran was determined by the methods of Ou et al. (2002). Phenolic compounds were analyzed using the modified Folin-Ciocalteu method with gallic acid as the standard (Awika 2000, Kaluza et al. 1980).

Bread Formula. Breads were baked using a straight-dough procedure (AACC 10-10B), with the ingredients listed in Table III. Sorghum bran, flax, and soy flour replaced a percentage of the wheat flour. Functionality of each ingredient is described in Table IV.

TABLE III
Nutraceutical Bread Mix Formula

Ingredient	Baker's %	Weight (g)
Superlative bread flour	100	515
Sugar	6	30.9
Salt	1.5	7.7
Yeast	0.75	3.9
Oil	3	15.5
Vital wheat gluten	1	5.2
Sodium stearoyl lactylate (SSL)	0.03	1.5
Glycerol monostearate (GMS)	0.02	1
Xylanase	50 ppm	0.03
L-cysteine	30 ppm	0.03
Water	62	289.3
Sorghum bran	12	
Soy flour	0-6	
Flax	5	

TABLE IV
Functionality of Ingredients in Nutraceutical Bread Mix

Ingredient	Functionality
Superlative bread flour	Bread structure
Sugar	Flavor, fermentable carbohydrate source
Salt	Flavor, dough strengthener
Yeast	Leavening agent, flavor
Oil	Antistaling agent, plasticizer, increased loaf volume
Vital wheat gluten	Increased dough strength
Sodium stearyl lactylate (SSL)	Surfactant, dough strengthener
Glycerol monostearate (GMS)	Surfactant
Xylanase	Dough strengthener
L-cysteine	Reducing agent
Water	Plasticizer, solvent
Sorghum bran	Increased water absorption, fiber, antioxidants
Soy flour	Increased water absorption
Flax	Increased water absorption, fiber, omega-3 fatty acids

Bread Baking. Two-pound loaves were baked in six TR800 Breadman Plus automatic bread machines (Salton/MAXIM Housewares, Inc., Mt. Prospect, IL). Loaves were baked for 3 hours and 10 minutes using the “normal” cycle and “medium crust” settings. Loaves were removed from pans and cooled on racks for one hour before evaluation.

Bread Quality Evaluation. Loaf volume was determined by rapeseed displacement. Specific volume was calculated as loaf volume (cm³) divided by loaf weight (g). Moisture was determined using the two-stage oven method (AACC 44-15A). Crumb firmness was evaluated 2 and 24 hours after baking using the TA.XT2i

Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) (AACC 74-09). Bread was sliced into 1 inch (25 mm) thick slices and analyzed with a flat 36 mm diameter probe (Fig. 6). For crumb firmness, six replications per loaf were conducted. Bread was stored at room temperature in low-density polyethylene bags. Crust and crumb color were determined using a Chroma Meter II Minolta Colorimeter (Osaka, Japan). Dietary fiber and ORAC values of a 56 g serving of bread were calculated. A nutrition label was generated using Food Processor v. 7.81 software (ESHA 2001).



Fig. 6. Use of the Texture Analyzer to determine crumb firmness.

Sensory Evaluation. An untrained sensory panel evaluated the optimum formula for flavor/aroma, texture, and overall acceptability using a continuous hedonic scale.

Experimental Design

- 1) Previous research recommended a bread mix containing 6% sorghum bran, 5% barley flour, and 5% flax (Rudiger 2003). Because barley flour is relatively low in dietary fiber (13% dietary fiber), it was removed from the formula and replaced by increased amounts of sorghum bran (containing 45% dietary fiber) to increase the total fiber content of the bread mix.

In preliminary trials, sorghum bran was substituted for 8, 10, 12, 14, 16, 18, and 20% of total flour weight while flaxseed remained constant at 5%. Physical and nutritional characteristics of each loaf were evaluated. Fig. 7 illustrates the effect of increasing levels of sorghum bran on loaf color and volume. The loaf containing 12% sorghum bran and 5% flaxseed was chosen as the optimum formula because it contained high levels of dietary fiber and antioxidants without adversely affecting loaf volume and texture.

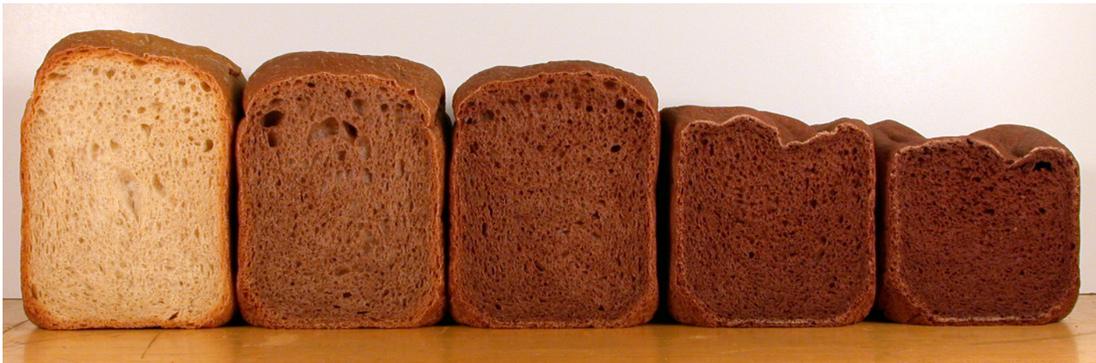


Fig. 7. Bread containing 5% flax and 0, 5, 10, 15, and 20% sorghum bran.

- 2) Preliminary trials suggested that small amounts of soy flour significantly improved the texture of day-old baked bread. In addition, the nutty flavor of 20 PDI soy flour was reported compatible with variety breads (Limpert 2004). Therefore, 20 PDI soy flour was substituted at 0, 2, 4, and 6% of wheat flour in the optimum formula of 12% sorghum bran and 5% flaxseed mentioned above. The highest level of soy flour that produced no adverse sensory effects and created the greatest improvements in textural qualities was chosen as the optimum substitution level.

Statistical Analysis

Averages, standard deviations, analysis of variance (ANOVA), and Fisher's least significant differences (LSD) with a confidence level of 95% were determined using SPSS 11.5 (Chicago, IL).

Results and Discussion

The proximate composition for bread flour, sorghum grain, sorghum bran, golden flaxseed, vital wheat gluten, and soy flour are presented in Table V. The sorghum bran was high in dietary fiber, while the flaxseed was high in fat and dietary fiber. The wheat flour used for baking contained 13.7% protein (DMB). The vital wheat gluten contained 80.6% protein, and the soy flour contained 53.2% protein.

TABLE V

Proximate Analysis Values and Dietary Fiber Levels for Raw Materials (% , DMB)^a					
Sample	Crude Protein	Crude Fat	Ash	Dietary Fiber	Moisture
Bread Flour	14.7	ND ^b	2.2	ND	15.1
Sorghum Grain ^c	9.7	2.8	1.4	11.8 ^d	13.2
Sorghum Bran ^e	12.7	10.4	5.1	45.0	11.2
Flaxseed	26.3	43 ^e	3.5	27 ^f	6.0
Vital Wheat Gluten	80.6	1.6	1.6	1.1 ^g	7.0
Soy Flour	53.2	ND	7.6	16 ^h	9 ^h

^a Dry matter basis.

^b Not determined.

^c Analysis of whole sorghum grain from similar research (Rudiger 2003).

^d Serna-Saldivar and Rooney (1995).

^e Brown sorghum bran analyzed and used in similar research (Gordon 2001).

^f Pizzey's Milling (Angusville, MB, Canada); typical value for dietary fiber.

^g Crude fiber (Rudiger 2003).

^h Proximate composition for ExPress Soy Protein Flour, Insta-Pro International.

Specific Volume. Increasing levels of soy flour decreased specific volume of bread containing 12% sorghum bran and 5% flaxseed (Fig. 8). With 2% soy flour, no significant decrease in specific volume was seen compared to the control. The 4% and 6% soy flour loaves had slightly decreased specific volumes, resulting in a more dense product. This result was expected, as soy flour contains no gluten and relatively high levels of dietary fiber. Fiber-containing ingredients can act to dilute the gluten matrix and decrease the dough's ability to retain gas, thereby reducing loaf volume.

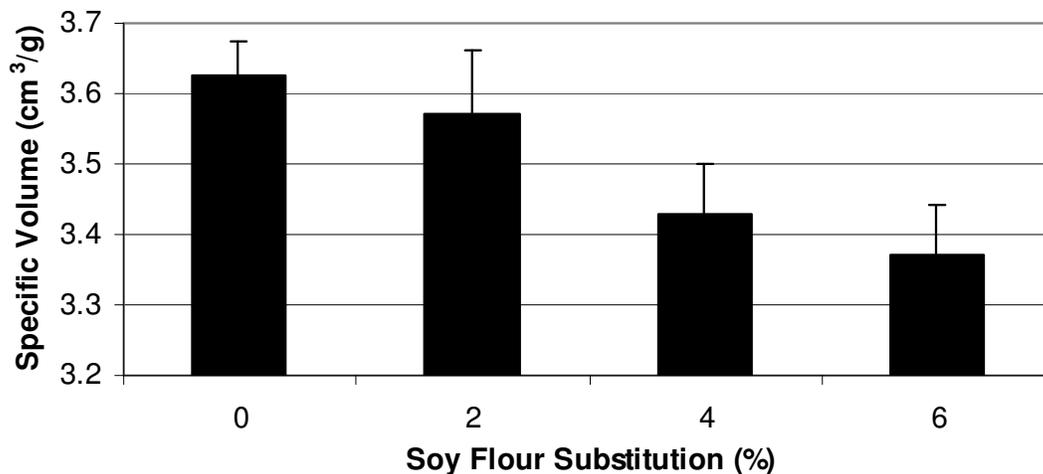


Fig. 8. Effect of soy flour substitution on specific volume of nutraceutical bread containing 12% sorghum bran and 5% flax.

Crumb Firmness. Increasing levels of soy flour substitution also increased crumb firmness (Fig. 9). The 2% soy flour loaf was not significantly different than the

control, but the 4% and 6% soy flour loaves were considerably firmer at 2 and 24 h after baking.

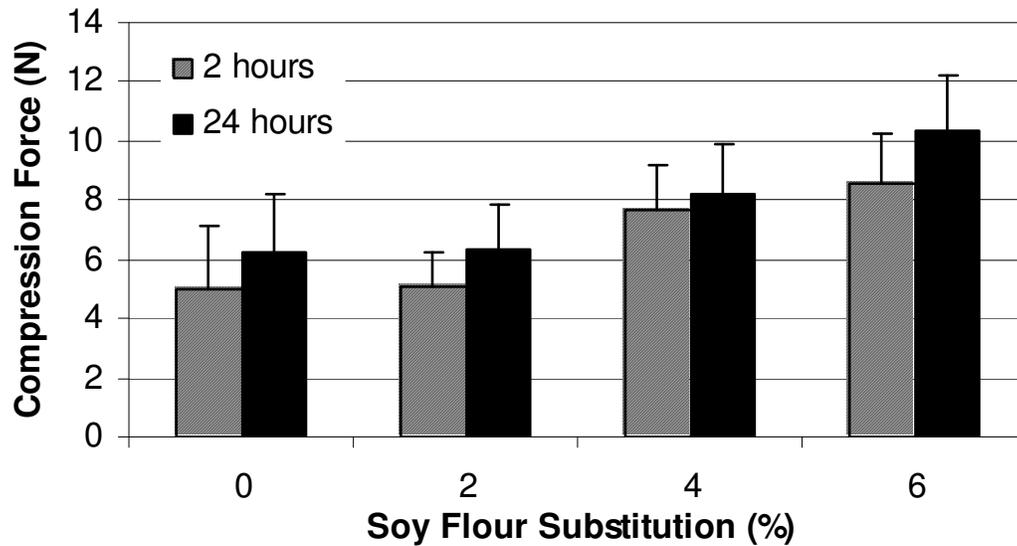


Fig. 9. Effect of soy flour substitution on crumb firmness in nutraceutical bread containing 12% sorghum bran and 5% flax.

Crust and Crumb Color. No significant differences in crust or crumb color were found for any level of soy flour substitution (Fig. 10). Because soy flour is a light-colored ingredient, it was not expected to make any significant difference in the color of the already dark bread.

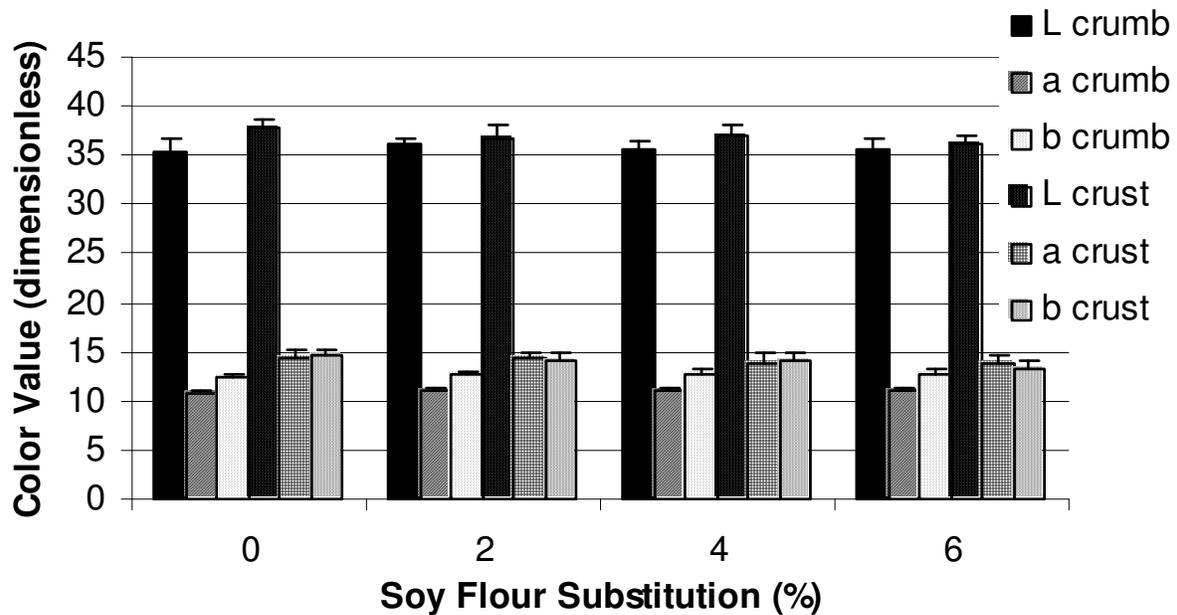


Fig. 10. Effect of soy flour substitution on crust and crumb color of nutraceutical breads containing 12% sorghum bran and 5% flax.

Moisture. No significant differences were seen in the moisture levels of the soy flour breads (Fig. 11). All breads were baked using 62% water. The higher levels of soy flour substitution did not appear to significantly increase the bread's ability to retain additional moisture. Therefore, the hypothesis that increased levels of soy flour would increase moisture levels was disproved in this bread system.

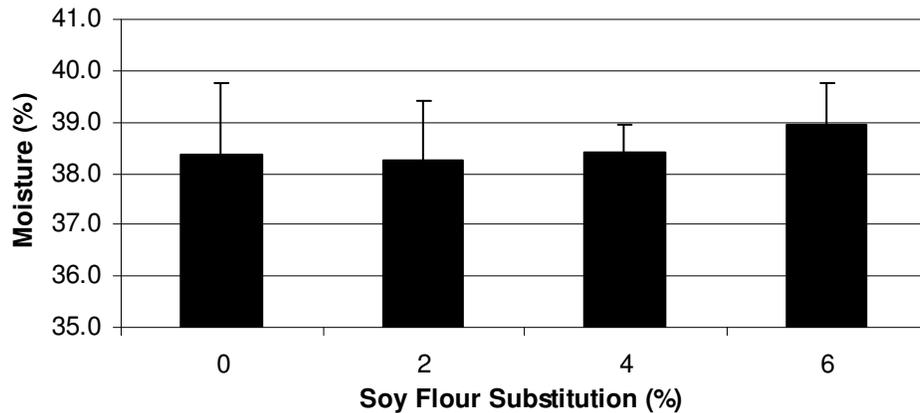


Fig. 11. Effect of soy flour substitution on moisture of nutraceutical breads containing 12% sorghum bran and 5% flax.

