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The development of gluten-free milk-free French bread

Annette Camnetar Bentley
Louisiana State University and Agricultural and Mechanical College, abentl1@tigers.lsu.edu

THE DEVELOPMENT OF GLUTEN-FREE MILK-FREE FRENCH BREAD

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Annette Camnetar Bentley
B.A. Rutgers University, Newark, New Jersey, 1991
M.S. New Jersey Medical School, 2003.
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ABSTRACT

Approximately 6% of children and approximately 4% of adults in the western countries including the United States (US) have food allergies. Milk allergy is reported to be one of the most common food allergies affecting as high as 7% in the US. Celiac disease affects approximately 1% of the US population. Such individuals are required to maintain diets restricting milk and gluten. Autism is estimated to affect over 673,000 in the US. A milk-free gluten-free diet is recommended for autism. The gluten grains identified are wheat, oats, barley and rye and any by-products or cross-bred grains of these products. Foods containing milk and milk by-products include those with casein, whey, curds, and glycomacropeptide (GMP). Literature search indicates problems including high cost, labeling, difficulty in finding specialty items, and quality contributed to difficulties maintaining a gluten-free diet.

A study of several local specialty stores and groceries to establish the availability of gluten-free food products that were also milk-free was performed. Celiac consumers were asked to complete a survey on the gluten-free products found in the store survey. The survey was to identify product problems. The conclusion from these two studies was that gluten-free bread products was the most unsatisfactory and there was a need to develop a desirable bread product.

Two gluten-free milk-free French breads were developed comparable to wheat French bread. Several gluten-free flours and combination gluten-free flours were tested using the Rapid Visco Analyzer (RVA). Texture, color, microbiological analyses and gluten testing procedures were performed. General and target sensory population studies were performed. The non-Celiac population results revealed marginal acceptability. The Celiac population sensory study rated the gluten-free milk-free breads as acceptable. Intent to purchase both gluten-free loaves of bread was rated acceptable.

CHAPTER 1. INTRODUCTION

1.1 GLUTEN-FREE MILK-FREE NEED AND JUSTIFICATION

There is a serious need for prepared food items that are both milk-free and gluten-free. Gluten-free items often contain milk. The products created as milk-free are not necessarily gluten-free. The need has arisen because of individuals that have food allergies and/or intolerances.

Approximately 6% of children and approximately 4% of adults in the western countries including the US have food allergies (Gonipeta and others 2009). Milk is the most common of food alergies reported to affect up to 7% of the population in the United States (Agamy E, 2007). Approximately 1% of the US population has Celiac disease (Alvarez-Jubete and others, 2010 and Fasano and others, 2005). This condition results because of an immune reaction to the gliadin contained in the protein of wheat and similar grains (Alvarez-Jubete and others, 2010 and Fasano and others, 2003). The toxicity of the gluten grains has been identified in the gliadin protein, which is found in wheat, triticale, rye, barley, and oats (Charbonnier and others, 1980 and Ylimarki and others, 1989). Autism is estimated to affect 1 in 88 children (Velasquez-Manoff, 2012). It is recommended that individuals with autism be on a combined gluten-free milk-free diet. The proposed ruling of the FDA suggested that products be labeled gluten-free if the final product does not contain any ingredients that include or are derived from wheat, barley, rye or a cross of these grains, e.g. triticale. Another stipulation is that the final product contain <20 parts per million of gluten. The FDA has not made a final ruling on this time (Thompson and Mendez, 2008).

The result of consuming the wheat protein causes the enzyme tissue transglutaminase to modify he protein and the immune system reacts by causing an inflammatory reaction

(Niewinski, 2008). This causes the destruction of the lining (the villi) of the small intestine which interferes with the absorption of nutrients. The only treatment for this problem is to avoid consuming products that contain gluten for life. Lessof (1985) reported that cow's milk provokes gluten enteropathy. It is strongly recommended Celiacs avoid any products with milk because of the absence of microvilli and villi. Villi are the mechanism by which gluten absorbs into body through the intestinal tract. The gluten flattens the villi and the individual cannot process gliadin protein from the grains. The microvilli are the mechanism for processing the milk sugars and proteins.

Te major concern of individuals with intestinal problems resulting from milk protein intolerance, lactose intolerance, milk sensitivity and/or milk allergies who are advised to avoid milk products is that they will not have a sufficient amount of calcium in their diets (Buckowski and others, 2010). The fact remains that milk is not the only source of calcium. Calcium is present in multiple food items. Such foods include vegetables (avocados, broccoli, Chinese cabbage, collard greens, kale, rhubarb, and turnip greens), fish/seafood (oyster, salmon, sardine and shrimp), fruit (apple, apricots, banana, dates, and oranges), rice, and other products including molasses and tofu (Buchowski and others, 2010 and USDA, 2010). A second concern is how efficient are the milk substitute products in preparing recipes that require milk. The milk substitutes including soy, rice, coconut milk, almond, and others for the most part easily work in preparing most recipes that call for milk including pastry products, casseroles, and even are used as a cheese (Miñarro and others, 2010).

