1-1-2011

Formulation and Sensory Evaluation of Gluten-Free Muffins Containing Flax

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FORMULATION AND SENSORY EVALUATION OF GLUTEN-FREE MUFFINS CONTAINING FLAX

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Nutrition
in the Department of Food Science, Nutrition, and Health Promotion

Mississippi State, Mississippi

August 2011

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Title of Study: FORMULATION AND SENSORY EVALUATION OF GLUTEN-FREE MUFFINS CONTAINING FLAX

Pages in Study: 58

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Celiac disease is characterized by an allergic reaction to gluten that causes inflammation of the small intestine and can lead to malabsorption and malnutrition. Gluten-free products are being developed that meet dietary needs of individuals with celiac disease. However, these products often lack whole grains and fiber. Fortification of gluten-free products with flax can increase nutritional value and alleviate inflammation. Sensory analysis (N=152) was conducted to evaluate the acceptability of gluten-free muffins with moderate (3.8%) and high (7.4%) amounts of added flax. Results indicated that consumers preferred ($p<0.05$) the muffin without flax or the high-flax muffin more than the muffin with the moderate-flax treatment. The high-flax and control treatments were rated 6.7, between like slightly and moderately like; the muffins from the moderate-flax treatment were rated 6.4 on a nine-point hedonic scale. Producers of gluten-free products could potentially formulate muffins that include flax and are acceptable to consumers.

ACKNOWLEDGEMENTS

.I am heartily thankful to Diane Tidwell my major professor, whose encouragement, guidance and support in the subject has allowed me the opportunity to learn more myself. Her genuine interest in my project along with Lynn Burney, and Michelle Lee has helped me to understand the importance of research not only in an academic standing, but in clinical practice in the field of dietetics. I would also like to especially thank Wes Schilling for his explanation of statistical methods and food science methods that have allowed me to understand the data that were obtained. It is with the most sincere gratitude that I thank these people as without their continued support, this project would not have been possible.

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CHAPTER I

INTRODUCTION

Celiac disease and gluten-sensitive enteropathy are becoming more prevalent worldwide (Logan & Bowlus, 2010) as dietary patterns and genetic predisposition become more widespread. These conditions are characterized by chronic allergic reaction to common cereal proteins that causes inflammation of the small intestine (Owens & Greenson, 2007). This chronic inflammation can lead to underlying malabsorption and malnutrition. Gluten-free products are currently a popular trend not only in terms of nutrition, but in terms of food product and market development (Lee, Ng, Zinvin, & Green, 2007). There are currently hundreds of new food products that cater to individuals with celiac disease and gluten allergy. While complete avoidance of gluten can be obtained through gluten-free products, nutritious fortified products are usually lacking in the marketplace. Gluten-free products generally are devoid of whole grains. Fortification of gluten-free products with other naturally occurring gluten-free ingredients can allow for greater variety of nutrient-rich foods, and also help alleviate chronic symptoms associated with celiac disease inflammation.

Flax, *Linum usitatissimum*, has recently been classified as a functional food because of its high content of lignans, polyunsaturated fatty acids, and alpha-linolenic acid, an essential fatty acid (Pan, Yu, Demark-Wahnefried, Fanco, & Lin, 2009). These compounds are associated with reduction in cardiovascular disease and cancer, and have anti-inflammatory properties (Heller, Rossler, Stehr, Heller & Koch, 2006). While

numerous benefits of flax have been studied, many of these applications are often not useful to individuals with gluten-sensitive enteropathy or celiac disease because flax is generally utilized in baked products containing gluten. The purpose of this study was to develop an acceptable gluten-free muffin with added flax. The objectives were to 1) determine an acceptable amount of flax to fortify gluten-free muffins, and 2) develop an acceptable gluten-free muffin that would provide the nutritious benefits of flax and also be rated favorably by consumers.

CHAPTER II

REVIEW OF LITERATURE

Celiac Disease

The occurrence and treatment of celiac disease is becoming more common in the field of medicine, nutrition, and epidemiology. Also known as celiac sprue or gluten-sensitive enteropathy, celiac disease is a malabsorption syndrome that involves a severe allergic reaction following the ingestion of wheat or gluten-containing compounds. It, however, should not be confused for a simple food allergy. Food allergies are IgE mediated (Mahan & Escott-Stump, 2004) and symptoms generally subside after the causative protein is removed. In celiac disease, the gluten protein causes an initiation of immune response that affects the body in several ways including malabsorption and inflammation. Celiac disease is a self-perpetuating condition and recuperation from symptoms can take weeks to years.

It has been suggested that 1:100 people in Western Europe and North America have celiac disease. Genetics plays a large part in regional susceptibility in addition to normal dietary patterns (Owens & Greenson, 2007). However, as people and cultural eating practices become more integrated and widespread, so does the incidence of celiac disease; prevalence is growing in Eastern cultures of Asia and the Caribbean. Even with increased public awareness, it is hypothesized that a majority of the cases worldwide are still under-diagnosed (Reeves & Leibovici, 2005). The worldwide incidence of celiac disease has climbed from 0.1% to 1% in the past decade, probably as a result of the

medical community's awareness and improved testing techniques (Kaukinen, Lindfors, Collin, Koskinen, & Maki, 2010). The fact that the incidence has risen so quickly in a relatively short period of time indicates that genetics is not the only factor involved. Kaukinen and colleagues (2010) postulate that the increase in celiac disease is not only related to genetics but also infant feeding practices, rotavirus infection, socioeconomic status, and environmental exposure to antigens.

Introduction of gluten before four months of age has been associated with increased risk of developing celiac disease (Norris, et al., 2005). In Sweden, there was an increased incidence of two-year-old children diagnosed with celiac disease in 1985-1987 and a subsequent decline in 1995-1997, which has been suggested to be related to popular infant feeding practices of the time (Lohi, et al., 2007), length of breastfeeding, and initial age of adding gluten into the diet (Sollid, 2000). The Hygiene Hypothesis theorizes that as our world has industrialized, we are more sheltered from microbial and antigen contact as children and unfortunately this has led to decreased humoral response. This can perhaps explain the body's up-regulation of food allergies later in life (Kaukinen, et al., 2010). The incidence of celiac disease has risen at a linear rate that is similar to autoimmune disorders such as insulin-dependent diabetes mellitus (Lohi, et al., 2007) with women comprising roughly 75% of newly diagnosed adult cases (Corrao, et al., 2001).

Gluten is a grass protein that consists of specific peptide fractions in wheat, rye, and barley (Mahan & Escott-Stump, 2004). The alcohol-soluble fractions of gluten are gliadin in wheat, hordeins in barley, and secalins in rye and are causative antigens in the celiac disease immune response. Wheat is a staple of the Western diet with the amount of gluten consumed per individual at approximately 10-20gms/day (Kaukinen, et al.,

2010). Gluten is also found in commonly used products such as cosmetics, medicines, emulsifiers, beer, and many processed foods. With the widespread usage of gluten in foods and other products, a diet completely eliminating gluten may not be possible (Storsrud, Yman, & Lenner, 2003); however, a safe threshold has been 10-50µg/day, much less than the average consumer intake in western culture (Catassi & Fasano, 2008). Unfortunately, the exact peptide sequence that causes an immune reaction has yet to be discovered (Owens & Greenson, 2007), but it is known that the peptide sequence is rich in glutamine and proline amino acids.

For people with celiac disease, an immune response occurs when gluten is ingested. Symptoms may be mild to severe depending on the age of the individual, duration of contact with gluten, and the extent of long-term damage to the small intestinal mucosa (Bassotii, et al., 2007). The extent of symptoms is not directly correlated with severity of mucosal damage but rather the quantity of damaged mucosal tissue (Kaukinen, et al., 2010). The symptoms are characteristic of gastritis such as abdominal discomfort, bloating, constipation, nausea, and/or diarrhea. Often times, the symptoms are overlooked as indigestion, food intolerance or irritable bowel syndrome (IBS) (Kaukinen, et al., 2010). External symptoms can also occur such as skin and liver conditions. Dermatis herpetiformis is common and involves large blisters on the skin and usually is not accompanied with abdominal discomfort. These blisters itch and most commonly appear on the scalp, elbows, and buttocks (Kaukinen, et al., 2010). Hepatopathy generally is mild and subsides immediately after removal of gluten from the diet. The symptoms can however take up to three months to fully dissipate (Owens & Greenson, 2007).