Sensory Evaluation. Two hours after baking, breads were evaluated on a nine point hedonic scale for flavor, texture, and overall acceptability (n=23). The substitution level of soy flour caused no significant differences in ratings for any of the attributes (Fig. 12). The inability to detect significant differences among these breads indicates that up to 6% soy flour could be used in bread without causing sensory changes detectable by an untrained sensory panel. The 2% soy flour loaf did have the highest numerical sensory scores in flavor, texture, and overall acceptability (Fig. 13). Several panelists indicated that the breads containing soy flour seemed to have a less astringent taste. It may be that the soy flour decreased the intensity of the astringent flavor contributed by the sorghum bran and resulted in a more acceptable flavor.

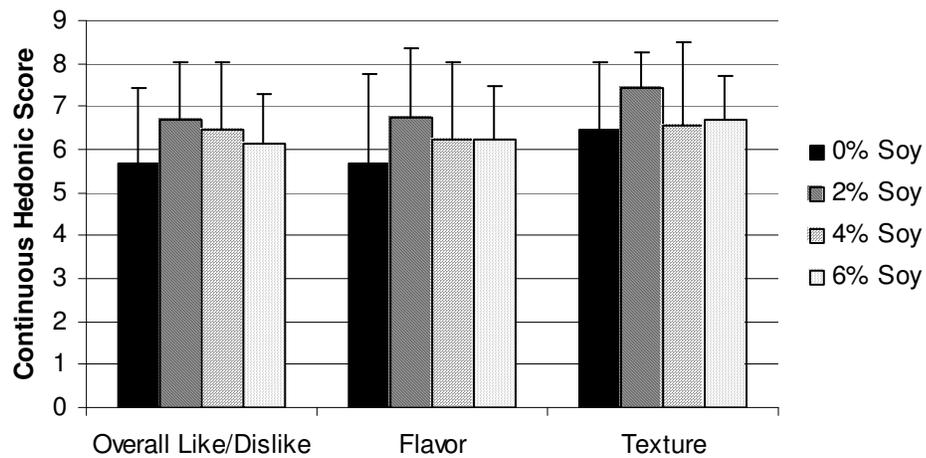


Fig. 12. Sensory scores of nutraceutical breads containing sorghum bran, flax, and soy flour.



Fig. 13. Nutraceutical bread containing 12% sorghum, 5% flax, and 2% soy flour.

Nutrition Content of Nutraceutical Breads Containing Sorghum Bran, Flax and Soy Flour. The addition of soy flour slightly increased dietary fiber levels and also contributed isoflavones and a small amount of soy protein. The amount of soy protein provided was insignificant with regards to labeling, as 6.25 g/serving is required for a product to bear the FDA health claim “reduces risk of heart disease.” The control formula containing 12% sorghum bran and 5% flaxseed was a good source of dietary fiber (3.1 g/serving, Table VI). The bread containing 6% soy flour contained the highest level of dietary fiber, with 3.4 g per serving.

The flaxseed contributed 396 mg alpha-linolenic acid (ALA) per serving of bread. A review by Gebauer et al. (2006) showed that intakes of ALA ranging from 0.58 to 2.81 g/day were associated with a reduced risk of CVD all-cause mortality. One slice of bread containing 5% flaxseed provided nearly all of the ALA at the lower end of this range. Two servings of the nutraceutical bread exceeded the amount of ALA required to meet the lower end of the range and thereby reduce the risk of CVD mortality.

The sorghum bran in the nutraceutical bread provided approximately 3417 μmol TE of antioxidants per serving. Previous work by Awika (2003) reported an ORAC of 2848 μmol TE for bread containing 10% high tannin sorghum bran. Rudiger (2003) reported an ORAC of 1940 μmol TE for bread containing 6% high tannin sorghum bran, 5% flax, and 5% barley flour. Prior and Cao (2000) estimated that consumption of 3000-3600 μmol TE per day would be needed to elicit the health benefits of fruit and vegetable consumption. Consequently, one slice of the nutraceutical bread could help

consumers to significantly increase antioxidant intakes and achieve health benefits associated with fruit and vegetable consumption.

As shown in Fig. 14, the bread containing 12% sorghum bran, 5% flaxseed, and 2% soy flour contained 130 kcal, 2 g fat, and 3.2 g dietary fiber per (56 g) serving. It also contained significant levels of ALA and antioxidants as described in Table VI.

Conclusions and Discussion. Contrary to the hypothesis, increased levels of soy flour increased crumb firmness, decreased specific volume, and had no appreciable effect on total moisture or crust and crumb color. The nutraceutical bread reached a maximum moisture level due to the increased water absorption properties of the sorghum bran and flax. Apparently, the addition of small amounts of soy flour did not increase moisture levels significantly enough to produce the expected result. However, it was notable that the 2% soy flour loaf was preferred in sensory evaluation over the

TABLE VI

Nutrient Content of One (56 g) Serving of Nutraceutical Bread Containing 12% Sorghum Bran, 5% Flaxseed, and Various Levels of Soy Flour

Percent Soy (%)	Dietary Fiber (g)	Isoflavones (mg) ^a	ORAC (μmol TE)	Soy Protein (g)	Alpha linolenic acid (mg)
0	3.1	0	3417 ^b	0	396
2	3.2	3	3417	0.2	396
4	3.3	6	3417	0.7	396
6	3.4	9	3417	1.0	396

^aDaidzein, genistein, and glycitein (Insta-Pro International 2003).

^bAntioxidant activity of brown sorghum bran in bread; extrapolated from Awika (2003).

Nutrition Facts	
Serving Size (56g)	
Servings Per Container	
Amount Per Serving	
Calories 130	Calories from Fat 15
% Daily Value*	
Total Fat 2g	3%
Saturated Fat 0g	0%
Cholesterol 0mg	0%
Sodium 190mg	8%
Total Carbohydrate 26g	9%
Dietary Fiber 3g	12%
Sugars 3g	
Protein 4g	
Vitamin A 0%	• Vitamin C 0%
Calcium 2%	• Iron 10%
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:	
	Calories: 2,000 2,500
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram:	
Fat 9 • Carbohydrate 4 • Protein 4	

Fig. 14. Nutrition facts label for one (56 g) slice of nutraceutical bread containing 12% sorghum bran, 5% flax, and 2% soy flour.

control. Regardless of its insignificant effect on physical characteristics of the bread, the addition of 2% soy flour may modulate the astringent taste of the sorghum bran, resulting in a product with more acceptable flavor to consumers. Nutrition information for one slice of bread containing 12% sorghum bran, 5% flax, and 2% soy flour is presented in Table VII.

TABLE VII
Dietary Fiber, Antioxidant, and Omega-3 Fatty Acid Content of One (56 g) Slice of Nutraceutical Bread Containing 12% Sorghum Bran, 5% Flax, and 2% Soy Flour

Nutraceutical Component	Recommended Intake (per day)			Actual Intake in US (per day)	Amount in 56 g serving of bread
	Age	Men	Women		
Dietary Fiber	<50	38 g ^a	25 g ^a	14-15 g ^b	3.2 g
	>50	30 g ^a	21 g ^a		
Antioxidants (ORAC)	3000-3600 $\mu\text{mol TE}^c$			1200-1640 $\mu\text{mol TE}^d$	3417 $\mu\text{mol TE}$
α -linolenic acid (ALA)	0.58-2.81 g/day ^e			Men 1.7 g ^f Women 1.3 g ^f	0.396 g

^aDietary Reference Intakes for dietary fiber (Institute of Medicine 2005).

^bAmerican Dietetic Association (2002).

^cEstimated amount required to elicit beneficial effects of fruit and vegetable consumption (Prior & Cao 2000).

^dFrom fruits and vegetables (Prior & Cao 2000).

^eStudies show intakes in this range are associated with reduced risk for all-cause CVD mortality (Gebauer et al. 2006).

^fMean intake of ALA (Gebauer et al. 2006).

CHAPTER IV

IMPROVEMENT OF GLUTEN-FREE BREAD WITH SORGHUM BRAN, FLAXSEED, AND INULIN

Materials

Sorghum flour was milled from white food grade sorghum (10% decortication of bran). Combined with the sorghum flour, tapioca flour (Bob's Red Mill, Milwaukie, OR), native corn starch, and native potato starch were used to make a gluten-free composite flour. Xanthan gum (Keltrol[®]) was obtained from CP Kelco (Chicago, IL). Bran was milled from high tannin sorghum (CSC3 x R28) grown in College Station, TX in 2001. Select Grād[™] milled golden flaxseed was obtained from Pizzey's Milling (Angusville, Manitoba, Canada), and Oliggo-Fiber[™] Instant Inulin was provided by Cargill, Inc. (Minneapolis, MN). Sugar, eggs, butter, vinegar, honey, salt, instant yeast (Fleischmann's), non-fat dry milk, and unflavored gelatin were purchased from H-E-B Grocery Company in College Station, TX.

Methods

Milling of Sorghum Bran. Sorghum bran was milled by the same method as described for the nutraceutical bread.

Analysis of Raw Materials. Sorghum flour, sorghum bran, sorghum grain, flaxseed, and inulin were evaluated for moisture, protein, crude fat, and ash according to standard AOAC procedures (Table VIII). Dietary fiber was determined by the Prosky method (1985). Particle size distributions for flours (Table IX) were conducted using the same methods as described for the nutraceutical bread.

TABLE VIII

Proximate Analysis and Dietary Fiber Levels of Raw Materials (% DMB)^a

Sample	Crude Protein	Crude Fat	Ash	Dietary Fiber	Moisture
Sorghum Flour	7.6	ND ^b	0.7	ND	10.1
Sorghum Grain ^c	9.7	2.8	1.4	11.8 ^d	13.2
Sorghum Bran ^c	12.7	10.4	5.1	45.0	11.2
Flaxseed	26.3	43 ^f	3.5	27 ^f	6.0
Inulin	ND	ND	0.5 ^g	90.0 ^g	ND

^a Dry Matter Basis.

^b Not determined.

^c Analysis of whole sorghum grain from similar research (Rudiger 2003).

^d Serna-Saldivar and Rooney 1995.

^e Tannin sorghum bran analyzed and used in similar research (Gordon 2001).

^f Typical analysis for dietary fiber; Pizzey's Milling (Angusville, MB, Canada).

^g Typical analysis; Cargill, Inc. (Minneapolis, MN).

TABLE IX

Particle Size Distribution of Sorghum and Rice Flours

US Screen #*	Percentage (%) of Sample Above Screen	
	Sorghum Flour	Rice Flour
80	<1	34.7
100	<1	9.3
120	93.3	44.2
140	<1	<1
Thru 230	4.1	5.6

*#80 screen = 177 microns, #100 screen = 149 microns, #120 screen = 125 microns, #140 screen = 105 microns, #230 screen = 63 microns

Bread Formula. The gluten-free composite flour was made up of sorghum flour, tapioca flour, corn starch, and potato starch (see footnote on Table X). Breads were baked using a straight-dough procedure (Table X). Sorghum bran, flax, and inulin were substituted for a percentage of the gluten-free composite flour. Water was adjusted for increasing levels of ingredient substitution.

TABLE X
Gluten-Free Bread Control Formula

Ingredient	Baker's %	Mass (g)
Gluten-free composite flour*	100	270
Xanthan gum	1.7	4.6
Unflavored gelatin	1.3	3.5
Salt	1.3	3.5
Sugar	7.4	20
Nonfat dry milk	6.6	17.8
Instant yeast	2.7	7.3
Eggs	26	70.2
Butter	8.5	23
Vinegar	1.4	3.8
Honey	7	18.9
Water	86	232.2
Total	249.9	675

*Composed of 30% sorghum flour, 30% tapioca flour, 30% cornstarch and 10% potato starch.

Bread Baking. Wet ingredients were combined in the bowl of a Hobart mixer (Hobart Mfg. Co., Troy, OH) and mixed until the butter was dispersed (Fig. 15). Dry

ingredients were then added and mixed for 30 sec on speed 1, 1 min on speed 2, and 1.5 min on speed 3. Three hundred grams (300 g) of batter was scaled into greased and floured pup loaf pans (bottom: 12.5 x 6.5 cm; top: 14 x 8 cm; height: 5.5 cm; volume: 520 cm³). The surface of the batter was smoothed with a wet spatula to create a uniformly flat surface, and the pans were placed in a proofing chamber (86°F, 99 % RH) (Piper Products Inc., Cudahy, WI) until the batter reached the top of the pan (approximately 60 min). The loaves were baked for 45 min at 375°F in a reel-type oven (Reed Oven Co., Kansas City, MO) using steam during baking. After baking, loaves were cooled on racks at room temperature 60 min before evaluation.

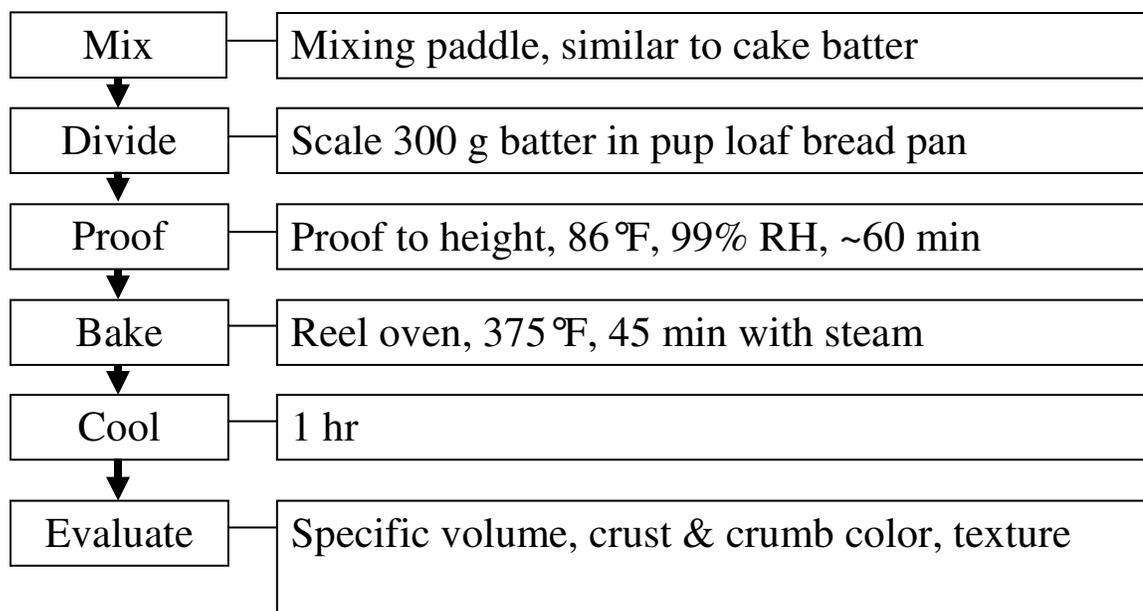


Fig. 15. Procedure for gluten-free bread baking.

Bread Quality Evaluation. Bread quality was evaluated by the same methods as described for the nutraceutical bread. When evaluating crumb firmness, only three replications per loaf were conducted due to the smaller size of the pup loaves.

Optimum Levels of Substitution. Optimum levels of substitution for sorghum bran, flax, and inulin were determined based on bread qualities (specific volume, crumb firmness, moisture, and color) and nutritional content to maximize dietary fiber, antioxidants, and omega-3 fatty acids.

Sensory Evaluation. Informal sensory evaluation was done by the researcher and colleagues.

Experimental Design

- 1) An acceptable base formula was created using a composite flour of sorghum flour, corn starch, tapioca flour, and potato starch (modified from Hagman 1999).
- 2) Using the base formula (Table X), sorghum bran, flax, and inulin were each substituted individually for 5% and 10% of the composite flour. Three replications of two loaves each were conducted. Effects on physical and nutritional properties of the gluten-free bread were evaluated.
- 3) Sorghum bran (2.5-5%), flax (2.5-5%), and inulin (5-10%) were combined in various ratios and were substituted for part of the composite flour in the base formula. Again, three replications of two loaves each were conducted. Bread quality was then evaluated based on specific volume, crumb firmness, moisture, and color. Nutritional content was also evaluated.

- 4) The optimum formula was compared to commercial gluten-free bread mixes based on bread quality and nutritional parameters.

Statistical Analysis

Statistical analysis was conducted by the same methods as described for the nutraceutical bread.