The integrity of the small intestine is compromised when symptoms are present. This unfortunately decreases the absorption of not only the offending gluten peptides, but other nutrients as well. Celiac disease is characterized by chronic inflammation that can cause nutrient sludge in the gut. This sludge then passes through the ileum to the colon and then out of the body with poor absorption of energy nutrients, vitamins, and minerals. Steatorrhea occurs when the fecal material has large concentrations of maldigested fat. This quick passage of material through the alimentary canal does not allow adequate time for nutrient uptake and can cause the individual to be deficient in energy, carbohydrates, protein, essential fatty acids, fat soluble vitamins A, D, E and K, and also folate, iron, calcium, and vitamin B12. Over time, symptoms of malnutrition may become apparent such as hypoalbuminemia, growth delay, weight loss, and failure to thrive. Megaoloblastic anemia may also occur as adequate folate is not absorbed, and iron deficiency anemia may occur as well. Many times adult patients do not present to their physicians with classic symptoms of celiac disease, but rather symptoms of anemia or poor coagulation from inadequate vitamin K absorption (Owens & Greenson, 2007). Inappropriate calcium absorption can lead to rickets in pediatric patients (Ensari, 2010) and decreased bone mineral density and osteoporosis in adults (Capriles, Martini, & Areas, 2009). Children with prolonged malnutrition from poor absorption are at risk for delayed puberty and failure to reach full growth potential (Owens & Greenson, 2007). Other complications of malabsorption and nutrient deficiency are infertility, neurological deficits, decreased skin integrity, increased risk for Non-Hodgkin's lymphoma, gastrointestinal cancer, and mortality (Sollid, 2000).

The susceptibility to gluten antigens relies heavily on genetic makeup. The human leukocyte antigen (HLA) haplotypes, HLA-DQ2 and HLA-DQ8, are associated

with manifestation of celiac disease (Green & Cellier, 2007; Plenge, 2010). Individuals that have the HLA phenotype possess the allele to synthesize and express HLA-DQ2 or HLA-DQ8 proteins. Karell and colleagues (2003) reported that there is increasing evidence for the presence of additional risk variants on different chromosomes. Individuals that do not have the HLA halpotype cannot express these proteins, and it is unlikely that they are susceptible to gluten antigens, which makes HLA-typing useful for exclusion purposes (Karell, et al., 2003). Unfortunately, 40% of the general population is HLA-positive so this genetic sequencing cannot be used for definitive diagnosis of celiac disease. The primary haplotype expressed in individuals with celiac disease is HLA-DQ2 (88%) and the remainder is HLA-DQ8 (Sollid, 2000). This haplotype is also associated with type 1 diabetes, Addison syndrome, and Sjögren's syndrome (Haines, Anderson, & Gibson, 2008). Homozygocity for the HLA phenotypes is much more efficient at expressing antigens to gluten than heterozygous HLA, which may explain the varying degree and severity of symptoms in active celiac disease (Vader, et al., 2003).

Historically characterized as a childhood disease, the age of celiac disease diagnosis has shifted upward over the past twenty years (Kaukinen, et al., 2010). Susceptibility to a wheat protein immune response can occur at any age. The diagnosis was first characterized by Dr. Samuel Gee, an English pediatrician, in 1888. It originally presented in children with diarrhea, lassitude, malabsorption, and failure to thrive (Kaukinen, et al., 2010). In general, infants and children can present with diarrhea, abdominal distention, failure to thrive, vomiting, irritability, anorexia, and constipation. Infants are usually diagnosed between nine and twenty-four months of age (Capriles, et al., 2009), and symptoms worsen as more cereals are introduced into their diets (Owens & Greenson, 2007). Toddlers and children generally present with extraintestinal skin

anomalies, short stature, neurological deficits, and anemia (Green & Cellier, 2007). Early intervention with children usually results in complete mucosal healing (Lanzini, et al., 2009), and if detected before puberty, peak bone mineral density can still be reached (Capriles, et al., 2009). Adolescents generally present with symptoms of dermatis herpetifromis and symptoms of abdominal discomfort; they have a good chance of bone remineralization assuming they follow a gluten-free diet (Capriles, et al., 2009). Unfortunately, adolescences have the lowest level of diet compliance of any age group (Mayer, Greco, Troncone, Auricchio, & Marsh, 1991).

Adults generally present with less common symptoms such as hypocalcaemia, elevated liver function tests, and hypoalbuminemia as well as diarrhea, abdominal pain, constipation, and weight loss (Green & Cellier, 2007). Diagnosis of celiac disease is often made in the third and fourth decades of life (Capriles, et al., 2009) as an overlying diagnosis to several nonspecific symptoms. Bone mineral density rarely returns to normal levels in adults with chronic celiac-related malabsorption simply because the small intestinal mucosa has lost functionality due to continuous inflammation. Geriatric patients surprisingly present with milder symptoms of abdominal discomfort and flatus. A study by Mukherjee, Luyer, Heineman, and Buurman (2010) compared clinical presentation, bone mineral density, duration, and small intestinal histology of individuals with celiac disease of two different age groups of active celiac disease patients: ≥65 years old and 18-30 years old; they found that both groups were histologically similar with no difference in degree of villous atrophy regardless of duration of diagnosis. This study suggested that most individuals with celiac disease have had it their entire life and did not develop it over time (Makherjee, et al., 2010).

Celiac disease is a T-cell mediated disease. Innate immunity via T-lymphocytes plays a part in intestinal damage and malabsorption (Murray, 1999). When gluten is ingested, it enters the alimentary canal and is broken into its component peptides, including gliadin (Shan, et al., 2002). Gliadin is then taken up by HLA^+ cells and presented on their surface in the lamina propria. In the lamina propria, gliadin can exist in the native form or the deamidated form by tissue transglutaminase (tTG). When gliadin is presented on the HLA in the lamina propria, it simultaneously activates the $CD4^+$ T-lymphocytes (Ensari, 2010) and activates the expression of interleukin-15 (Sollid, 2000). The activated $CD4^+$ T-lymphocytes release cytokines and interleukins, and then migrate to the surface. The expression of interleukin-15 (IL15) activates intraepithelial lymphocytes (IELs) when the T-lymphocytes reach the surface of the epithelium, which is the cause of inflammation (Owens & Greenson, 2007).

Humoral response is activated when gliadin is deamidated by tTG. The B-lymphocytes produce and secrete IgA and IgG antibodies into the intestinal lumen aimed at destroying gliadin, tTG, and connective tissue auto-antigens including antiendomysial cells (Owens & Greenson, 2007). The endomysium is a sheath of connective tissue and reticular fibrils that surround the small intestinal muscle fibers. Antiendomesial antibodies and tTG correlate to the amount of tissue damage.

Overall, celiac disease is a disorder of the proximal small intestine that presents in several patchy areas of inflammation along the intestinal brush border (Murray, 1999). The inflammation is contained along the absorptive surfaces, or villi, and is the main site of malabsorption in the small intestine. Under normal circumstances, the intestinal villi are small finger-like projections that are equipped with digestive enzymes on their surface. These enzymes help break the fragments of food particles into smaller entities

that can enter circulation. The villi are also equipped with a network of lymphatic vessels that provide direct access to circulation (Owens & Greenson, 2007). However, when a person with celiac disease comes into contact with gluten protein fractions, the immune response produces a cascade of biochemical reactions that not only target the offending allergen, but also causes damage to the body's own tissues in an effort to destroy the gluten antigen. Upon ingestion of gluten, T-cells release inflammatory cytokines to the duodenum. When this happens, the villi become inflamed and are not able to function properly. The villi are unable to absorb nutrients because of decreased exposure of villous mass. This causes delayed transit time of the small intestine and lack of exposure to digestive enzymes. Over time, this can lead to deficiencies in nutrients including the essential alpha-linolenic and linoleic fatty acids (Solakivi, Kaukinen, Kunnas, Lehtimaki, Maki, & Nikkari, 2009).

The level of duodenal damage relies heavily on the genetic susceptibility of the person, the amount of gluten exposure, and the duration of exposure. Small intestinal damage has been described by Marsh (1992) in what is known as the Marsh Criteria. Marsh (1992) classified celiac-related inflammation into four categories: Types 0-III. Type 0 is described as the absence of lesions; this is descriptive of a small intestine of an individual without celiac disease or a person with celiac disease with a tightly controlled diet void of gluten. Type I lesions are also known as the infiltrative state. At this point of gluten exposure, gastrointestinal symptoms and malabsorption are not present; there is simply an infiltration in the villi of intraepithelial leukocytes. After further exposure, Type II, or hyperplasic lesions develop in which the IELs are still present and crypt spaces have enlarged. Type III lesions are considered completely atrophied with persistent IEL presences.

While the Marsh Criteria are helpful in confirmation of celiac disease, histology is not the final word in diagnosis. The symptoms of villous atrophy and crypt cell hyperplasia are not pathognomonic to the condition. These pathological findings are characteristic, but non-specific (Green & Cellier, 2007). Other conditions with similar presentation to celiac disease include rotavirus infection and giardiasis (Kaukinen, et al., 2010). It has been hypothesized that celiac disease is often mistakenly suspected by patients who suffer from chronic *Helicobacter pylori* infections, other food allergies, and Crohn's disease; all of these have similar histology to celiac disease (Ensari, 2010). It has been demonstrated that the amount of bacteria present in the small intestine of active celiac patients is up to 30% higher than controlled celiac disease (Sollid, 2000). The general consensus is that the body's immune system is compromised to a level that cannot keep bacteria such as *H. pylori* to manageable levels because of the gluten exposure and diminished immune function.

Correct diagnosis of celiac disease is essential because it requires that the individual follow gluten-free protocols for the rest of his or her life (Bhatnagar & Tandon, 2006). It is important that the medical practitioner consider the diagnosis of celiac disease seriously because compliance with the gluten-free diet is not only expensive, it is socially inconvenient (Green & Cellier, 2007). Patients often present with generic symptoms of gastrointestinal complications; many patients self-diagnose themselves with celiac disease and begin following a gluten-free diet before seeking medical attention. Many patients do not experience classic symptoms of celiac disease (Fasano, et al., 2003). Resolved symptoms after following a gluten-free diet is not sufficient evidence to make a definitive diagnosis because the symptoms of celiac disease are similar to that of IBS, Crohn's disease, and ulcerative colitis (Campanella, Biagi,

Bianchi, Zanellati, Marchese, & Corazza, 2008). The severity of the disease in a prolonged active state necessitates early diagnosis. Normalization of the small intestine is more likely to occur for individuals that have not had prolonged gluten exposure and villous inflammation (Lanzini, et al., 2009).

Diagnostic criteria involved in the diagnosis of celiac disease include serological antibody testing, histological evidence, and reduction of symptoms after following a gluten-free diet. Unfortunately, there is not currently a consensus in the medical community on precise criteria needed for a celiac disease diagnosis. The American Gastroenterological Association mandates that biopsies be preformed regardless of serological findings, in any patient that is suspected to have gluten-sensitive enteropathy. The U.S. National Institutes of Health, however, only recommends biopsies to confirm positive serological findings. Serological testing relies on antibodies produced in response to gluten ingestion. Recent advances in serological antibody testing have shown that celiac disease is much more common than once thought (Haines, et al., 2008). Presence of specific antibodies can reveal that the body has produced an immune response to gluten. Humoral response to gluten produces antibodies against the offending gluten molecules and antibodies against the body's own tissues. It is possible to detect antibodies specific to gluten molecules. This method uses an assay to detect native gliadin antibodies but is no longer useful for diagnostic practices because the test lacks sensitivity and specificity, and the chances for false negatives and false positives are too high (Kaukinen, et al., 2010). Early serological diagnostic for celiac disease auto-antibodies involving immunofluorescent assay (IFA) can be utilized to test for anti-endomysial antibodies. This test is highly specific to antigens only produced in active

celiac disease. However, this test requires a trained technician and relies on subjective interpretation of results for diagnosis (Ensari, 2010).

It is now customary to use an enzyme-linked immunosorbent assay (ELISA) method to test for tTG antibodies (Owens & Greenson, 2007). The ELISA test for tTG antibodies can be performed in an office and does not require subjective assessment (Green & Cellier, 2007). This test is also more sensitive than the IFA endomysial antibody test and is much less expensive. Often times, the IFA test is used in conjunction with the ELISA test for confirmatory purposes (Kaukinen, et al., Maki, 2010). Positive serological testing with negative histological findings may be an indicator of early celiac disease. While serotesting has been essential in the diagnosis of celiac disease, it does not provide enough evidence for a diagnosis of celiac disease except in pediatric patients (Kaukinen, et al., 2010); a presumptive diagnosis can be made with positive serotesting but histological evidence is a must.

A diagnostic tool that is considered by some as the "gold-standard" is through pathological studies of the small intestine. Given the patchy nature of celiac disease inflammation, it is recommended that four to six endoscopic tissue samples be biopsied from the duodenum. These samples can be evaluated for villous atrophy and crypt cell hyperplasia (Kaukinen, et al., 2010). The presence of intraepithelial lymphocytes within the villous crypts can also be detected histologically and are indicative of celiac disease (Owens & Greenson, 2007). A final diagnosis of celiac disease cannot be made until there have been positive serological tests confirmed by histological evidence along with resolved gastrointestinal symptoms after following a gluten-free diet. Once a diagnosis is determined, the patient should be assessed for nutritional deficiencies and should undergo nutrition counseling by a registered dietitian and, if possible, attend celiac disease support

group meetings. Customary treatment of celiac disease is lifelong adherence to a gluten-free diet. The patient should be reassessed and re-biopsied in four to six months to monitor for new inflammation and intestinal damage (Kaukinen, et al., Maki, 2010). Serological testing is useful for determining compliance in the avoidance of gluten. It is important to note that approximately 5% of patients do not respond to gluten-free diets and are classified as having refractory sprue (Schuppan, Dennis, & Kelley, 2005); the prognosis for refractory sprue is poor.

Much research has been conducted on the effectiveness of gluten-free diets. It is hypothesized by some that gluten does not actually cause tissue injury itself, but inflammatory chemokines cause the most damage (Owens & Greenson, 2007). However, gluten avoidance is currently the only effective treatment for the management of celiac disease. It is generally accepted that not only can gluten avoidance improve symptoms, but also improve nutrition status related to absorption and prevent long-term complications of persistent inflammation. The focus of gluten-free diets is to avoid all varieties of wheat including smelt, kamut, and triticale as well as derivatives such as rye and barley (Schuppan, et al., 2005). Gluten-free diets are generally successful in reducing gastrointestinal distress in patients with celiac disease. In severely ill patients, symptoms subside dramatically (Bassotii, et al., 2007). Resolved symptoms however do not reflect complete healing of the small intestine. Avoidance of gluten decreases the number of IELs and allows for reconstitution of the intestinal villi (Owens & Greenson, 2007). Healing of the small intestine usually occurs in a distal to proximal direction (Ensari, 2010). However, the linkage to intestinal damage and resolution of symptoms are strongly evident in symptomatic celiac disease patients (Carroccio, et al., 2008). One study reported patients that followed gluten-free diets always had partial remission

periods but still expressed high numbers of IELs (Lanzini, et al., 2009). In the retrospective study, Lanzini and colleagues (2009) also found that architectural normalization occurred but mucosal abnormalities still persisted in adults with celiac disease; they proposed that complete normalization occurs in only 8% of adults while 65% had periods of normalization with remission, and 1% had complete deterioration of the villi even after following a gluten-free diet. A study by Basotti et al. (2007) determined patients with celiac disease did not return to normal levels of mucosal function in adults even after twenty-four months on a gluten-free diet.

Gluten-Free Diet

Research in the area of gluten-free dietary practices has shifted focus. Formerly, the point-of-interest was pinpointing specific peptides to be avoided so as to prevent celiac disease exacerbation. The new focus has moved to improving the nutritional quality of the gluten-free diet. Exclusion of gluten proteins, which are a staple in many parts of the world, may lead to exclusion of essential nutrients. Many gluten-free products, such as cereals, pastas, and breads, are not enriched or fortified with vitamins and minerals. Overall, folate content was lower in gluten-free breads when compared to breads containing gluten (Yazynina, Johansson, Jägerstad, & Jastrebova, 2008). Thompson (2000) also reported that only three gluten-free cold cereals were enriched with folic acid out of 58 gluten-free products, which included breads, pastas, and cold cereals. Additionally, only nine of the 58 products were enriched with iron (Thompson, 2000). This affects the dietary intake of people with celiac disease. Stanley, Gonzales, Carroll, Nelson, and Hakkak (2010) reported significantly less niacin and carbohydrate intakes in people following a gluten-free diet when compared to national averages as

determined by data from the National Health and Nutrition Examination Survey (NHANES). Females on a gluten free diet also had less thiamin and iron when compared to NHANES than females not on a gluten-free diet. It was concluded that additional focus was needed on obtaining adequate amounts of B vitamins, fiber, iron, and carbohydrate intakes for individuals following gluten-free diets (Stanley et al., 2010).