Results and Discussion

Development of Control Formula. In preliminary trials, two formulas were evaluated (Schober 2005 and Hagman 1999) to determine an acceptable control (Fig. 16). Schober's control formula was baked according to the procedures he described (2003). Two variations on this formula were also tested as described by Schober (2005): one with the addition of 1% xanthan gum and the other with the addition of 1% non-fat dry milk. A formula described by Hagman (1999) was also evaluated. A variation of Hagman's formula was tested by replacing rice flour with white sorghum flour. All three breads baked with Schober's formula were dense, hard, and had poor crust color with multiple surface cracks. The addition of 1% xanthan gum to Schober's bread reduced specific volume and resulted in poor quality bread with a bright white crust. The addition of 1% non-fat dry milk to Schober's bread resulted in increased crust browning but did not significantly improve bread quality. Based on specific volume, flavor, and subjective texture analysis, Hagman's formula was superior (2.85 cm³/g) over all other treatments. However, the variation on Hagman's formula containing sorghum flour in place of the rice flour was quite acceptable despite decreased specific

volume ($2.4 \text{ cm}^3/\text{g}$). Because one of the objectives of this project was to develop a gluten-free bread containing sorghum ingredients, this formula was chosen as the control for the remainder of the experiments.

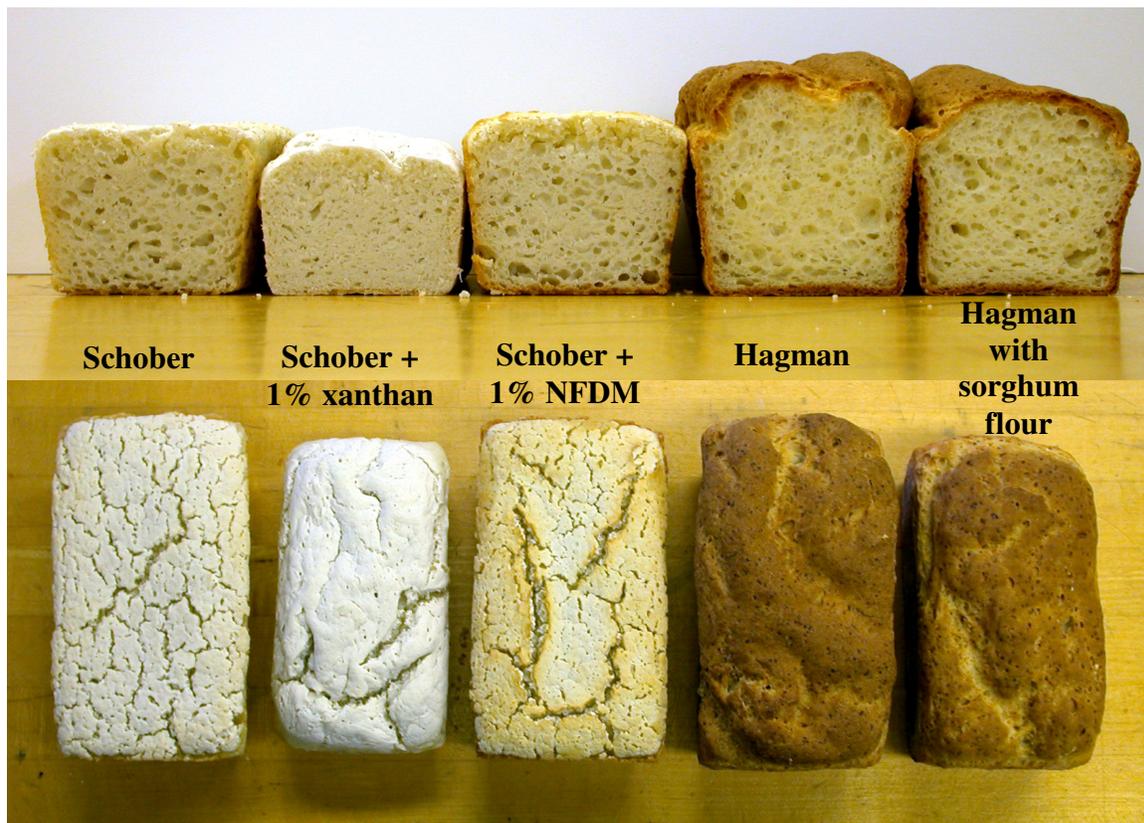


Fig. 16. Side and top view. Left to right: Schober control, Schober formula + 1% Xanthan gum, Schober formula + 1% nonfat dry milk, Hagman formula, and Hagman formula with sorghum flour instead of rice flour.

Fiber Substitution Trials. With the control formula established, the next phase of the project determined the effects of 5 and 10% substitution of sorghum bran, flax, and inulin individually for part of the composite flour (Fig. 17). Each ingredient had different nutritional and functional properties. The goal was to first determine the effect of each ingredient separately in the gluten-free bread and then combine them in an optimum ratio to provide the best quality bread with enhanced nutritional value. At the 5% substitution level, 86% water (composite flour basis) was used. At the 10% substitution level, it was necessary to increase water to 105% to achieve the same batter consistency (subjective evaluation) due to the increased absorptive capacity of the fiber-containing ingredients. Fig. 18 illustrates the differences in moisture of the baked breads.

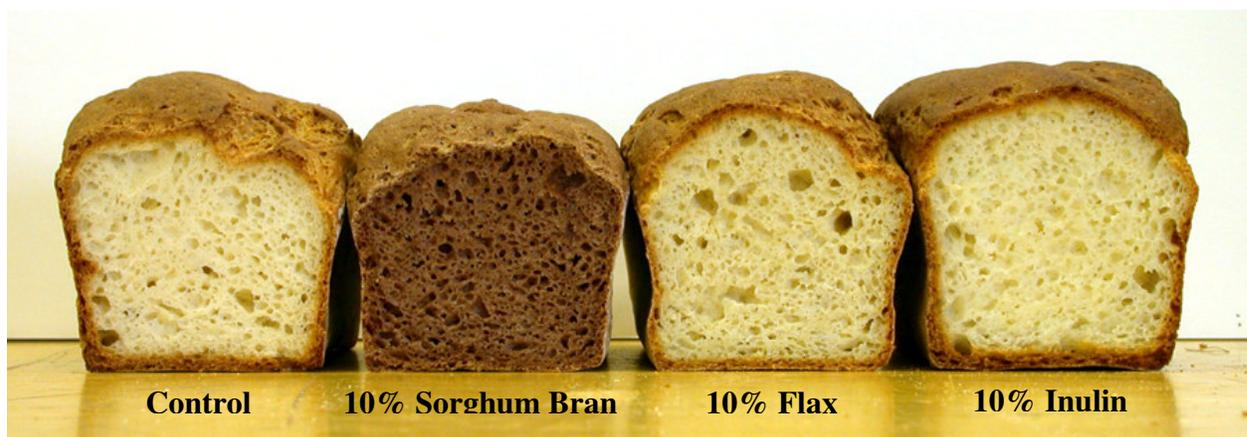


Fig. 17. Breads containing sorghum bran, flax, or inulin substituted for 10% of the composite flour.

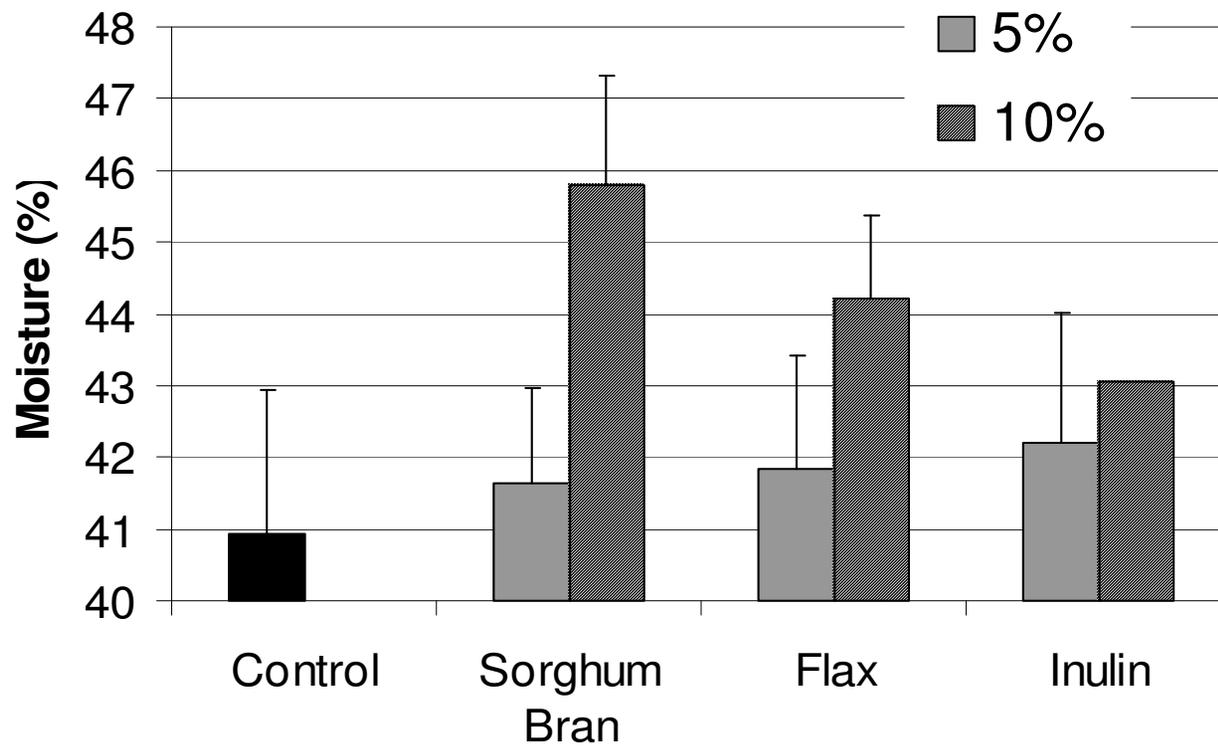


Fig. 18. Moisture of gluten-free breads containing 5% and 10% sorghum bran, flax, or inulin.

Effect on Specific Volume. Sorghum bran was substituted for 5% and 10% of the composite flour weight. As was observed by Rudiger (2003) in wheat based breads, the substitution of 5% sorghum bran decreased specific volume over the control (Fig. 19). However, at the 10% substitution level, specific volume was the same as the control. This observation was surprising, considering that increasing levels of sorghum bran substitution are known to decrease specific volume in wheat-based breads. In wheat breads, high levels of bran can act to dilute the gluten matrix and reduce the ability of bread to retain gas during baking (Rudiger 2003). However, in gluten-free bread, there is no gluten matrix development and the primary source of structural integrity in this formula comes from xanthan gum, eggs, and gelatin.

Flax was also substituted for 5% and 10% of the composite flour weight. At both 5 and 10% substitution levels, specific volume was increased over the control, although the greatest effect was seen at the 10% level. Flax contains ~27% dietary fiber and has been observed to increase specific volume in wheat bread. One third of the fiber in flax is soluble. The soluble fiber may have contributed gas-retaining properties to the gluten-free batter, resulting in greater specific volume.

Ten percent inulin substitution showed the greatest improvement in specific volume over the control compared to sorghum bran or flax at either level (Fig. 19). Five percent inulin improved specific volume over the control, but had less of an effect than the 10% level. Inulin contains ~90% fiber, all of which is soluble. The soluble

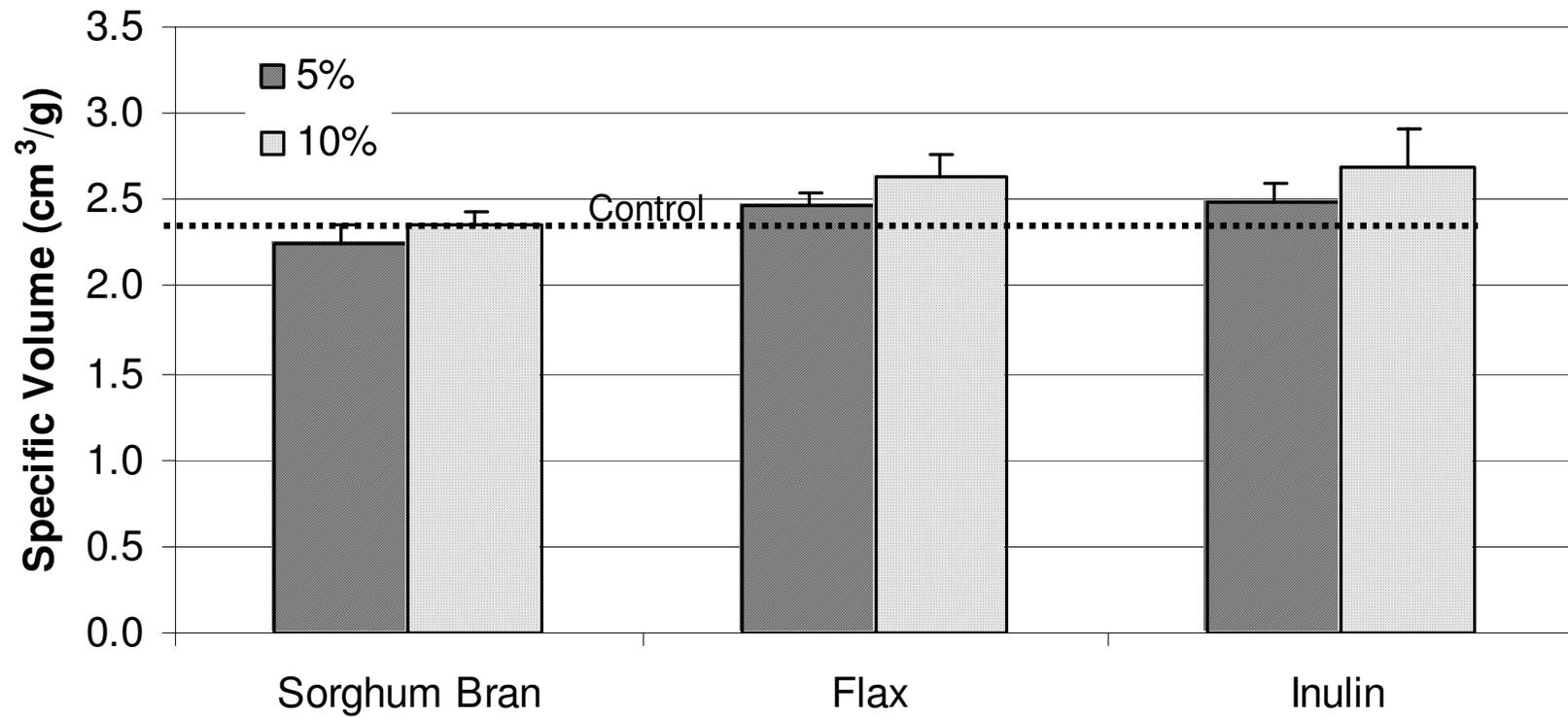


Fig. 19. Effect of 5% and 10% substitution of sorghum bran, flax, and inulin on specific volume of gluten-free bread. Control contains no sorghum bran, flax, or inulin (Table X).

fiber contributed by the inulin may have increased viscosity in the batter, which could have improved gas bubble retention, thereby improving loaf volume.

Effect on Crumb Firmness. In general, bread tends to stale during and after baking via retrogradation. In this process, amylose and especially amylopectin recrystallize, resulting in increased crumb firmness over time (Hosney 1994). The staling process occurs much more quickly in gluten-free breads than in wheat breads. As expected, the addition of 5% sorghum bran produced bread that was firmer than the control at both 2 and 24 h (Fig. 20). However, addition of 10% sorghum bran actually resulted in softer crumbs at both 2 and 24 h after baking.

The loaves containing 5% flax were softer than the control at both 2 and 24 h. However, the loaves containing 10% flax were significantly softer than both the control and the 5% loaves at 2 and 24 h. It is significant that any level of a fiber-containing ingredient reduced crumb firmness, and even more significant that the higher substitution level reduced crumb firmness more effectively.

The 10% inulin loaves were significantly softer than the loaves containing 5 or 10% sorghum bran or flax. At 2 h after baking, the 10% inulin loaves were much less firm than the control and only slightly firmer than white pan bread. At 24 h after baking, the 10% inulin loaf increased in firmness but was still the least firm of all the treatments and the control at 24 h.