Adhering to a gluten-free diet is difficult as gluten is a widely used protein in commercial bakery and food production. Not only does it serve as the main structural protein of dough, it gives bread products their characteristic texture (Brown, 2008). Gluten is also employed as a filler, thickener, binder, and stabilizer in ready-to-eat products and convenience foods (Catassi & Fasano, 2008). Products that are devoid of gluten have altered shape, quality, smell, and taste. Current practice in gluten-free product production is to simply substitute gluten flour with maize, potato, or rice flour. Pseudo-cereals such as amaranth, buckwheat, and quinoa are also employed, and are used to make bread products with different crumb textures and tastes. With increasing incidence of celiac disease, the demand for gluten-free products is growing. Currently, the market offers limited variety, and gluten-free products are more expensive then gluten-based products such as macaroni, cake mixes, cookies, breakfast cereals, and bread products. A study from the British Journal of Nutrition found substantial differences in price evident for gluten-free products when compared to their gluten-base counterparts (Lee, et al., 2007). Gluten-free products are most readily available via the Internet, followed by health-food stores, upscale grocery stores, and lastly in supermarkets. This availability is also reflected in product prices. Supermarkets are the most affordable source of gluten-free products; however, they also offer the least variety of products (Lee, et al., 2007). The study also found regional differences in availability.

The Northwest United States offers the most availability while the Southern region offers the least availability of gluten-free products.

The gluten-free diet has different definitions depending on geographic location, which affect the type and variety of products that are sold. In the United States, there is currently no federal definition of gluten-free; however, the U.S. Food and Drug Administration proposes that less than 20 ppm of gluten as safe for people with celiac disease (Niewinski, 2008). In the United States, gluten-free diets are based on complete elimination of wheat, rye, barley, and sometimes oats from the diet. It focuses on naturally gluten-free products such as fruits, vegetables, and unprocessed meats. It also focuses on incorporating different grains as substitutions for the gluten proteins in products that are normally wheat-based such as pastas, breads, cereals, and crackers. In the United Kingdom, gluten-free products focus on the biochemical elimination of gliadin molecules from wheat-based foods. Most European nations follow the *CODEX Alimentarius Food Trade Guidelines* that specify less than 20 ppm of gluten in naturally gluten-free products as safe and less than 200 ppm of gluten in products containing ingredients from wheat, rye, or barley as safe for people with celiac disease (Hall, Rubin, & Charnock, 2009; Niewinski, 2008). It is considered safe to consume wheat, rye, and barley products assuming they have been modified to deamidate the prolamine fractions (Gallagher, Gormley, & Arendt, 2004).

Wheat is a staple food in many regions of the world (Catassi & Fasano, 2008). Complete elimination of gluten from the diet is practically burdensome, restrictive, and expensive. Approximately 60% of individuals with celiac disease are noncompliant, or at least partially noncompliant, in following a strict gluten-free diet (Hall, et al., 2009). It is difficult to determine the root cause of noncompliance with the gluten-free diet.

Accidental ingestion of gluten is also a strong possibility since gluten is often used in products that are not considered food such as cosmetics, lipstick, and toothpaste (Kaukinen, et al., 2010). Noncompliance has been linked to factors such as cognitive, emotional, and socio-cultural influences. It has also been shown that access to registered dietitians and celiac disease support groups greatly enhances adherence to the diet (Hall, et al., 2009). Many patients however do not trust their health care professional and continue trying home methods to eliminate symptoms. Another factor affecting compliance is availability of gluten-free products (Lee, et al., 2007). Compliance to the gluten-free diet is lowest among ethnic minorities and teenagers (Hall, et al., 2009). It has also been shown that children diagnosed with celiac disease have good compliance as their meals are controlled by their caregivers; compliance tends to wane in these children as they reach adolescence and adulthood. Compliance of the gluten-free diet is important for individuals with celiac disease to control symptoms and prevent damage to the small intestine.

Flax

Flax (*Linum usitatissimum*) and flaxseed oil have been important crops in agriculture for thousands of years and are cultivated around the world. It has been used not only for its nutrient properties as food, but also for its functional fibers. The use of flax dates to as early as 5000 B.C. as one of the first crops to be domesticated by man. Worldwide, it has been employed for not only its nutritional properties, cloth-making and medicinal properties, but also for industrial capabilities. This crop is thought to have originated in the Mediterranean areas and northern Africa. The ancient Egyptians used it to produce fine linens that were used for preserving their dead. It is documented that

Hippocrates used flax for the treatment of abdominal pain and as a laxative. Charlemagne was so impressed by the nutritional properties of flax that he made it law that his subjects consume it every day. There are also accounts of the use of flax and flaxseed oil for cough relief, as a diuretic, wound-healing, and some gynecological treatments (Tolkachev & Zhuchenko Jr., 2000).

The versatility of flax and flaxseed oil comes from its biochemical and structural properties. Flax is an annual flower that produces seeds that posses a nutrient dense chemical breakdown. The seeds have a chewy texture and nutty flavor (Carter, 1993). Two varieties of seeds are available: yellow and brown, which are similar in nutrient profile. Oil can be extracted from the seeds using a cold-press method and has many useful applications. It is also possible to use a cold carbon dioxide treatment to extract the oils (Tolkachev & Zhuchenko Jr., 2000).

Flax has many uses in the chemical industry and agriculture. Industrially, flaxseed oil is known as linseed; this oil can be used to formulate asphalt, paints, varnishes, and linoleums. Other products made from flax include paper, fishnets, and personal products such as hair gel and soap. Until the invention of the cotton gin, it was a primary source of fibers for fabric production. Flax is also a popular additive to livestock feed because of its relative low cost and high protein content. Cheap and high in protein, flax can also allow for change in nutrient ratio of agricultural output. The addition of flax to livestock feed is common because it improves the coat luster of show animals. There was demonstrated improvement in the ratio of linoleic to alpha-linolenic acid in eggs when hens were fed flaxseed (Sims, McGregor, Plessers, & Mess, 1961).

Flax has recently been classified as a functional food (Pan, et al., 2009) because it has a high content of fiber and the essential fatty acid alpha-linolenic acid. It also is a

rich source of polyunsaturated fats and protein. The nutrient analysis of flaxseed is 41% fat, 20% protein, and 28% fiber. The fatty acid composition of flax is unique and contains a relatively low amount of saturated fat (9%), 18% monounsaturated fat, and the remaining is polyunsaturated fat with 16% linoleic acid and 57% alpha-linolenic acid (McDonald, 1994). The ratio of linoleic acid to alpha-linolenic acid is approximately 0.3:1. It contains the highest amount of alpha-linoleic acid of any of the major seed oils. The protein component of flax is approximately 20gm/100gms of flax and has a biological value similar to that of soy. It contains all essential amino acids for humans. Flax is also a rich source of potassium, with equivalence to bananas per serving. It is also a good source of magnesium, iron, copper, and zinc. Flax provides 28gm/100gms of fiber with approximately 66% water insoluble cellulose and lignin. The water soluble portion of flax fiber comes from the gum mucilage of the seed coat (Carter, 1993). Flax also has a high content of lignans, which are structural phytochemicals that exert weak estrogenic activity.

The plant material lignan in flax owes its functional properties partially to secoisolariciresinol diglycoside (SDG). This phytochemical is present in the husk coating of the flax plant and is in similar plants such as soybeans, seeds, whole grains, barley, buckwheat, millet, and legumes. However, flax is the richest source of this compound. When digested, gut flora are able to ferment SDG into enterolactone and enterodial. These compounds have been shown to have anti-cancer effects in estrogen-related cancers (Thompson, Robb, Serraino, & Cheung, 1991). They are able to block cellular pathways that lead to the production of mutated cells and are also able to inhibit protein synthesis that leads to metastasis of cancer in different types of tissue including breast, colon, ovary, and prostate (Adolphe, Whiting, Juurlink, Thorpe, & Alcorn, 2010).

Similar pathways have indicated that lignan compounds down-regulate gene expression of other cancerous growth factors by competitive inhibition (Kaminski, Jendraschak, Kiefl, & von Schacky, 1993).

Alpha-Linolenic Acid

The fatty acid component of flax gives it many functional properties. It is a rich plant source of polyunsaturated fatty acids, mainly alpha-linolenic acid. This omega-three fatty acid is essential to the diet. It cannot be endogenously synthesized and must be obtained from the diet. Food sources of alpha-linolenic acid include leafy green vegetables, cold water fish, flax, nuts, and vegetable oils; however, the concentration of alpha-linolenic acid is low in green plants. Deficiency in alpha-linolenic acid or linoleic acid (an omega-six fatty acid), can have adverse consequences on cell membrane integrity. It can present in patients with impaired growth, dermatitis, and neurological disorders (Steel, Ryd, Ascher, & Strandvik, 2006) and reproductive disorders. Maternal deficiency during pregnancy can lead to fetal brain and retinal deformities. It is especially common in individuals with chronic gastrointestinal disorders such as celiac disease. In this case, lipid malabsorption is not only the leading cause of deficiency, but occurs secondarily to this deficiency since there is a decrease in absorptive integrity. In this way, essential fatty acid deficiency is self-perpetuating.

While both of the essential fatty acids, alpha-linolenic and linoleic acids, are important in the diet, an increasing body of evidence is suggesting that the Western diet receives an excess amount of linoleic acid and quantities of alpha-linolenic acid are too low. In the Paleolithic Age, these essential fatty acids were consumed in equal amounts, but as dietary practices have changed (even over the past one hundred fifty years), a

substantial change in ratio has occurred (Simopoulos, 1996). This conversion to a higher amount of linoleic acid has been associated with chronic degenerative diseases such as rheumatoid and osteo-arthritis because of its relationship essential to inflammation. Prostaglandins are formed from cell membrane phospholipids when injury occurs. Different prostaglandins are formed from alpha-linolenic and linoleic acids. The ones formed from linoleic acid are pro-inflammatory and those formed from alpha-linolenic acid are inflammatory to a lesser extent and even possess anti-inflammatory properties. This gives strength to the argument for supplementation of omega-three, or alpha-linolenic, acid.

Flax has a high correlation to anti-inflammation due to its content of polyunsaturated fatty acids, which control the metabolism of prostaglandins mainly due to its alpha-linolenic acid content (Mostofsky & Yehuda, 1997). The alpha-linolenic acid present in flax exhibits anti-inflammatory properties when available in the body. Inflammation is the body's response to stress and involves several reactions and biomarkers. The usual components of inflammation are increased blood flow, and increased permeability across capillary walls allowing antibodies and cytokines to travel from the blood to the infected tissue. Inflammation can be local or systemic. When systemic inflammation gets out of control, a condition called systemic inflammatory response syndrome (SIRS) is present; when this occurs, cytokines tissue necrosis factor (TNF), interleukin-6 (IL6), and interleukin-8 (IL8) are out of control and destroy the body's own tissues. This systemic or local inflammation, while having a main goal of protecting the body, can have deleterious effects over time. Chronic inflammation has been linked to degenerative joint disease, systemic lupus erythemis, asthma, food allergies, and heart disease (Allan & Devereux, 2011). The management of inflammation

with diet has been studied. Kelly et al. (1991) noticed that these oils are useful in suppressing cell-mediated immunity without affecting the humoral response, which would allow the body to still defend itself from foreign bodies.

Alpha-linolenic acid affects immune response in several ways. First, it affects inflammation within the cellular membrane. The components of the cell membrane are phospholipids; the chemical breakdown of each cell membrane is affected by the fatty acids obtained from the diet. The alpha-linolenic acid to linolenic acid ratio of fatty acids in the membrane affects the permeability of cytokines that promote inflammation. A higher concentration of alpha-linolenic acid in the phospholipid membrane physically inhibits the action of some cytokines. Current dietary consumption patterns of linoleic acid to alpha-linolenic acid are approximately 20-30:1 (Simopoulos, 1999). This change in membrane composition also gives rise to different eicosanoids. Eicosanoids are inflammatory and immune signaling molecules, derived from 20-carbon polyunsaturated fatty acids that are produced on demand from phospholipid membranes when injury occurs. Eicosanoids then trigger inflammatory markers called prostaglandins. These eicosanoids are derived from alpha-linolenic acid and linolenic acid. Arachonidoic acid is an eicosanoid derived from linoleic acid phospholipids and eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) is an eicosanoid derived from alpha-linolenic acid. In general, arachonidoic acid has proinflammatory functions while EPA and DHA have anti-inflammatory properties. Increased alpha-linolenic acid concentration in the phospholipid membrane allows greater production of anti-inflammatory eicosanoids.

Linoleic acid and its derived eicosanoids function in immune response by promoting inflammation. If linoleic acid is excessive in the diet, there will be a direct proportional excess in the cell membrane. This allows for the creating of arachonidoic

acid, which is the dominant substrate when eicosanoids are produced. Large amounts of omega-six fatty acids can cause chronic inflammation in states of stress and can exacerbate other states of inflammation. Hyperinflammation can even occur, especially in immunosuppressed individuals. The signal for the production of cytokine TNF is stimulated by prostaglandins, which are arachonidoic acid metabolites. This TNF up-regulates inflammatory leukocytes leading to further local inflammation and possibly systemic response syndrome (Tull, et al., 2009). C-reactive proteins, which are markers for inflammation, have been demonstrated to be reduced with supplementation of alpha-linolenic acid (Renaud & Lanzmann-Petithory, 2002). The use of omega-three fish oils and plant-derived flax oils are currently being employed in situations of artificial nutrition (Calder, 2010). Essential fatty acids have been incorporated into enteral formulas and parenteral intravenous nutrition regimens. It was reported that the essential fatty acids induced a decline in antigen-presenting cells and demonstrated clinical improvement in surgical patients requiring either enteral or parenteral nutrition (Calder, 2010).

Consumption of alpha-linolenic acid demonstrated many benefits to heart health such as positively affecting serum cholesterol levels, lowering blood pressure in hypertensive individuals, decreasing platelet-cell aggregation, anti-arrhythmic effects, and electrolyte stabilization. Anti-atherosclerotic effects are also demonstrated by decreased incidence of inflammation. A meta-analysis by Pan et al. (2009) on the effects of flax supplementation revealed that dietary supplementation with alpha-linolenic acid from flax in food products consistently had a major impact on cholesterol and cholesterol ratios. Researchers found a consistent decrease in LDL cholesterol when patients were supplemented with 15gms of flaxseed oil-containing products (Pan, et al., 2009).

However, with flax oil, they noted that HDL and total cholesterol were only marginally decreased. Researchers noted that there was a consistent substantial decrease in LDL and total cholesterol when whole or milled flax seed was involved. This is probably due to additional functional characteristics present in the flaxseed such as lignans and fiber. The fiber is soluble, which has a known cholesterol lowering effect. These results were most significant in those with mildly elevated blood lipids, men and postmenopausal women (Pan, et al., 2009). Bierenbaum, Reichstein, and Walkins (1993) reported similar results when participants consumed bread containing 15gm of flax/day for three months. After three months, the participants total cholesterol concentrations decreased by 10%, and HDL was not significantly affected.

Perhaps one of the most well known studies on dietary effects on heart health was the Lyon Diet Heart Study: a randomized secondary prevention trial by de Lorgeril, Salen, Martin, Monjaud, Boucher, and Mamelle (1998). The study did not focus on flax in general, but the Mediterranean diet, which is rich in alpha-linolenic acid. This study found that a Mediterranean-style diet not only was preventative in the development of coronary artery disease, but also significantly prevented future occurrences after a cardiac event. While the benefits of the Mediterranean diet cannot be fully attributed to alpha-linolenic acid, several other components of the diet are represented in flax such as fiber, natural antioxidants, and low linoleic acid/alpha-linolenic acid ratios. It has also been demonstrated that the high potassium intake of a flax-containing diet was inversely related to stroke incidence, blood platelet aggregation, and presence of oxygen-scavenging free radicals in blood (Carter, 1993). It has been suggested that those at risk or with family history risk factors of coronary artery disease should adopt a Mediterranean-style diet pattern (de Lorgeril, Salen, & Matin, 1996). While the full

benefits are not clearly understood, as well as the single effect of alpha-linolenic acid, there are substantial positive and relatively no deleterious side effects. A meta-analysis by Howe (2006) reported blood pressure in individuals (both men and women) was positively affected from diets rich in polyunsaturated fats. A -3.4/-2.0mmHg decrease was noted in hypertensive subjects that consumed 5.6gms of alpha-linolenic acid per day (Howe, 2006). In comparison of linoleic and alpha-linolenic acids, alpha-linolenic acid has the ability to act as a vasodilator. Howe (2006) also contributes the increased vascular surface area to decreased production of arachonidoic acid, which is inhibited by diets with favorable linoleic acid/alpha-linolenic acid ratios.

The increase in the alpha-linolenic acid to linoleic acid ratios seems to have the greatest effect on blood and blood systems as well as endothelium membrane integrity (Simopoulos, 2008). Cawood and colleagues (2010) found that vessels with higher levels of alpha-linolenic acid in the actual atherosclerotic plaque were less inflamed than vessels with low alpha-linolenic acid-plaque concentration. This decrease in inflammation leads to more stable vessels that are less likely to rupture spontaneously. Histologically, the alpha-linolenic acid content was inversely related to the amount of T-cells present in the vessels (Cawood, et al., 2010).