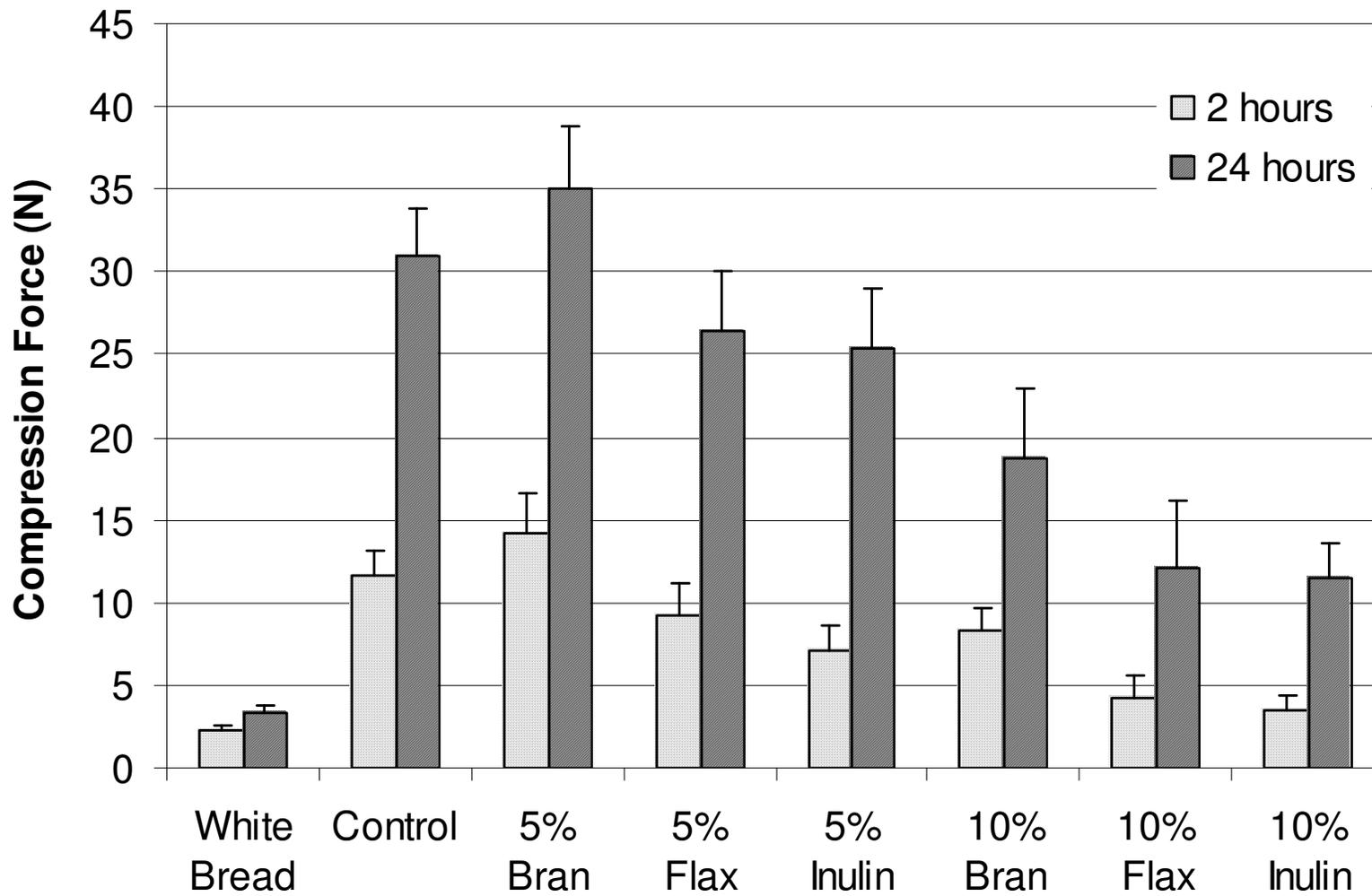


Fig. 20. Effect of 5% and 10% substitution of sorghum bran, flax, and inulin on crumb firmness at 2 and 24 hours.

Effect on Crust and Crumb Color. The most significant color difference in the breads containing sorghum bran was seen in the crumbs. At both 5 and 10% sorghum bran, the crumbs were significantly darker, with more red and blue tones than the control (Figs. 22 and 24). As was expected, the crumb of the 10% sorghum bran bread was darker than the crumb of the 5% sorghum bran bread. The color values for the crusts were not significantly different from the control (Figs. 21 and 23). This is likely because the Maillard browning that occurred in the crust of the breads masked any color differences contributed by the ingredients.

There was little difference between the 5% flax loaves and the control with regard to crust and crumb color. At 10% substitution, both the crust and crumb L values were lower than the control, indicating a darker color.

At the 5% substitution level of inulin, there was no significant difference in crust or crumb color from the control. At the 10% substitution level, the crust was darker than the control and the crumb was lighter. The darker crust was likely a result of increased Maillard browning caused by an increased availability of reducing sugars present in inulin. The 10% inulin loaf contained more fiber than any of the other treatments but was comparable in color to white pan bread.

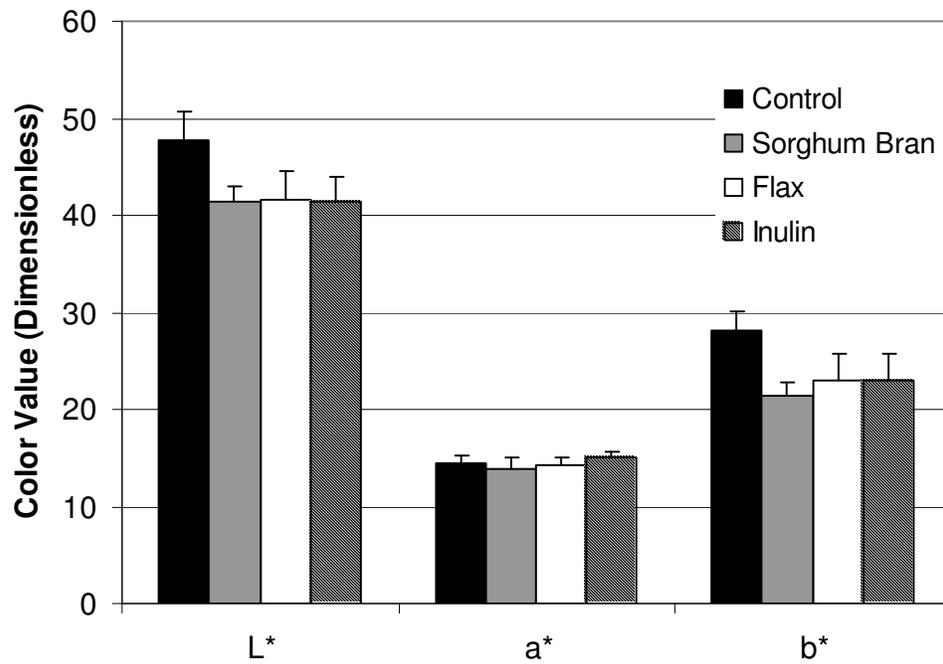


Fig. 21. Lightness and color of gluten-free bread crusts containing 5% sorghum bran, flax, or inulin.

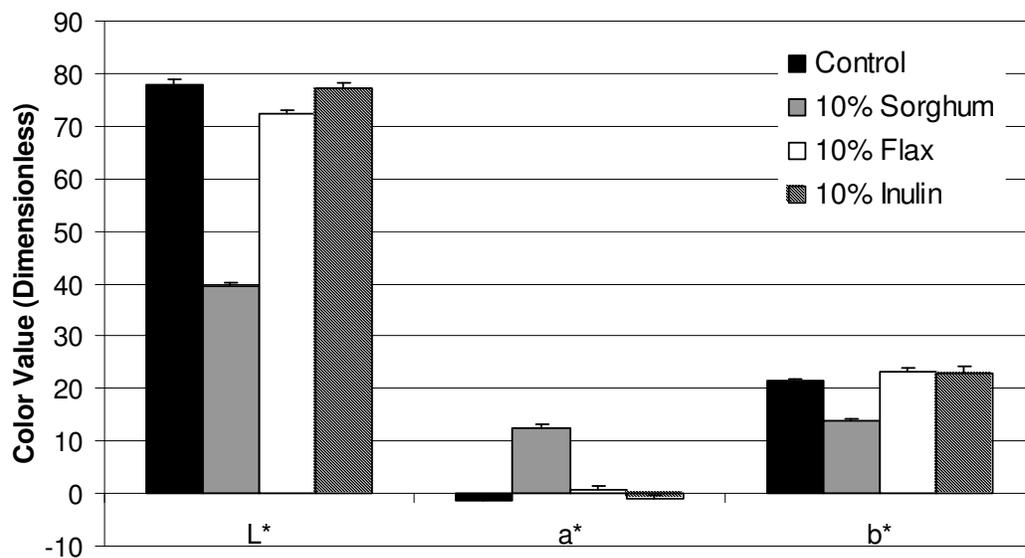


Fig. 22. Lightness and color of gluten-free bread crumbs containing 5% sorghum bran, flax, or inulin.

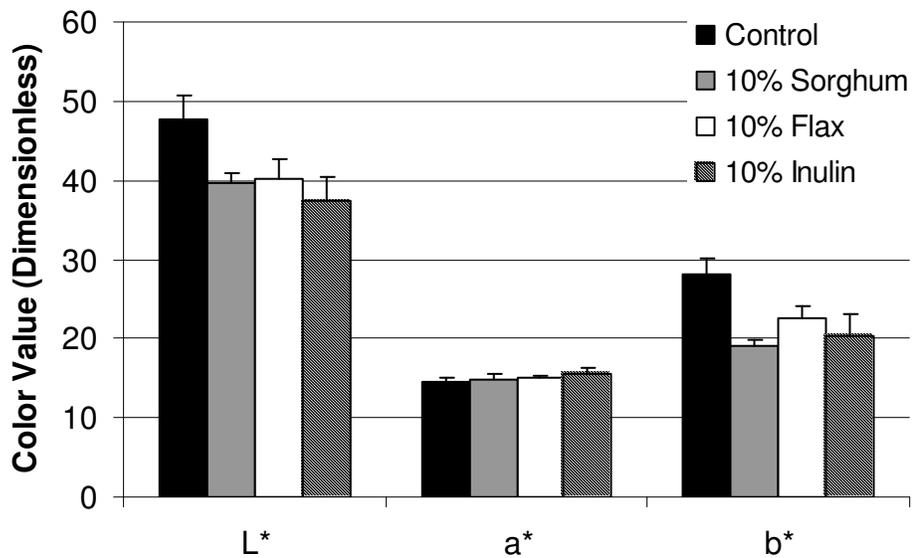


Fig. 23. Lightness and color of gluten-free bread crusts containing 10% sorghum bran, flax, or inulin.

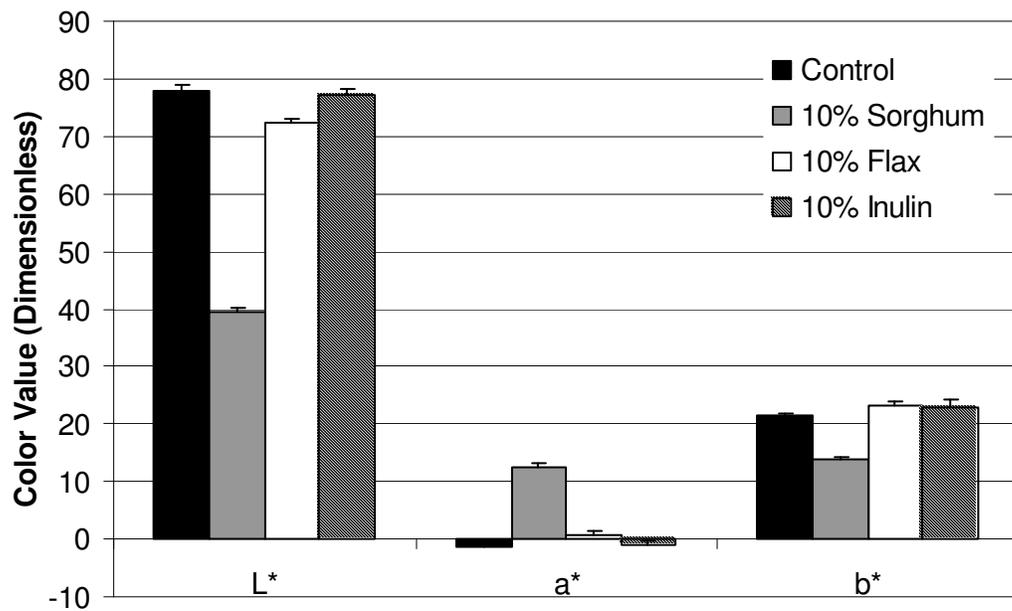


Fig. 24. Lightness and color of gluten-free bread crumbs containing 10% sorghum bran, flax, or inulin.

Dietary Fiber Content of Breads. The dietary fiber content of each formula was calculated for a standard 56 g serving using data from proximate compositions of raw materials (Table XI). The control formula contained 1.2 g dietary fiber per slice, similar to many commercially available gluten-free breads. Sorghum bran contained 45% dietary fiber, while flax and inulin contained 28% and 90% fiber, respectively. Therefore, when compared to equal levels of sorghum bran or flax substitution, formulations that contained inulin provided more dietary fiber. The 10% inulin bread provided the highest level of dietary fiber, with 3.1 g/56 g serving. ORAC was calculated using data on brown sorghum bran assuming 60% retention of antioxidant activity in bread after baking as reported by Awika (2003). Breads containing flaxseed provided omega-3 fatty acids, while those containing sorghum bran provided high levels of antioxidants.

TABLE XI
Dietary Fiber, Alpha-Linolenic Acid, and ORAC Content of 56 g Serving of Bread Containing 5 and 10% Sorghum Bran, Flax, or Inulin

Treatment	Dietary Fiber (g)	ALA (mg)	ORAC μ mol TE (from sorghum bran)
Control	1.2	0	0
5% Sorghum Bran	1.7	0	355
5% Flax	1.5	288	0
5% Inulin	2.3	0	0
10% Sorghum Bran	2.1	0	656
10% Flax	1.6	527	0
10% Inulin	3.1	0	0

Comparison of Sorghum Bran, Flax, and Inulin Substitution in Gluten-Free Bread. Of all the treatments evaluated, the 10% inulin loaf was superior in specific volume and crumb firmness. At 2 h after baking, its crumb firmness was comparable to that of white pan bread and staled at a much slower rate than any of the other treatments. It had the highest loaf volume, and its crumb color was similar to that of white pan bread. In addition, it contained far more dietary fiber than any of the other treatments. This treatment was judged best by an informal sensory panel.

Combining Sorghum Bran, Flax, and Inulin in Gluten-Free Bread. After determining the effects of each ingredient at 5 and 10% substitution levels, sorghum bran, flax, and inulin were combined in different ratios with the goal of optimizing nutritional benefits while minimizing quality losses. It was hypothesized that because inulin produced a loaf with increased specific volume, it might be able to “carry” other nutritionally desirable ingredients that were known to have less desirable effects on loaf volume, thereby producing a superior bread. Inulin was substituted at 5-10% (composite flour basis), and flax and sorghum bran were added at 2.5-5% (Fig. 25). Table XII outlines the percentages of sorghum bran, flax, and inulin used in the four combination loaves. The control was the modified Hagman formula containing sorghum flour in place of rice flour.