Flax owes much of its anti-carcinogenic capabilities to its lignan content. Lignans are phytoestrogens, or plant-based materials that have weak estrogenic activity in mammals. These compounds are also found in soybeans, barley, buckwheat, millet, and oats; however, flax is the richest plant source. The functional component of phytoestrogen in flax is SDG, which is a precursor for estrogen-like materials. This compound is ingested and fermented by gut flora into enterodial and enterolactone in the colon. These metabolites are structurally and chemically similar to estrogen and can bind

normal estrogen receptors. This can cause the inhibition of estrogen-stimulated cancer cells such as breast cancer (Thompson, et al., 1991). Enterodial and enterolactone reduce the risk of cancer by preventing precancerous cellular changes and preventing cell proliferation. The cancerous cells can then be destroyed by the immune system. They can also prevent the metastasis of cancer cells to different tissues and organs (Adolphe, et al., 2010). Thompson and Cunnane (2003) noted increased urinary excretion of enterolactone and enterodial, which was directly related to dietary intake. This urinary excretion was noted to be reduced in women with breast cancer whereas consumption of flax increased urinary concentration of lignans (Thompson & Cunnane, 2003).

While lignans play an important role in reducing the risk of cancers, their anti-inflammatory properties can also be credited with tumor cell prevention. Dietary alpha-linolenic acid has been shown to down-regulate gene expression of potent carcinogenic growth factors (Kaminski, et al., 1993). In a study by Cave (1991), it was demonstrated that supplemental alpha-linolenic acid suppressed tumor growth in experimental models. Cave (1991) also demonstrated that diets high in linoleic acid were able to enhance tumor cell development. Tumor growth requires specific conditions to grow and metastasize. It requires immune factors such as leukotrienes and prostaglandins and specific cellular environments to compete their pathway to spreading. Luekotrienes and prostaglandins are inflammatory markers that are related to tumor cell growth and inflammation. These can be modified by supplementation with alpha-linolenic acid and a decrease in consumption of linoleic acid.

While benefits to cardiovascular health, cancer, and inflammation are key benefits of flax consumption, various other health benefits have been noted. Laxation and bowel movement frequency were shown to improve in elderly patients after consuming flax

muffins for seven days (Hamadeh, Liede, Sanguli, Wolever, & Cunnane, 1992). This improvement is also partially due to the high amount of insoluble fiber present in the flax seed. Some studies have linked flax to improved glucose control. Post-prandial blood glucose levels were reduced 27% in response to consumption of glucose and flax simultaneously (Cunnane, Hamadeh, Liede, Thompson, & Wolever, 1995).

Flax as an Ingredient

Flax is a versatile food ingredient that has been used in cookery for thousands of years with applications in whole form and in oil (Dremucheva, Plandova, & Bessonova, 1995). It has a high protein, fiber, and oil content making it an excellent choice as a food additive. Currently it is used in products such as breakfast cereals, salad dressings, meat extenders, crackers, soups, bagels, and cakes (Carter, 1993). One of the most common vehicles for flax employment is baked products (Craig, 1999). Pseudo-flour can be formed from the milling of flax seeds. This flour provides a strong texture and a rich nutty flavor that works well in enhancing the flavor of leavened breads, waffles, and pancakes when it comprises approximately 6-8% of the dry ingredients (Carter, 1993). Flax is also a vegetarian-friendly source of protein that has functional properties similar to that of soy protein isolates. The protein fraction of flax has a digestibility of 85-90%.

Flax can also be used as an egg substitute in bakery products providing a cholesterol-free fat additive with similar chemical qualities (Meuller, Eisner, Yoshie-Stark, Nakada, & Kirchhoff, 2010). While not useful for deep frying (due it its relatively low smoke point of 225°F), the use of flax oil for stir frying is common practice in China (Pan, 1990). While "generally recognized as safe (GRAS) status" has not yet been reached by flax, the U. S. Food and Drug Administration has made no objection in its use

up to 12%. Flaxseed's unique chemical and nutritional profiles make it an excellent food ingredient. The pseudo-flour derived from flax has been noted to have a gummy texture (Carter, 1993). This unique texture allows for enhanced viscosity and can be seen as favorable in certain products. Flaxseed products also have an excellent water and oil binding capacity that allows them to maintain moisture and freshness (Krause, Schultz, & Dudek, 2002). Unfortunately, this causes flax to have a relatively short shelf-life. In terms of nutritional quality stability, flaxseed-derived SDG was stable in baked products with no significant losses during processing or storage for one week at room temperature, or frozen for one to two months (Hyvarinen, et al., 2006).

While an increasing number of studies involving flax-fortified products are beginning to be seen in the literature, there are also studies involving sensory properties of gluten-free products. However, to date, no studies have been identified that focus on gluten-free products fortified with flax. It was demonstrated that the muffin was an excellent vehicle for flax supplementation of up to 50gm/day (Cunnane, et al., 1995). One study from Trinidad and Tobago found that flax decreased consumer acceptability of muffins; however, the products tested were not gluten-free (Ramcharitar, Badrie, Mattfeld-Beman, Matsuo, & Ridley, 2005). Another study reported that there was limited to no difference in consumer acceptability of banana nut muffins and oatmeal cookies fortified with flax (Alpers & Sawyer-Morse, 1996). To date, no studies have been identified that evaluated the sensory properties of gluten-free flax-fortified products.

Sensory Analysis

Sensory analysis is a method used to test human perception of a product. Product perception not only induces a consumer to initially purchase the product, but to also

purchase the product again. This technique allows products to be developed that will likely be sold based on the theory that the perception of a sample of individuals tasting a product can be extrapolated to a population (Meilgaard, Carr, & Civille, 2007). Sensory analysis generally involves placing participants in a private area and providing them with variable treatments of the product being tested. The panelists are often asked to complete a survey of their perception of each sample. Common perceptional inquiries include flavor, aroma, texture, and appearance (Meilgaard, et al., 2007), which can serve as dependent variables. This can allow the researcher to change specific attributes (in this case, the presence and amount of flax) and determine how the specific amounts being tested relate to sensory acceptability.

CHAPTER III

MATERIALS AND METHODS

Muffin Formulation

A basic gluten-free muffin recipe was adapted from Bob's Red Mill™ website (Bob's Red Mill Natural Foods, n.d.). This recipe was formulated using gluten-free baking flour formulated by Glutino™. This baking mix was a formulation of cereals including white rice flour, potato starch, and tapioca starch to provide structural proteins. It was decided to develop plain spice muffins in order to eliminate pre-conceived consumer biases related to additional ingredients commonly associated with muffins (frosting, fruit, etc). The ingredients of the recipe were listed in English standard measurements, and thus were converted to metric units. Table 1 lists the ingredients included in the muffins.

Preliminary testing was conducted in the Garrison Sensory Evaluation Laboratory in the Department of Food Science, Nutrition, and Health Promotion at Mississippi State University in order to determine appropriate amounts of milled flax to add to the muffins. It was determined that a 3.8% milled-flax concentration would serve as an appropriate moderate amount in muffins and 7.4% milled-flax concentration would serve as an appropriate high-flax muffin based on previous literature (Cunnane, et al., 1995; Ramcharitar, et al., 2005) along with perception of the preliminary panelists. These amounts of flax provided a noticeable difference and still had an acceptable taste. This

study incorporated flax into gluten-free muffins in order to incorporate alpha-linolenic acid for supplementation into baked products.

Table 1 Ingredients for gluten-free muffins with added flax.

Ingredients	Brand	Amount
Gluten-free all purpose-flour	Glutino Gluten Free Pantry®	400gm
Sugar	Kroger® brand	150gm
Baking powder	Clabber Girl®	20gm
Nonfat dry milk	Kroger® brand	20gm
Dried Egg White Powder	Deb El™	10gm
Cinnamon	Kroger® brand	4gm
Nutmeg	McCormick®	2gm
Cloves	McCormick®	2gm
Salt	Morton®	2gm
Water		550mL
Canola Oil	Kroger® brand	80gm
Vanilla Extract	Simply Organic®	10gm
Milled Flax Seed	Bob's Red Mill®	0gms, control (0.0%) 50gms, moderate-flax (3.8%) 100gms, high-flax (7.4%)

To prepare the muffins, the muffin method was utilized (Labensky & Hause, 2006) and all ingredients were gathered in a *mis-en-place* fashion. A Viking™ gas-range dual-burner convection oven was preheated to 325°F (163°C). A convection oven was employed to produce even heating of the muffins and a more consistent product (Molt, 1997). Three mini-muffin pans were greased with Pam™ No-stick cooking spray providing a total of 72 muffin slots. The dry ingredients were weighed on a tared digital kitchen scale (Denver Inst. Company TL-603) including the gluten-free flour, sugar, baking powder, egg white powder, nutmeg, cloves, salt, and cinnamon. The independent variable was flax and muffins containing flax included 50gms and 100gms of milled flax,

respectively. Once weighed, the ingredients were placed in a large mixing bowl and gently sifted. The wet ingredients included canola oil, vanilla extract, and water. The canola oil and vanilla extract were weighed into a separate bowl and 550mL of water were added. A well was made in the center of the dry ingredients and the liquid solution was added. In order to avoid tunneling, the batter was stirred a total of fifty times. It was still lumpy, a trait consistent with the muffin method. A #16 dipper was used to place approximately 2 ounces of batter in the muffin wells (Molt, 1997). The muffin wells were approximately 2/3 full and each batch of batter produced 72 small muffins. The muffins were then baked for 17 min. At the end of 17 min, a toothpick was inserted into the center of a muffin to ensure doneness of the batter. The muffins were left to cool in the wells for 10 min. After 10 min, they were placed into small plastic ramekins with their assigned 3-digit random number.

Nutrient analysis of the muffins was performed using the U.S. Department of Agriculture nutrient database for standard reference (U.S. Department of Agriculture, Agricultural Research Service, 2010).

Institutional Review Board Approval

Approval from the Mississippi State University Institutional Review Board was obtained (#10-161) on October 11, 2010 (Appendix A). This was completed to ensure protection of human subjects that would be participating in taste panels. Written consent was provided from all panelists prior to tasting the muffins (Appendix B). This consent expressed the purpose of the research project, confidentiality policies, notification of voluntary participation, and contact information of the researchers. A complete list of ingredients was also provided to ensure protection against food-related reactions.

Sensory Analysis

The sensory panels were set-up in assembly-line fashion. The muffin samples were placed into three plastic ramekins with their perspective random number printed on the side of the ramekin. The tasting booths were private and panelists could push a button that lit up on the other side of the panel booth if they had questions. Sensory analysis of the muffins was performed at the Garrison Sensory Evaluation Laboratory at Mississippi State University. The panelists provided consent upon entry to the laboratory. The panelists entered the booth and were presented three different muffin samples, a glass of water, an expectorant cup, a napkin, a pencil, and a score sheet/survey (Appendix C). The lighting condition in the booths was white overhead lighting.

The panelists were asked to provide demographic information including gender, age, frequency of consumption of gluten-free products, preferences of muffins, and muffin-type preference. They were then instructed to taste each sample and rate appearance, aroma, flavor, texture, and overall acceptability on a scale of 1-9. A Hedonic rating scale was employed as follows: 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely (Meilgaard et al., 2007). Score sheets/surveys listed the three samples in different order to provide more consistent ranking among all three trials and to prevent bias. A space for additional comments was also provided on the score sheet/survey (Appendix C). The samples presented contained a control muffin, a moderate-flax muffin, and a high-flax muffin. The samples were placed in covered plastic ramekins and assigned randomly-generated 3-digit numbers to control for consumer number association. The random numbers are presented in Table 2. Between tasting each sample, panelists were asked to expectorate the sample in a cup

provided or swallow the sample. This taste panel was repeated on three different days from approximately 10:30am-1:00pm. Each trial consisted of at least 50 panelists.

Table 2 Random numbers assigned to each treatment.

Treatment	1st panel	2nd panel	3rd panel
Control	131	335	245
Moderate-flax	498	154	416
High-flax	389	928	795

Statistical Analysis

Statistical analysis of the data was performed using SAS (version 9.1.2, 2005). A randomized complete block design with three replications/panels ($n \geq 50$ per replication/panel) was used to determine if differences existed among treatments. Significance was determined at a p value of less than 0.05. When significant differences occurred ($p<0.05$), the Fisher's Protected Least Significant Difference (LSD) test was used to separate treatment means. Cluster analysis was conducted to group consumers together according to their preference and liking of treatments. Consumers were grouped into clusters based on results of the dendrogram and dissimilarity plot and differences ($p<0.05$) were determined between treatments among clusters. Demographic data were analyzed using SPSS (version 18.0, 2010). Age of participants is reported as years $\pm$ standard deviation (SD).

CHAPTER IV

RESULTS AND DISCUSSION

The survey (Appendix C) inquired basic demographic information including age and gender. Other inquiries on the survey included consumption of gluten-free products, likeness of muffins, and types of muffins liked. Participants (N=152) ranged in age from 19 to 63 years old with a mean age of 32.2 years old $\pm$ 12.5 SD. Of the panelists, 70.2% were female. When asked whether or not you consume gluten-free products, 20.9% of the participants indicated "yes." The panelists were also asked how well they like muffins in general (Table 3); the mean score was 4.6 ± 0.6 SD on a five-point Likert Scale (1=No, dislike very much, 3=neither like nor dislike 5=Yes, like very much). A majority (96%) of the panelists indicated that they either like muffins "very much" or "like somewhat." Only two panelists indicated that they had any distaste for muffins and four panelists indicated that they "neither like nor dislike" muffins.

Table 3 Panelists' responses to: "Do you like muffins?"

	n	%
Very much	95	63
Like somewhat	49	33
Neither like nor dislike	4	3
Dislike somewhat	1	1
Dislike very much	1	1

(1=No, dislike very much, 3=neither like nor dislike, 5=Yes, like very much)

The panelists were asked which types of muffins they liked (Table 4). It was determined that blueberry and cake-like muffins were ranked the highest with 77.0% and

67.1%, respectively. Banana-nut muffins were followed with 62.5% of panelists indicating that they liked these. Spice muffins, the muffin vehicle for this study, ranked next with 42.8% of panelists indicating predilection. Bran muffins followed by plain muffins received the lowest levels of consumer propensity with 38.2% and 32.9%, respectively, indicating that they also liked these muffins. Other muffins liked were indicated by 23.7% panelists and not listed on the survey (Appendix C), but were written in by panelists and included sweet potato muffins, oat muffins, and lemon-poppyseed muffins. Based on this data, the panelists tended to like sweeter-tasting muffins with added ingredients such as fruit and sugar over traditional dinner muffins.

Table 4 Percentage of consumers that like different types of muffins.

	Yes (n)	Yes (%)	No (n)	No (%)
Plain Muffin	50	32.9	102	67.1
Bran Muffin	58	38.2	94	61.8
Cake-like	102	67.1	50	32.9
Banana Nut	95	62.5	57	37.5
Blueberry	117	77.0	35	23.0
Spice	65	42.8	87	57.2
other	36	23.7	116	76.3

Differences ($p<0.05$) in sensory properties were observed in appearance, flavor and overall acceptability of the gluten-free muffins with added flax, while no differences ($p>0.05$) were observed in aroma or texture (Table 5). On average, the panelists liked the appearance of the control muffins more ($p<0.05$) than muffins from the moderate- and high-flax treatments. This indicates that use of flax has a slightly negative impact on the acceptability of the muffins appearance. However, the appearance of all muffin treatments were liked moderately, indicating that there was minimal practical difference

in the appearance of muffins. The flavor of the control and high-flax muffins were liked more ($p<0.05$) than the muffins from the moderate-flax treatment. The flavor of the muffins was ranked lower in the moderate-flax treatment ($p<0.05$) but there was no difference ($p>0.05$) between the control (no flax) and the high-flax muffin. Addition of milled flax generally gives baked products a rich, nutty flavor which is considered favorable in leavened breads and also quick breads, including muffins (Carter, 1993). On average, the mean scores for overall acceptability were consistent with flavor ratings in subsequent treatments. The moderate-flax muffins were ranked lower ($p<0.05$) than the control and high-flax muffins, but there was no difference ($p>0.05$) between the control and high-flax muffins. These results are similar to those from a previous study in which a 7.3% flax concentration was rated similarly to a 0% (no flax) control muffin (Ramcharitar, et al., 2005). These results indicate that a higher concentration may suggest an "all-or-nothing response" to the addition of milled flax to gluten-free muffins for flavor and overall acceptability.

No difference ($p>0.05$) was observed between treatments with respect to the acceptability of aroma and texture (Table 5). This is surprising considering that the addition of milled flax generally produces a stronger, firmer texture when added to baked products (Carter, 1993). Perhaps the oil-binding capacity of the milled flax allowed for the softening characteristic of the fat to be perceived by consumers.

Table 5 Mean scores for consumer acceptability (N=152) of gluten-free muffins with three treatments.