TABLE XII
Ingredient Substitution Levels of Gluten-Free Combination Loaves

Treatment	% Sorghum Bran	% Flax	% Inulin
Control*	0	0	0
A	2.5	2.5	5
B	5	5	5
C	2.5	2.5	10
D	5	5	10

* Modified Hagman formula (Table X)

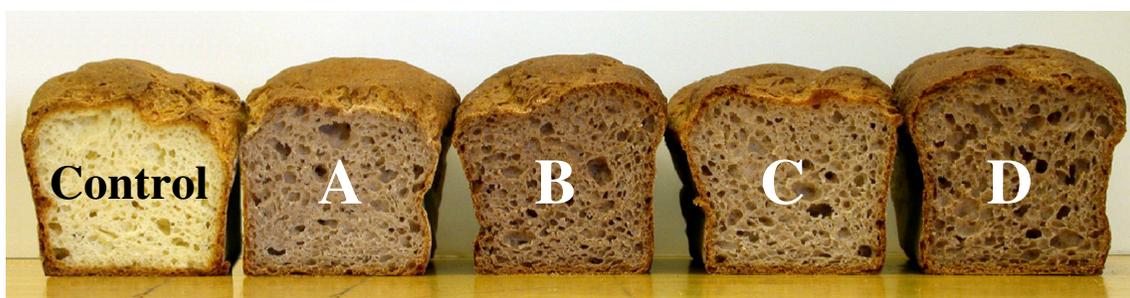


Fig. 25. Photograph of gluten-free breads containing combinations of sorghum bran, flax, and inulin. A = 2.5% bran, 2.5% flax, and 5% inulin. B = 5% bran, 5% flax, and 5% inulin. C = 2.5% bran, 2.5% flax, and 10% inulin. D = 5% bran, 5% flax, and 10% inulin.

Specific Volume. As expected, all of the combination loaves resulted in lower specific volumes than the 10% inulin loaf (Fig. 26). However, all were greater than or equal to the specific volume of the control ($2.4 \text{ cm}^3/\text{g}$). The specific volume of loaf C (2.5% sorghum bran, 2.5% flax, and 10% inulin) was only slightly lower than the 10% inulin loaf. This difference was not statistically significant. When compared to equal levels of sorghum bran and flax substitution, the loaves containing 10% inulin (loaves C and D) had higher specific volumes than the loaves containing 5% inulin (loaves A and

B). This was seen previously in the single-ingredient substitution of inulin, where higher levels of inulin resulted in increased specific volume. It is notable that even loaf D, which contained a combined total of 20% sorghum bran, flax, and inulin, did not have a significantly lower specific volume than the 10% inulin loaf.

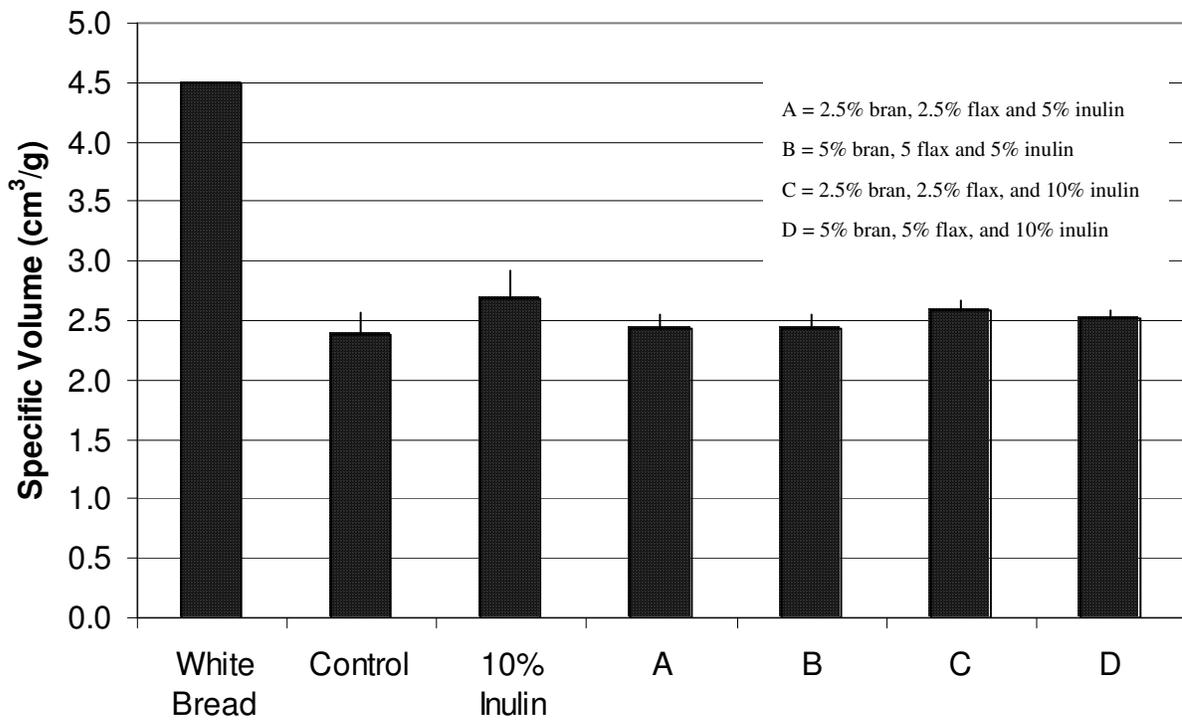


Fig. 26. Effect of sorghum bran, flax, and inulin combinations on specific volume of gluten-free breads.

Crumb Firmness. All of the combination loaves were slightly firmer than the 10% inulin loaf 2 h after baking, but all were less firm than the control (Fig. 27). Compared to the 10% inulin loaf, Loaf D (10% inulin, 5% flax, and 5% sorghum bran) resulted in a slightly firmer crumb 2 hours after baking, but was softer than the 10% inulin loaf 24 hours after baking. Of the four combination loaves, treatments C and D were the softest at both 2 and 24 hours after baking and were comparable to the 10% inulin loaf. Again, when comparing equal amounts of sorghum bran and flax substitution, the loaves containing 10% inulin were softer than those containing 5% inulin at both 2 and 24 h after baking. These results indicate that up to 5% each of sorghum bran and flax can be added to a gluten-free loaf containing 10% inulin without significantly affecting crumb firmness. This resulted in improved sensory quality, as well as increased levels of dietary fiber, antioxidants, and omega-3 fatty acids.

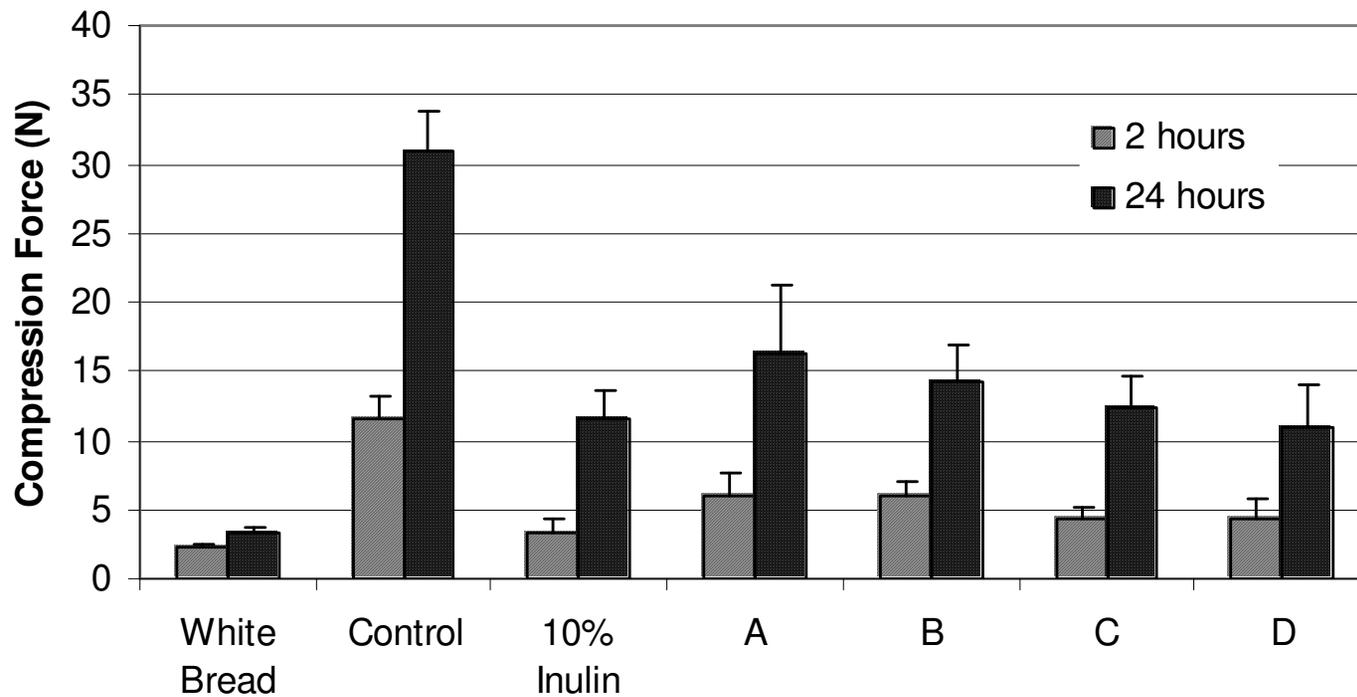


Fig. 27. Effect of sorghum bran, flax, and inulin combinations on crumb firmness of gluten-free bread at 2 and 24 h after baking. A = 2.5% bran, 2.5% flax and 5% inulin, B = 5% bran, 5 flax and 5% inulin, C = 2.5% bran, 2.5% flax, and 10% inulin, D = 5% bran, 5% flax, and 10% inulin.

Crust and Crumb Color. The lowest “L” value (indicating the darkest color) was seen in the crust of bread D (10% inulin, 5% flax, and 5% sorghum bran) (Fig. 28). This bread contained the highest level of sorghum bran substitution (5%), which was the darkest ingredient used. In addition, this loaf contained the highest amount of inulin (10%), which contained 10% mono- and disaccharides. These sugars likely contributed to increased Maillard browning. Consumers tend to identify dark-colored breads as more “healthy” products, which would be appropriate for this bread considering the nutraceutical ingredients it contains.

The lightest crust (highest “L” value) was observed in bread A (2.5% sorghum bran, 2.5% flax, and 5% inulin). This bread contained the least amount of sorghum bran, which contributed to its lighter color. Also, it had a lower level of inulin substitution (5%), which likely resulted in less Maillard browning. This bread’s crust was lighter than the loaf containing 10% inulin with no flax or sorghum bran. Even with the addition of 2.5% flax and 2.5% sorghum bran, the reduced level of inulin (5%) in loaf A resulted in less Maillard browning that produced an overall lighter crust.

All of the crusts had very similar “a” values, indicating a color that was more red than green. Loaf D (5% sorghum bran, 5% flax, and 10% inulin) had the lowest “b” value, indicating a more blue-colored crust. Loaf A (2.5% sorghum bran, 2.5% flax, and 5% inulin) had the highest “b” value, indicating a more yellow-colored crust.

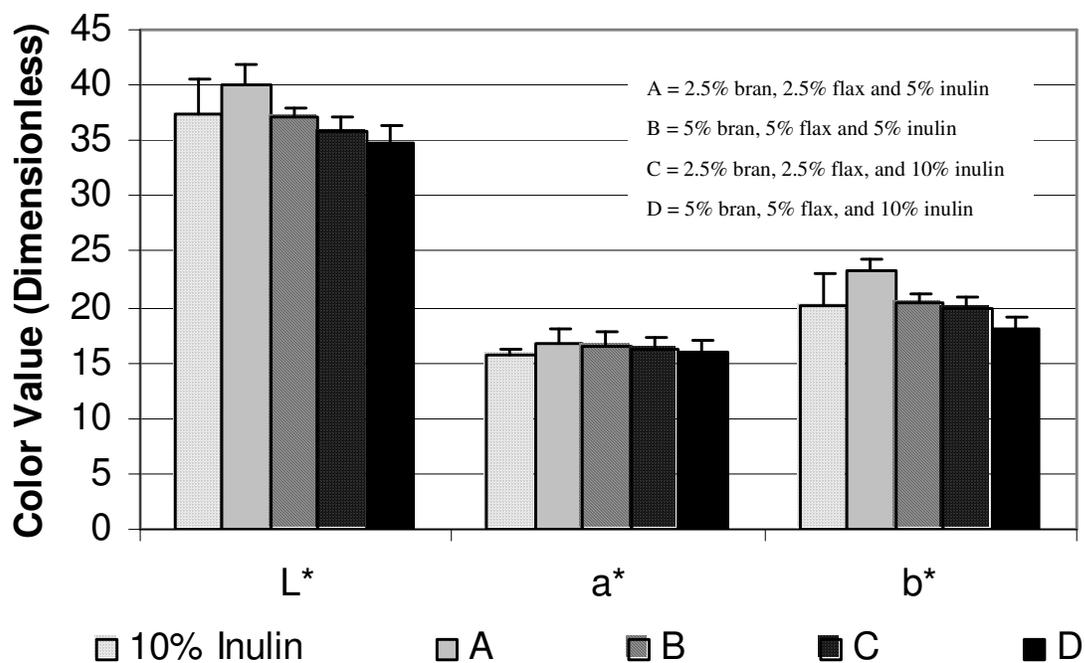


Fig. 28. Lightness and color of gluten-free bread crusts containing combinations of sorghum bran, flax, and inulin.

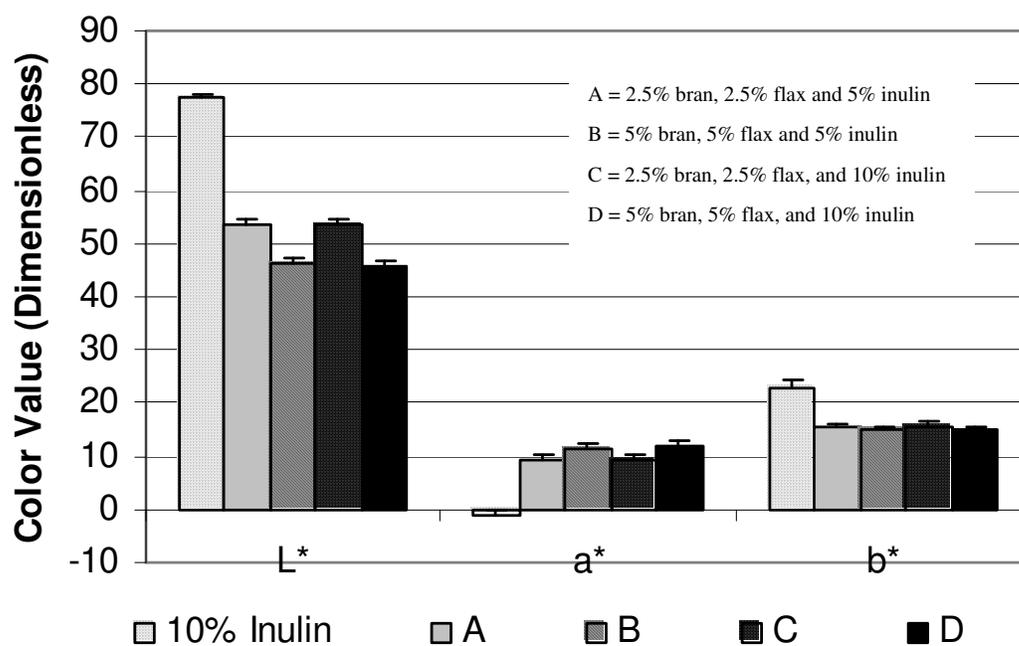


Fig. 29. Lightness and color of gluten-free bread crumbs containing combinations of sorghum bran, flax, and inulin.

More variation between samples was seen in the crumb color of the combination loaves than in their crusts (Fig. 29). With regards to “L” value, two groupings were seen. The lightest crumbs (highest “L” values) were in the breads containing 2.5% flax and 2.5% sorghum bran (loaves A and C), regardless of their level of inulin substitution. The darkest crumbs (lowest “L” values) were seen in the breads containing 5% flax and 5% sorghum bran (loaves B and D), regardless of their level of inulin substitution. This was to be expected, as sorghum bran and flax are dark colored ingredients. Inulin substitution did not have a noticeable effect on the crumb color as it is white in color and Maillard browning did not occur in the crumb. This same stratification was observed with regards to “a” value. Loaves that contained 5% bran and 5% flax (loaves B and D) were slightly redder (higher “a” values) than those that contained 2.5% bran and 2.5% flax (loaves A and C). Very little variation was seen in any of the “b” values measured in the combination loaves, indicating a crumb color that was consistently more yellow than blue.

Moisture Content of Combination Breads. All loaves were baked using 105% water (flour basis). The final moisture contents of all the combination loaves baked were similar, and no significant differences were seen. (Fig. 30).

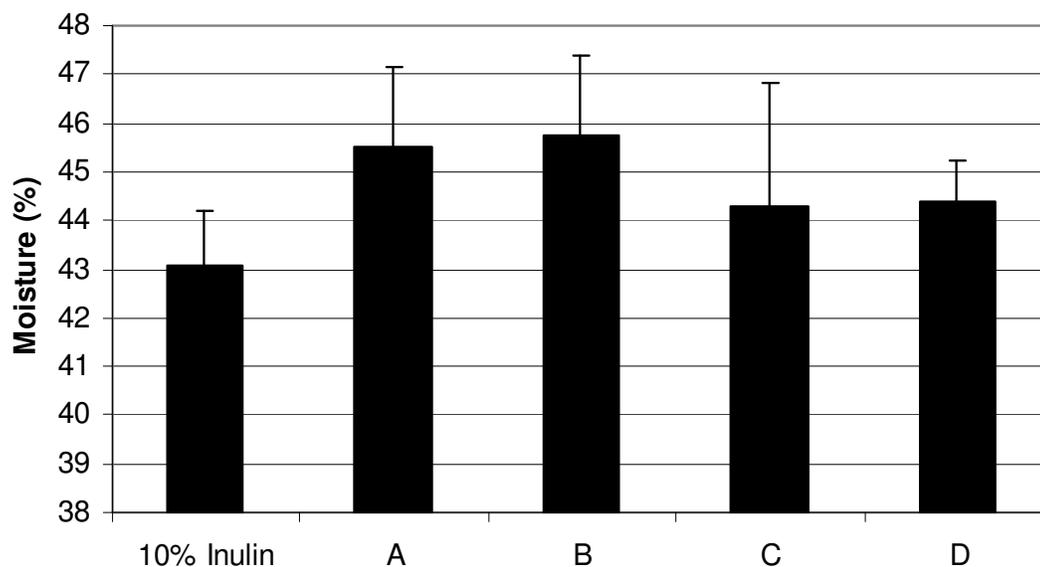


Fig. 30. Moisture levels of gluten-free breads containing combinations of sorghum bran, flax, and inulin. A = 2.5% bran, 2.5% flax, and 5% inulin. B = 5% bran, 5% flax, and 5% inulin. C = 2.5% bran, 2.5% flax, and 10% inulin. D = 5% bran, 5% flax, and 10% inulin.

Nutritional Content of Gluten-Free Combination Breads. Dietary fiber levels of gluten-free combination loaves were calculated based on known fiber contents of bread ingredients. Due to the high cost of dietary fiber analysis, only Bread C was analyzed for dietary fiber using the Prosky method. Data on actual dietary fiber for the remaining breads were extrapolated based on the ratio of expected versus actual dietary fiber of bread C. Calculated values of the combination breads ranged from 2.1 to 3.8 g per serving (Table XIII). However, actual dietary fiber content of the breads was lower, ranging from 1.0 to 1.7 g per serving. Bread A, with 2.5% sorghum bran, 2.5% flax, and 5% inulin contained the lowest level of dietary fiber per serving. This was expected, as

it had the lowest total percentage of sorghum bran, flax, and inulin substitution (10% total). Bread D, which contained 5% sorghum bran, 5% flax, and 10% inulin contained the highest level of dietary fiber. This was also expected, as 20% of bread D's gluten-free composite flour was replaced by fiber-containing sorghum bran, flax, and inulin. One slice of bread D provided 1.7 g dietary fiber per slice. Based on the FDA's food labeling rules, this value would be rounded up to 2 g per serving on the nutrition facts panel. This level is comparable to the amount of dietary fiber found in commercial gluten-free breads evaluated, which typically contained 2-3 g per serving.

ORAC values ($\mu\text{mol TE/serving}$) were calculated based on the ORAC of brown sorghum bran, assuming a 60% retention of antioxidant activity after baking, as reported by Awika (2003). Breads B and D contained 5% brown sorghum bran and therefore provided the highest levels of antioxidants (1882 $\mu\text{mol TE/serving}$). Breads A and C contained 2.5% sorghum bran and provided only about half the antioxidants per serving (941 $\mu\text{mol TE}$) as breads B and D. Prior and Cao (2000) estimated that antioxidant intakes of 3000-3600 $\mu\text{mol TE}$ per day would be necessary to produce health benefits associated with fruit and vegetable consumption. Two slices of bread B or D would exceed these antioxidant requirements.

Breads A and C contained 2.5% flaxseed and provided 144 mg omega-3 fatty acids in the form of alpha-linolenic acid. Breads B and D provided higher levels of ALA (287 mg/serving) as they contained 5% flaxseed. Two (56 g) slices of bread B or D would meet the lower end of the estimated required intake to reduce the risk of CVD mortality.

TABLE XIII**Nutritional Content of Gluten-Free Combination Breads (per 56 g serving)**

Bread	Calculated Dietary Fiber (g) ^a	Actual Dietary Fiber (g) ^b	ORAC (μmol TE)	Alpha-Linolenic Acid (mg)
A	2.1	1.0	941	144
B	2.6	1.2	1882	287
C	3.3	1.5	941	144
D	3.8	1.7	1882	287

^aBased on dietary fiber contents of sorghum bran, flax, inulin, and xanthan gum.

^bBread C analyzed for dietary fiber content. Actual dietary fiber content of breads A, B, and D extrapolated from data on bread C.

Selection of the Best Formula. Bread D was superior to the other formulas with regards to nutritional value. It contained the highest levels of dietary fiber and alpha-linolenic acid, and also provided the highest ORAC value per serving.

With regards to bread quality, bread D possessed the softest crumb 24 hours after baking. Considering that staling is a major problem in gluten-free breads, it was important to choose a formula where crumb firming after baking was minimized. The specific volume of bread D was also improved over the control. Both the crust and crumb of bread D were dark colored. This could be an advantage with consumers, who associate dark colored bread with a healthy product. Bread D was therefore chosen as the Best Formula and is compared with commercially available gluten-free breads in the following section.

Comparison with Commercial Breads. Seven commercially available gluten-free bread mixes were purchased from HEB Grocery Company and Whole Foods Market (Table XIV, Fig. 31 and Fig. 32). Because gluten-free breads stale quickly, it would not

have been reasonable to compare store-bought bread that had been prepared days earlier to the freshly baked gluten-free bread. Therefore, gluten-free bread mixes were baked in TR800 Breadman Plus automatic bread machines (Salton/MAXIM Housewares, Inc., Mt. Prospect, IL) and evaluated based on the same parameters as the experimental gluten-free bread. Breads were baked using the “rapid cycle” and “medium crust” settings. Results were compared to existing data for the Best Formula (loaf D; 5% sorghum bran, 5% flax, and 10% inulin), which was baked in a reel oven as described in the materials and methods section. Based on informal sensory evaluation by the researcher and colleagues, the “Favorite Sandwich Bread Mix” and “Tom’s Light Gluten-Free Bread” were the best commercially available breads with regards to texture, flavor, and overall acceptability.

TABLE XIV

Commercial Gluten-Free Bread Mixes Evaluated

Product Name	Brand
Homestyle White Bread Mix	Cause You're Special
Favorite Sandwich Bread Mix	Gluten Free Pantry
Bread Mix - Home Style	Authentic Foods
Whole Grain Bread Mix	Gluten Free Pantry
Multi-Grain Bread with Seeds	Gluten Free Pantry
Tom's Light Gluten-Free Bread Mix	Gluten Free Pantry
Homemade Wonderful GF Bread Mix	Bob's Red Mill

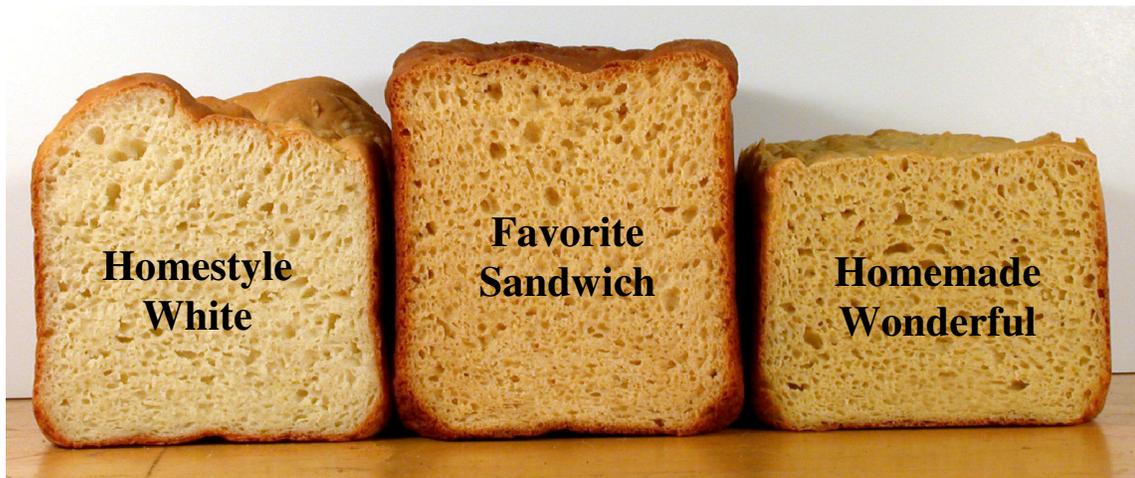


Fig. 31. Commercial gluten-free breads baked from mixes in automatic bread machines.

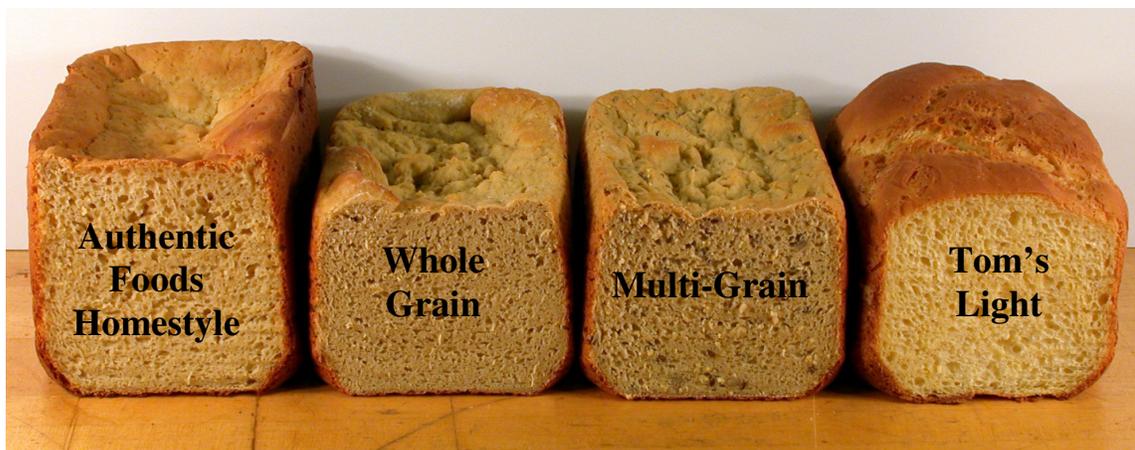


Fig. 32. Additional commercial gluten-free breads baked from mixes in automatic bread machines.

Specific Volume. The commercial gluten-free breads had specific volumes ranging from 1.7 cm³/g for the Multi-Grain and Whole Grain products to 3.0 cm³/g for Tom's Light Gluten-Free Bread (Fig. 33). In general, whole grain products are expected to have lower specific volumes than their refined grain counterparts. There was little difference in the dietary fiber levels of these breads (2-3 per serving), even though large differences in specific volume were seen. The Best Formula (bread D, containing 5% sorghum bran, 5% flax, and 10% inulin) had a specific volume of 2.5 cm³/g. This falls within the range of specific volumes of breads currently on the market. In fact, Tom's Light gluten-free bread was the only commercially available bread with a specific volume greater than that of bread D.

Crumb Firmness. There was a large amount of variation in crumb firmness among the commercially available breads (Fig. 34). Tom's Light Gluten-Free Bread had the softest crumbs at both 2 and 24 hours after baking (5.4 N, 17.1 N). The Whole Grain bread had the firmest crumb 2 h after baking (27.4 N). The Homemade Wonderful Bread was the firmest 24 h after baking (43.2 N), indicating a product that staled very quickly. The Best Formula (loaf D, containing 5% sorghum bran, 5% flax, and 10% inulin) produced bread with a much softer crumb than any commercially available product evaluated (4.3 N 2 h after baking and 10.8 N 24 h after baking). In comparison to white pan bread containing wheat flour, the Best Formula was about two times more firm 2 hours after baking and about 3 times more firm 24 hours after baking. Rapid staling is a major problem in gluten-free breads and severely limits the acceptable shelf

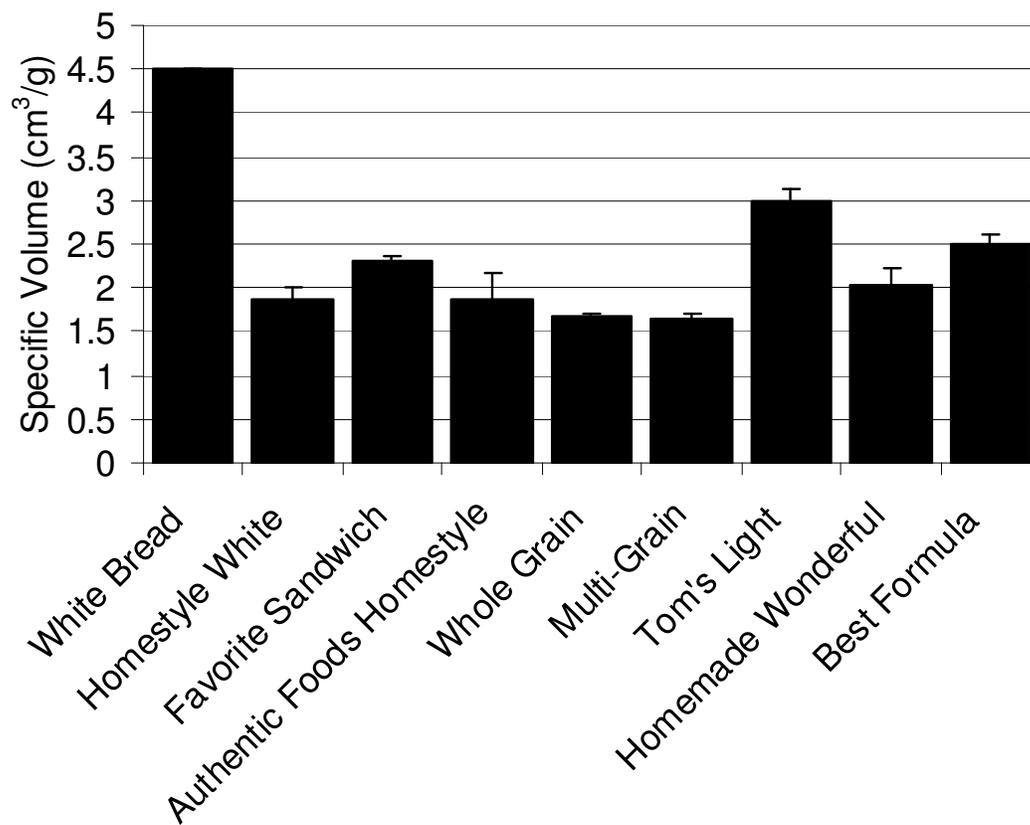


Fig. 33. Specific volume of commercial gluten-free breads compared to the Best Formula, containing 5% sorghum bran, 5% flax, and 10% inulin.