Treatment	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Control (0% flax)	7.3[a]	7.3[a]	6.7[a]	6.4[a]	6.7[a]
Moderate flax (3.8%)	7.0[b]	7.2[a]	6.3[b]	6.4[a]	6.4[b]
High flax (7.4%)	6.9[b]	7.4[a]	6.6[a]	6.6[a]	6.7[a]

[a-b] Mean scores within a column with different letters are significantly different ($p<0.05$). Scores were based on a Hedonic Scale: (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely).

Cluster Analysis

Agglomerative hierarchical clustering was performed to further determine attitudes of the consumers toward gluten-free muffins with added flax; this would allow for determination of consumer groups that have similar liking and preferences of muffins based on similar survey responses. A dendrogram and dissimilarity plot were generated to determine commonalities among panelists. It was determined by cluster analysis that there were five distinct groups of panelists (Table 6).

Table 6 Mean scores for consumer acceptability of gluten-free muffins according to different clusters of consumer segments.

Cluster	Consumers (n)	Consumers (%)	Control	Moderate-flax	High-flax
1	21	14.3	7.2[a]	5.1[b]	7.3[a]
2	79	52.7	7.5[a]	7.5[a]	7.5[a]
3	11	7.5	2.9	3.0	4.3
4	26	17.7	6.2[a]	5.5[b]	4.9[b]
5	10	6.8	4.4	5.7	7.0

Mean scores within a row with different letters are significantly different ($p<0.05$) Scores were based on a Hedonic Scale: (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely).

Panelists from cluster one (n=21, 14.3%) liked the control and high-flax treatments moderately and preferred ($p<0.05$) muffins from these treatments over the control treatment, which was neither liked nor disliked. Panelists from cluster two (52.7%) liked all muffin treatments between "like moderately" and "like very much." This was the largest group of consumers, and this group has a high degree of liking for muffins made with and without flax. Panelists from cluster three did not like muffins from any treatment, and is therefore not representative of consumers who like gluten-free muffins. Cluster four, which contained 17.7% of the panelists, preferred only the control muffins ($p<0.05$) over the flax treatments, and acceptability decreased as flax percentage increased in the sample. These consumers did not like the sensory properties that flax imparted to the muffins. Panelists from cluster five (6.8%) liked muffins more as flax percentage increased and therefore liked the sensory properties that flax imparted to the muffins. Results further indicate that 92% of the panelists that liked at least one muffin treatment liked the control muffins, 81% of the panelists that liked at least one muffin treatment liked the high-flax treatment, and 57% of these panelists liked the moderate-flax treatment. This indicates that a high percentage of consumers that like the muffin treatments would like muffins from the control or high-flax treatment.

Nutrient Analysis

The control muffins contained 69.3% carbohydrate, 4.0% protein, and 26.7% fat with relatively no fiber. The addition of milled flax in the moderate-flax treatment (3.8% milled flax) contributed relatively few kcal, grams of fat, protein, and small amount of fibrous carbohydrates changing the nutrient ratios to 65.7% carbohydrate, 5.5% protein, and 28.8% fat with additional 64mg alpha-linolenic acid per muffin. The final

composition of the moderate-flax treatment was 66% carbohydrate, 6% protein, and 28% fat. The high-flax treatment (7.4% milled flax) also contributed relatively few kcal, grams of fat, protein, and small amount of fibrous carbohydrates changing the nutrient ratios to 62.7% carbohydrate, 6.8% protein, and 30.5% fat with additional 129mg alpha-linolenic acid per muffin (U.S. Department of Agriculture, Agricultural Research Service, 2010).

Limitations of Study

While it was originally thought that a spice muffin would provide a neutral but well-accepted muffin for consumers, it may not have been the most appropriate choice for testing sensory qualities of added flax. Some of the write-in comments from the survey referred to "too strong nutmeg flavor" and "too much nutmeg." This flavor may have interfered with some panelists' ability to differentiate properties of the treatment being tested. Also, when questioned on types of muffins preferred, only 43% of the panelists indicated that they liked spice muffins. Perhaps a different type of muffin such as blueberry or banana nut would be appropriate vehicles for further study. Only 20.9% of the panelists indicated that they ordinarily consumed gluten-free products. This provides limitation because the target market for gluten-free products was not well represented in this sample. These data may indicate lack of consumer preference for gluten-free products, lack of consumer knowledge of gluten-free products, and lack of overall availability of the products as stated in previous studies (Lee, et al., 2007).

CHAPTER V

CONCLUSIONS

In this study, muffins were a successful vehicle for testing acceptance of flax (*Linum usitatissimum*) added to a gluten-free baked product. Consumers were grouped into five clusters based on how they rated the muffins for appearance, flavor, texture, aroma, and overall acceptability. Overall, consumers preferred gluten-free muffins without flax or antithetically high amounts of flax (7.4%); moderate amounts of flax (3.8%) were liked less by panelists ($p<0.05$). This can allow producers of gluten-free products to maximize nutritional properties along with consumer acceptance of gluten-free muffins. Applications for further study should include the use of different gluten-free flours. This study employed a blend of white rice flour, potato starch, and tapioca starch. The characteristic flavor of each of these flours may have affected sensory perception of the treatments. Also, it would be beneficial to experiment with different types of muffins to eliminate possible consumer bias.

The findings of this study can be helpful for medical practitioners as well as food manufacturers in recipe development for both home use and commercial preparation. Registered dietitians can provide celiac patients with recipes that can not only be tolerated without causing inflammation, but can incorporate nutrients into the diet that are often lacking in diets requiring strict avoidance of gluten. Flax is an excellent source of alpha-linolenic acid that can provide many nutritional benefits. Recipes with added flax can utilize the functional properties of alpha-linolenic acid for disease prevention such as

heart disease and cancer, and possible reduction in inflammation caused by gluten. Future development of the gluten-free market is not only wide open and versatile, but likely to receive more consumer attention considering the increasing incidence of celiac disease.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

MISSISSIPPI STATE UNIVERSITY

Compliance Division
Administrative Offices
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1207 Hwy 182 West
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October 11, 2010

Adam Woodyard
439 Mallory Lane
Starkville, MS 39760

RE: IRB Study #10-161: Consumer Acceptability of Added Flaxseed to Gluten-Free Muffins

Dear Mr. Woodyard:

The above referenced project was reviewed and approved via administrative review on 10/11/2010 in accordance with 45 CFR 46.101(b)(6). Continuing review is not necessary for this project. However, any modification to the project must be reviewed and approved by the IRB prior to implementation. Any failure to adhere to the approved protocol could result in suspension or termination of your project. The IRB reserves the right, at anytime during the project period, to observe you and the additional researchers on this project.

Please note that the MSU IRB is in the process of seeking accreditation for our human subjects protection program. As a result of these efforts, you will likely notice many changes in the IRB's policies and procedures in the coming months. These changes will be posted online at http://www.orc.msstate.edu/human/aahrpp.php. The first of these changes is the implementation of an approval stamp for consent forms. The approval stamp will assist in ensuring the IRB approved version of the consent form is used in the actual conduct of research. You must use copies of the stamped consent form for obtaining consent from participants.

Please refer to your IRB number (#10-161) when contacting our office regarding this application.

Thank you for your cooperation and good luck to you in conducting this research project. If you have questions or concerns, please contact me at cwilliams@research.msstate.edu or call 662-325-5220.

Sincerely,

For use with electronic submission

Christine Williams
IRB Compliance Administrator

cc: Diane Tidwell (Advisor)

APPENDIX B

INFORMED CONSENT FORM

Informed Consent Form (You must be at least 18 years old to participate)

Title of Study: Consumer acceptability of added flaxseed to gluten-free muffins

APPENDIX C

SURVEY

Evaluation of Muffins **Date:** _____

1. What is your gender? ____Female ____Male

2. What is your age? ____ years old

3. Do you consume gluten-free baked products: ___Yes ___No

4. If you answered "yes" above, why do you eat gluten-free products?

5. In general, do you like muffins?

___Yes, like very much
___Yes, like somewhat
___Neither like nor dislike
___No, dislike somewhat
___No, dislike very much

6. If you like muffins, which of the following do you like? (check all that apply)

___Plain muffins ___Blueberry muffins
___Bran muffins ___Spice muffins
___Chocolate, strawberry, or sweet flavored muffins (cake-like)
___Banana nut muffins
___Other, please list_____

Please evaluate each sample independently. After tasting, if you do not wish to swallow the sample, you may expectorate in the cup and rinse with the water provided.

Each column will need one check mark if you choose to evaluate all samples.

Sample 154	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Please turn over →

Sample 335	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 928	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Please feel free to provide any comments about the muffins_____

_____

Thank you for your participation