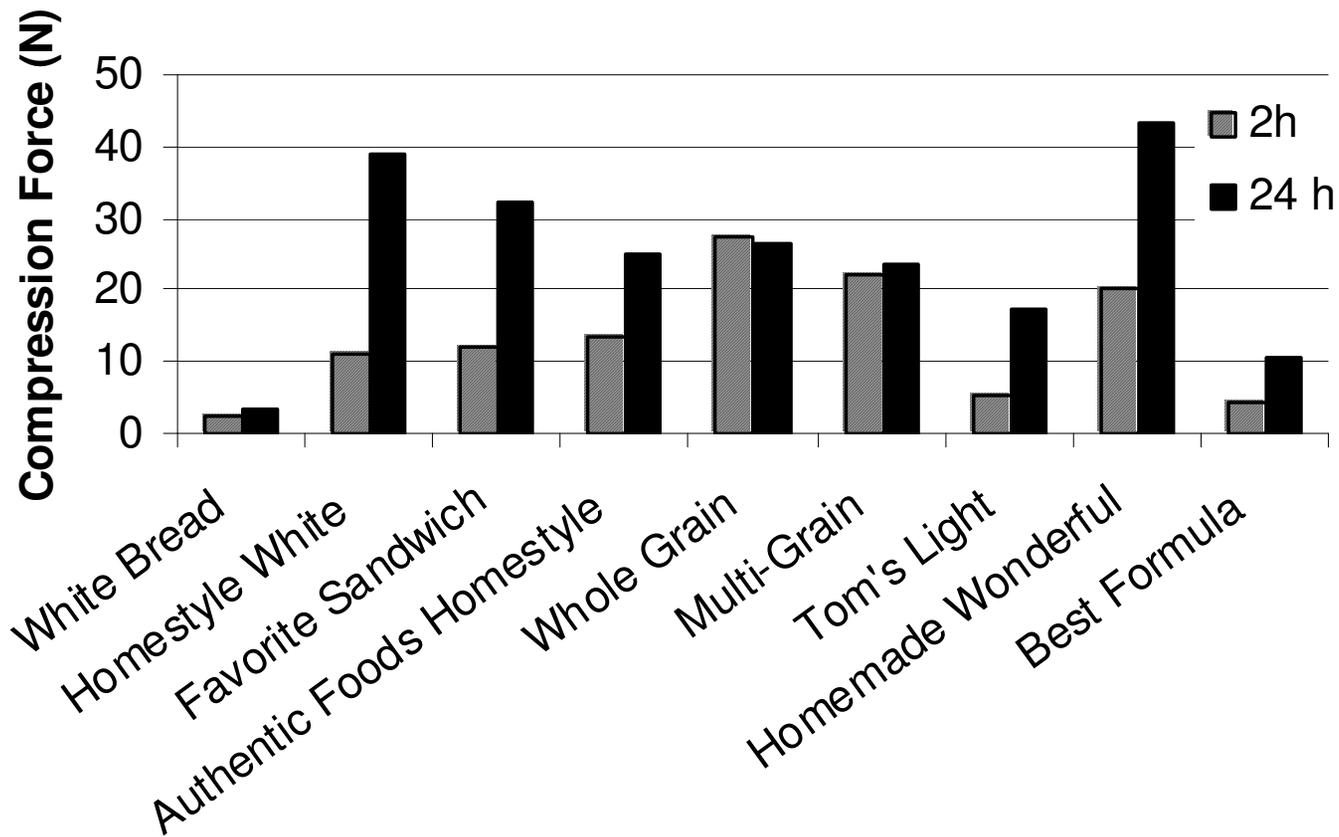


Fig. 34. Crumb firmness of commercial gluten-free breads compared to the Best Formula containing 5% sorghum bran, 5% flax, and 10% inulin.

life of these products once baked. The Best Formula was significantly softer 24 hours after baking and the texture of this “day old” bread would be more acceptable than any of the commercially available breads.

Color. The crust and crumb colors of the Best Formula were significantly different than all of the commercial products evaluated. All of the commercial breads baked were fairly light in color (high “L” values) with mostly yellow hues (high “b” values). The Best Formula had the darkest crust and crumb colors, with the lowest “L” values overall (Figs. 35 and 36). Even the Whole Grain and Multi-Grain breads that are generally expected to be darker in color were not as dark as the Best Formula. Dark colored breads are typically expected to be more “healthy” products and could be a visual indication to consumers of their high fiber and antioxidant content. The Best Formula also had much higher “a” values for both crust and crumb, indicating a product that was significantly more red than green when compared to the commercial products. The Best Formula had the lowest “b” values in both crust and crumb, indicating a color that was significantly bluer than any of the other commercial breads. These color differences could be an advantage when competing with other commercially available gluten-free breads as they may serve to differentiate between “white bread” types of gluten-free breads and the Best Formula, which contains nutraceutical ingredients and dietary fiber.

Moisture. The moisture content of the commercial gluten-free breads ranged from 40.6% for Tom's Light Gluten-Free Bread to 52.2% for the Whole Grain bread (Fig. 37). Tom's Light bread (40.6%) and the Favorite Sandwich bread (44.4%) tended toward the lower end of the range and were judged best overall. The Whole-Grain and Multi-Grain breads were at the upper end of the range for moisture (52.2% and 50.6% respectively) and were judged worst overall as they were significantly more dense with a sticky texture. The Best Formula had 44.4% moisture, which was consistent with the better commercial breads. Overall, the gluten-free breads had significantly higher moisture levels than whole wheat bread, which has a moisture content of about 38% (Gordon 2003).

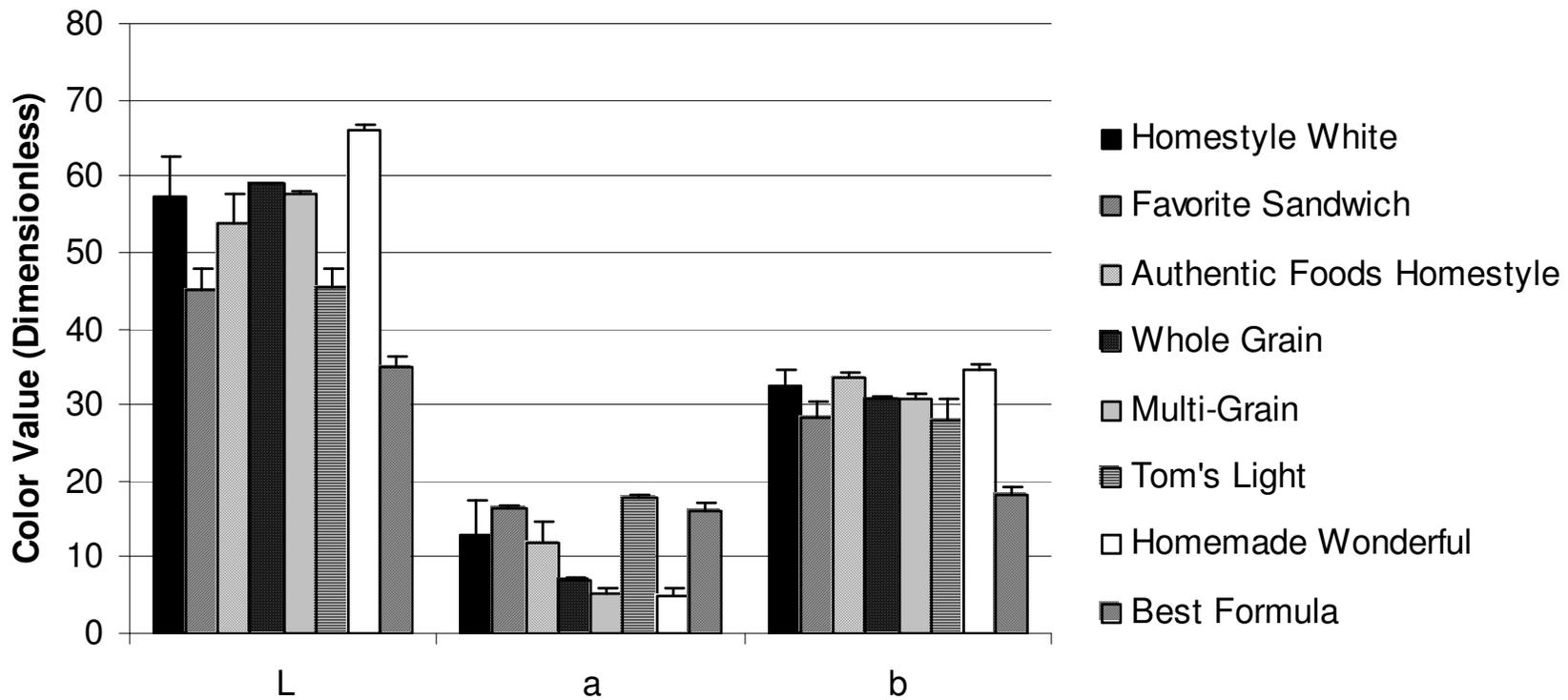


Fig. 35. Comparison of crust color of commercial gluten-free breads to the Best Formula containing 5% sorghum bran, 5% flax, and 10% inulin.

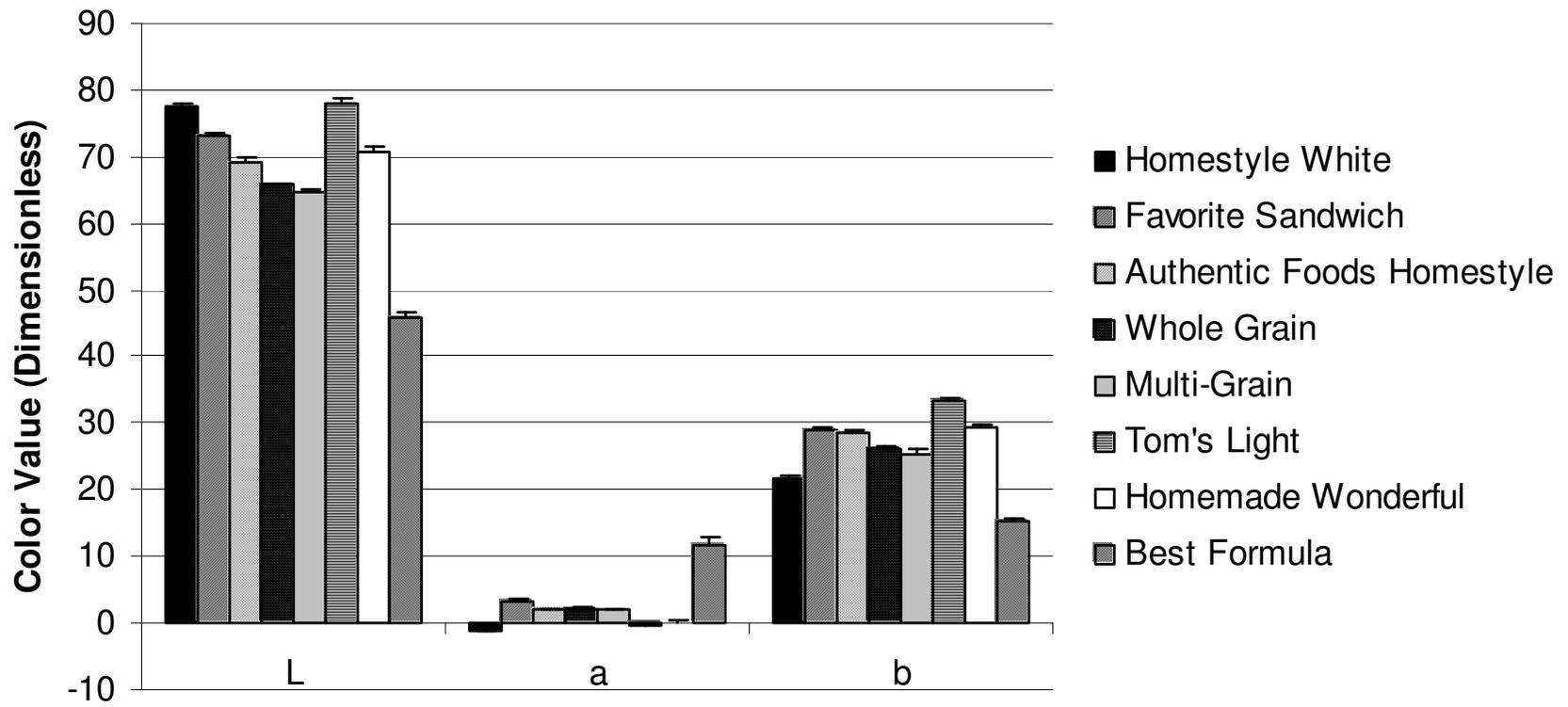


Fig. 36. Crumb color of commercial gluten-free breads compared to the Best Formula, containing 5% sorghum bran, 5% flax, and 10% inulin.

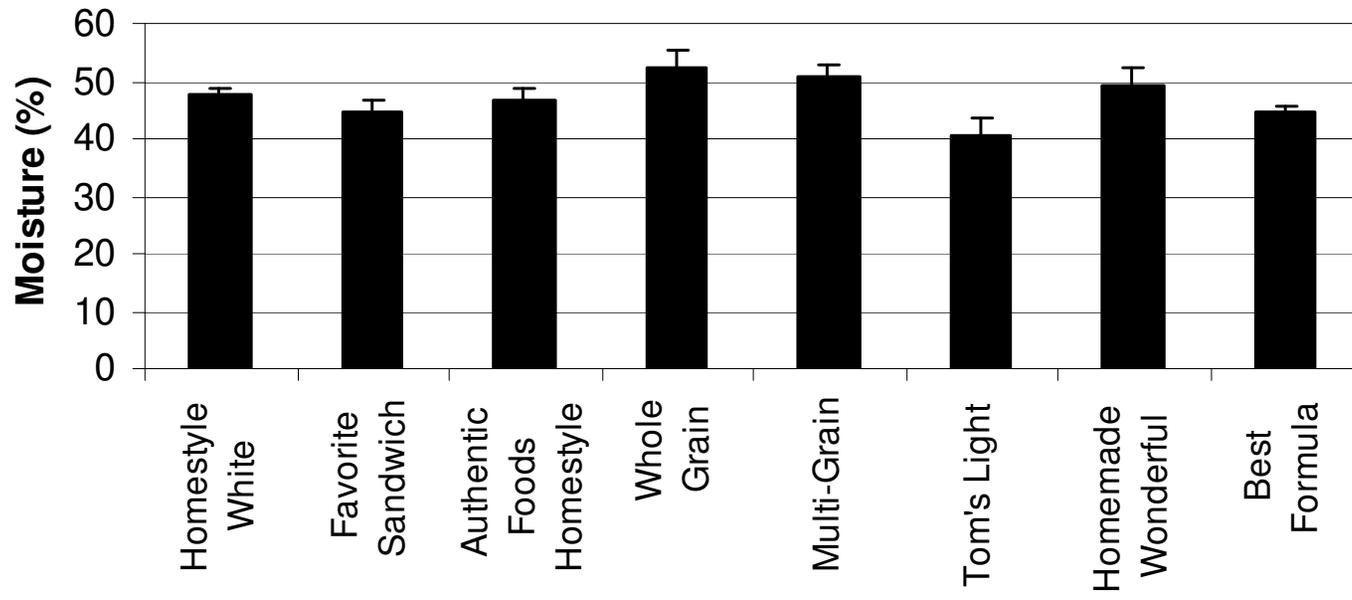


Fig. 37. Moisture content of commercial gluten-free breads compared to the Best Formula, containing 5% sorghum bran, 5% flax, and 10% inulin.

Mixing Characteristics and Crumb Grain. Due to the lack of gluten network formation, most gluten-free breads do not form a dough ball during mixing and more closely resemble cake batter in consistency. All of the commercial bread mixes resembled cake batter during mixing except for Tom's Light bread and the Homemade Wonderful bread. These formed wet, sticky balls in the bread machine that more closely resembled wheat bread dough. When the Best Formula was baked in the bread machine, it also formed a "dough ball" (Fig. 38). This may indicate that enough network formation occurred through the addition of gums, proteins, etc. to produce a cohesive ball during mixing. Only preliminary trials were conducted to bake the experimental gluten-free bread in a bread machine as opposed to the reel oven. While the appearance of the batter during mixing was encouraging due to the dough ball formation, the crust collapsed in the finished product (Fig. 39). More research needs to be done to make this product acceptable for use in automatic bread machines.

From visual inspection of the commercial gluten-free breads (Figs. 31 and 32) compared to the Best Formula (bread D in Fig. 25) it can be observed that the crumb grain of the commercial breads is tighter with fewer holes than that of the Best Formula. The use of emulsifiers and enzymes was not studied in this work and could possibly improve the crumb grain of the experimental formula.



Fig. 38. Photograph of the dough ball formed by bread C (2.5% sorghum bran, 2.5% flax, and 10% inulin) during mixing in the automatic bread machine.



Fig. 39. Photograph of bread containing 2.5% sorghum bran, 2.5% flax, and 10% inulin (Bread C) after baking in the automatic bread machine.

Ingredients of Commercial Gluten-Free Breads. The commercial gluten-free breads contained a wide variety of flours, including sorghum, rice, tapioca, potato, soy, fava, garbanzo, buckwheat, and corn starch (Table XV). With regards to gums, the commercial mixes were evenly divided between xanthan and guar gum. Three breads contained guar gum, three breads contained xanthan gum, and one bread contained both. In addition to the gums, eggs were a prevalent ingredient added by the consumer that contributed to network formation in the bread. Yeast was the leavening agent for all breads. The only bread that contained added bran was the Home Style bread mix, which contained rice bran. The Multi-Grain bread mix did contain whole millet, flaxseed, and sunflower seeds which contributed additional dietary fiber.

Nutritional Analysis. Serving size varied among the commercial gluten-free breads from 28 g to 71 g of dry mix per serving. For the majority of the breads, the serving size was about 30 g of dry mix. Because this was the most common serving size seen in commercial breads, nutritional information was standardized to 30 g of dry mix for all products (Table XVI). Nutrition information was also calculated for 30 g of the dry ingredients for the Best Formula (excluding eggs, butter, vinegar, and honey) in order to make a fair comparison. For most commercial mixes, nutrition information for the finished product was not included on the label. Because ingredients added by the consumer prior to baking included butter, oil, eggs, or milk, the finished product would be higher in calories, protein, and fat. The dietary fiber level would remain the same as

TABLE XV

**Ingredients of Commercial Gluten-Free Bread Mixes
Compared to the Best Formula**

Product Name	Flour/Starch	Gum	Leavening	Minor Ingredients	Added Ingredients
Homestyle White Bread Mix	Rice flour Potato starch Corn starch Soy flour	Xanthan	Yeast	Sugar Salt	Eggs Butter Water
Favorite Sandwich Bread Mix	Brown rice flour White rice flour Potato starch Cornstarch	Guar	Yeast	Brown sugar Non-fat dry milk Whey Salt	Oil Eggs Water
Bread Mix – Home Style	Rice flour Corn flour Potato flour Potato starch	Xanthan	Yeast	Evaporated cane juice Molasses Salt Butter flavor Almond flavor Egg white Citric acid Rice bran	Eggs Oil Milk
Whole Grain Bread Mix	Sorghum flour Buckwheat flour Soy flour Tapioca starch Potato flour	Guar	Yeast	Salt	
Multi-Grain Bread with Seeds	Sorghum flour Buckwheat flour Soy flour Tapioca starch Potato flour	Guar	Yeast	Sunflower Seeds Millet Flaxseed Salt	
Tom's Light Gluten-Free Bread Mix	Chick pea flour Cornstarch Tapioca starch	Xathan	Yeast	Sugar Salt Cream of tartar	
Homemade Wonderful GF Bread Mix	Garbanzo flour Potato starch Corn starch Sorghum flour Tapioca flour Fava flour Potato flour	Xanthan Guar	Yeast	Turbinado sugar Sea salt Soy lecithin	Milk Whole egg Egg whites Butter/oil Cider vinegar
Best Formula 5% Sorghum Bran, 5% Flax, and 10% Inulin	Sorghum flour Tapioca flour Corn starch Potato starch	Xanthan	Yeast	Gelatin Salt Sugar Non-fat dry milk Sorghum bran Flax Inulin	Eggs Butter Honey Vinegar Water

listed on the nutrition facts labels as no fiber-containing ingredients were added prior to baking.

TABLE XVI

**Nutrition Content of Commercial Gluten-Free Breads
Compared to the Best Formula Per 30 g Dry Mix**

Product Name	Calories (kcal)	Protein (g)	Dietary Fiber (g)	Price per Mix (\$)
Homestyle White Bread Mix	111	2	1	6.88
Favorite Sandwich Bread Mix	106	3	0	4.95
Bread Mix - Home Style	112	4	2	6.25
Whole Grain Bread Mix	110		3	4.39
Multi-Grain Bread with Seeds	105		0	4.95
Tom's Light Gluten-Free Bread Mix	118	3	2	4.95
Homemade Wonderful GF Bread Mix	107	2	3	3.95
Best Formula	95	2	2	

Gluten-Free Bread Summary. Substitution of inulin for 10% of the composite gluten-free flour provided high levels of dietary fiber while significantly improving bread quality. Specific volume was increased, while crumb firmness was reduced. Sorghum bran and flax contributed antioxidants, omega-3 fatty acids, and dietary fiber to the gluten-free bread. However, these ingredients had less beneficial effects on bread quality. The use of 10% inulin allowed the addition of up to 5% each sorghum bran and flax without detrimental effects on bread quality. The resulting gluten-free bread contained increased levels of dietary fiber, antioxidants, and omega-3 fatty acids.

Minimal decreases in specific volume were seen, while crumb firmness was improved even further over the 10% inulin treatment. The bread's crust and crumb were dark in color, providing a visual cue to its high levels of antioxidants and dietary fiber.

The Best Formula compared favorably to commercially available gluten-free bread mixes. Its specific volume was surpassed by only one commercially available product, and its crumb firmness was significantly better than any of the products evaluated. Rapid staling is a major problem in gluten-free breads, and a product that stales at a significantly slower rate would likely be well received by consumers. In addition to improved bread quality, the Best Formula provided comparable levels of dietary fiber, while supplying antioxidants and omega-3 fatty acids not normally found in gluten-free breads. Gluten-free breads containing nutraceutical ingredients are not widely available, and a product such as the Best Formula could likely compete in the gluten-free bread market. Some of the specialty ingredients used in the Best Formula are more costly than those found in traditional wheat bread. However, retail prices for gluten-free bread mixes ranged from \$4 to \$7 and indicate that consumers are willing to pay a premium for this specialty product. At this time, the Best Formula is only appropriate for use in a home oven. More research is needed to make the formula suitable for use in home bread machines

CHAPTER V

CONCLUSION

Nutraceutical Bread Summary

In preliminary research, bread containing 12% sorghum bran and 5% flaxseed was developed. This bread was high in dietary fiber, antioxidants, and omega-3 fatty acids. However, the bread staled quickly. It was hypothesized that small amounts of soy flour could be added to the formula to increase water absorption and decrease crumb firmness, thereby lengthening shelf-life of the baked bread. Soy flour was substituted for 0, 2, 4, and 6% the wheat flour in the bread containing 12% sorghum bran and 5% flaxseed. While no significant improvement was seen crumb firmness, sensory evaluation revealed that untrained panelists preferred the bread containing 2% soy flour. Although the addition of soy flour did not result in the expected quality improvements, it may improve consumer acceptance of bread containing sorghum bran and flax. The addition of soy flour could result in a more palatable means of delivering high levels of dietary fiber, antioxidants, and omega-3 fatty acids to consumers via bread.

Gluten-Free Bread Summary

Sorghum bran, inulin, and flaxseed were combined in gluten-free bread to create a product high in dietary fiber, antioxidants, and omega-3 fatty acids. The optimum combination was 10% inulin, 5% sorghum bran, and 5% flaxseed. Specific volume was increased over the control, while crumb firmness was significantly improved. The crust

and crumb were dark in color, providing a visual cue to the bread's high antioxidant content. One serving (56 g) of the Best Formula provided 2 g dietary fiber, 1882 μmol TE antioxidant value, and 287 mg omega-3 fatty acids. The Best Formula's quality compared favorably to commercially available gluten-free bread mixes. Its specific volume was higher than all but one commercial product. Its crumb remained significantly softer than any bread mix evaluated 24 hours after baking. The Best Formula's improved bread qualities and high levels of health-promoting nutraceutical ingredients could make this product an attractive choice for consumers with celiac disease.

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APPENDIX A

NUTRACEUTICAL BREAD DATA TABLES

TABLE A-I

Specific Volume of Soy Flour Breads	
Percent Soy	Specific Volume (cm ³ /g)
0	3.6
2	3.6
4	3.4
6	3.4

TABLE A-II

Crumb Firmness of Soy Breads 2 and 24 Hours After Baking

Percent Soy	2h	24h
0	5	6.2
2	5.1	6.3
4	7.7	8.2
6	8.6	10.4

TABLE A-III

Crumb Color of Breads Containing Soy Flour

Percent Soy	L	a	b
0	35.4	10.8	12.3
2	36.1	11.0	12.6
4	35.5	11.1	12.6
6	35.7	11.0	12.8

TABLE A-IV
Crust Color of Breads Containing Soy Flour

Percent Soy	L	a	b
0	37.8	14.4	14.6
2	36.8	14.3	14.0
4	36.9	13.9	14.1
6	36.1	13.8	13.4

TABLE A-V
Moisture of Breads Containing Soy Flour

Percent Soy	Total Moisture (%)
0	38.4
2	37.8
4	38.4
6	38.9

TABLE A-VI
**Sensory Scores of Nutraceutical Breads Containing
Sorghum Bran, Flax, and Soy Flour**

Percent Soy	Overall Acceptability	Flavor	Texture
0	5.7	5.7	6.5
2	6.7	6.7	7.4
4	6.5	6.2	6.6
6	6.1	6.2	6.7

APPENDIX B

GLUTEN-FREE BREAD DATA TABLES

TABLE B-I

Effect of 5% and 10% Substitution of Sorghum Bran, Flax, and Inulin on Specific Volume

	Specific Volume (cm ³ /g)
White bread	4.5
Control	2.4
5% Sorghum Bran	2.2
5% Flax	2.5
5% Inulin	2.5
10% Sorghum Bran	2.4
10% Flax	2.6
10% Inulin	2.7
LSD ($\alpha=0.05$)	0.16

TABLE B-II

Crumb Firmness at 2 and 24 Hours After Baking

Treatment	2h	24h
White Bread	2.3	3.3
Control	11.6	31.0
5% Sorghum Bran	14.2	35.1
5% Flax	9.2	26.4
5% Inulin	7.1	25.4
10% Sorghum Bran	8.2	18.7
10% Flax	4.3	12.2
10% Inulin	3.5	11.5

TABLE B-III
**Effect of 5% and 10% Substitution of Sorghum Bran,
 Flax, and Inulin on Crust Color**

Objective Color Measurements of Bread Crust			
Treatment	L Value ^a	a Value ^b	b Value ^c
Control	47.7	14.5	28.1
5% Sorghum Bran	41.3	19.9	21.4
5% Flax	41.5	14.3	23.1
5% Inulin	41.4	15.0	23.0
10% Sorghum Bran	39.7	14.9	19.0
10% Flax	40.1	15.0	22.6
10% Inulin	37.4	15.7	20.3
LSD ($\alpha=0.05$)	5.1	0.5	1.4

^aValues are from 0 (darkest) to 100 (lightest).

^bValues are + (red) to - (green).

^cValues are + (yellow) to - (blue).

TABLE B-IV
**Effect of 5% and 10% Substitution of Sorghum Bran,
 Flax, and Inulin on Crumb Color**

Objective Color Measurements of Bread Crumb			
Treatment	L Value ^a	a Value ^b	b Value ^c
Control	78.0	-1.2	21.5
5% Sorghum Bran	47.8	10.7	14.6
5% Flax	75.3	-0.2	23.8
5% Inulin	78.9	-1.1	22.2
10% Sorghum Bran	39.3	12.6	13.8
10% Flax	72.3	0.8	23.8
10% Inulin	77.3	-1.1	22.9

^aValues are from 0 (darkest) to 100 (lightest).

^bValues are + (red) to - (green).

^cValues are + (yellow) to - (blue).

TABLE B-V
Moisture Content of Gluten-Free Breads Containing 5-10% Sorghum Bran, Flax, or Inulin

Treatment	Moisture Content (%)
Control*	40.9
5% Sorghum Bran*	41.6
5% Flax*	41.8
5% Inulin*	42.2
10% Sorghum Bran**	45.7
10% Flax**	44.2
10% Inulin**	43.0

* 86% water (flour basis) used to bake bread.

** 105% water (flour basis) used to bake bread.

TABLE B-VI
Effect of Fiber Combinations on Specific Volume

Treatment	Specific Volume (cm ³ /g)
White Pan Bread	4.5
Control	2.4
10% Inulin	2.7
A	2.4
B	2.4
C	2.6
D	2.5

TABLE B-VII**Crumb Firmness of Fiber Combination Breads at 2 and 24 hours After Baking**

Treatment	2h	24h
White Bread	2.3	3.3
Control	11.6	31.0
10% Inulin	3.3	11.5
A	5.9	16.4
B	6.1	14.3
C	4.3	12.3
D	4.3	10.8

TABLE B-VIII**Crust Color of Gluten-Free Breads Containing Fiber**

Treatment	L Value ^a	a Value ^b	b Value ^c
10% Inulin	37.4	15.7	20.3
A	39.9	16.8	23.2
B	37.0	16.5	20.3
C	35.8	16.3	19.9
D	34.9	16.1	18.1

^aValues are from 0 (darkest) to 100 (lightest).

^bValues are + (red) to – (green).

^cValues are + (yellow) to – (blue).

TABLE B-IX
Crumb Color of Fiber Combination Bread Crumbs

Treatment	L Value ^a	a Value ^b	b Value ^c
10% Inulin	77.30	-1.11	22.88
A	53.41	9.46	15.31
B	46.34	11.53	15.06
C	53.31	9.44	15.74
D	45.94	11.80	15.21

^aValues are from 0 (darkest) to 100 (lightest).

^bValues are + (red) to - (green).

^cValues are + (yellow) to - (blue).

TABLE B-X
Moisture Content of Fiber Combination Breads

Treatment	Moisture Content (%)
10% Inulin	43.1
A	45.5
B	45.7
C	44.3
D	44.4

TABLE B-XI
Specific Volume of Commercial Gluten-Free Bread Mixes Evaluated

Product Name	Brand	Specific Volume (cm ³ /g)
Homestyle White Bread Mix	Cause You're Special	1.9
Favorite Sandwich Bread Mix	Gluten Free Pantry	2.3
Bread Mix - Home Style	Authentic Foods	1.9
Whole Grain Bread Mix	Gluten Free Pantry	1.7
Multi-Grain Bread with Seeds	Gluten Free Pantry	1.7
Tom's Light Gluten-Free Bread Mix	Gluten Free Pantry	3.0
Homemade Wonderful GF Bread Mix	Bob's Red Mill	2.0
Best Formula		2.5

TABLE B-XII
Crumb Firmness of Commercial Gluten-Free Breads
2 and 24 Hours After Baking

Product Name	2h	24h
Homestyle White Bread Mix	11	39.1
Favorite Sandwich Bread Mix	12.01	32.2
Bread Mix - Home Style	13.6	25
Whole Grain Bread Mix	27.36	26.6
Multi-Grain Bread with Seeds	22.35	23.6
Tom's Light Gluten-Free Bread Mix	5.38	17.1
Homemade Wonderful GF Bread Mix	20.27	43.2
Best Formula	4.3	10.8

TABLE B-XIII
Crust Color of Commercial Gluten-Free Breads

Product Name	L	a	b
Homestyle White Bread Mix	57.3	13.0	32.6
Favorite Sandwich Bread Mix	44.9	16.3	28.2
Bread Mix - Home Style	53.8	12.0	33.5
Whole Grain Bread Mix	58.9	7.1	30.8
Multi-Grain Bread with Seeds	57.6	5.1	30.9
Tom's Light Gluten-Free Bread Mix	45.3	17.7	27.8
Homemade Wonderful GF Bread Mix	65.9	4.8	34.7
Best Formula	34.9	16.1	18.1

TABLE B-XIV

Crumb Color of Commercial Gluten-Free Breads			
Product Name	L	a	b
Homestyle White Bread Mix	77.5	-1.4	21.9
Favorite Sandwich Bread Mix	73.0	3.4	29.1
Bread Mix - Home Style	69.1	2.2	28.5
Whole Grain Bread Mix	65.8	2.2	26.0
Multi-Grain Bread with Seeds	64.7	1.9	25.2
Tom's Light Gluten-Free Bread Mix	77.9	-0.3	33.3
Homemade Wonderful GF Bread Mix	70.9	0.2	29.4
Best Formula	45.9	11.8	15.21

TABLE B- XV**Moisture Content of Commercial Gluten-Free Breads**

Product Name	Moisture
Homestyle White Bread Mix	47.9
Favorite Sandwich Bread Mix	44.4
Bread Mix - Home Style	46.5
Whole Grain Bread Mix	52.2
Multi-Grain Bread with Seeds	50.6
Tom's Light Gluten-Free Bread Mix	40.6
Homemade Wonderful GF Bread Mix	49.0
Best Formula	44.4

VITA

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