There is a serious need to provide a quality and comprehensive supply of specialty food products that are both gluten-free and milk-free to meet the need of individuals with a combined intolerance or allergies to milk and gluten. Currently, there is a limited amount of prepared food items that are both milk-free and gluten-free. There is a growing market for such products. Celiac disease affects 1% of individuals in the US. Celiac disease is believed to be currently under diagnosed (Fasano and others, 2003) and such individuals are highly recommended to be on a gluten-free and milk-free diet. Autism is estimated to affect 1 in 88 children (Velasquez-Manoff, 2012). It is recommended that individuals with autism be on a combined gluten-free milk-free diet which has been documented to improve autistic problems such as learning and behavior (Johnson, 2009).

Individuals having gluten intolerance, such as Celiac disease, have damaged villi. Therefore, the microvilli on these villi are non-existent. For this reason, besides avoiding gluten-gliadin grains, it is necessary for them to avoid milk produce. Currently, there are products labeled gluten-free, however all are not milk-free. Some are not pleasing to the palate and consequently, make it difficult for individuals on such a diet to continue eating such a diet.

Other studies have looked at the availability of gluten-free products. This research includes gluten-free products that are also milk-free because of the need to be on the combined diet at least initially by all Celiac persons and for many life-long.

The first objective of this study was to determine the availability to consumers in the local market of products that are gluten-free and milk-free. The second objective was to determine what key sensory problems existed with gluten-free products. The third objective was to develop a gluten-free milk-free French bread. There is an inadequate supply of quality tasting gluten-free products that are also milk-free to meet the needs of consumers on special diets, even something as basic as French bread. Using alternative gluten-free ingredients it may be possible to develop gluen-free milk-free French bread. The gluten-free ingredients do not have the elasticity and texture of the gluten grains so creating similar products may take several ingredients to create the same texture and taste.

This thesis has five chapters. Chapter one provides a brief introduction including the objective, hypothesis, assumptions, limitations, and justification of the study. Chapter two covers a literature review including key related similar studies. Chapter three provides the materials and methods used including two surveys, product development, sensory analyses and statistical analyses. Chapter four provides the results and discussion of the surveys, sensory evaluation and statistical analyses. Chapter five provides the conclusion of the thesis. The final section includes a copy of the consumer questionnaire, the research consent forms, analyses codes and other items. The last page consists of a brief VITA of the authors work.

CHAPTER 2 LITERATURE REVIEW

2.1 RELATED STUDIES

2.1.1 Celiac Disease

Celiac disease is not the only one of the reasons for maintaining a gluten-free and in many cases also a milk-free diet. Many other medical problems require such a diet, such as allergies, intolerances, autism, and others (Gonipeta and others 2009 and Velasquez-Manoff, 2012). The prevalence of diagnosed Celiac disease in the US is reported to affect 1 in 130 (Fasano and others, 2003). Rubo-Tapia and others (2009) reported a dramatic increase incidence and mortality of Celiac Disease in the past 50 years. There is a discrepancy in the prevalence of Celiac disease because symptoms are often attributed to other medical problems and usually delays the diagnosis for many years (Green and Jabri, 2003). The classical symptoms associated with Celiac disease include: abdominal pain and bloating, cramping, chronic diarrhea, vomiting, constipation, and failure to thrive. Other symptoms exhibited include fatigue, behavior changes, irritability, depression, bone and joint pain, dental problems, infertility, osteoporosis, skin problems and a host of other medical problems (Ferrell and Kelly, 2002). Another important fact is that Celiac disease in an autoimmune disease and a genetic disorder. Therefore, individuals with Celiac disease have a higher probability of having other autoimmune diseases and more than one family member may have this problem (Catassi and others, 2002). The only recognized acceptable treatment for Celiac disease is maintaining a gluten-free diet for life. The result of consuming the gluten grains is damage to the intestinal villi. There has been considerable controversy as to what is the acceptable amount of gluten that may be consumed by individuals with Celiac disease that would not cause more damage to the intestinal mucosa.

2.1.2 Milk Allergies or Intolerances

The most common food allergy is to cow's milk reported to affect from 0.3 to 7% of the population (El-Agamy, 2007). Reactions can be immediate or delayed. Allergy is a reaction to the foreign protein. Allergies can affect the skin or mucous membrane if entering the blood stream. The main causes of milk allergy are the casein and β-lg in cow's milk. Other problems noted in patients with persistent allergies the five IgE-binding epiopes. (El-Agamy, 2007). The treatment for cow's milk allergy is the elimination of milk from the diet (Tuokkola and others, 2010). Asero and others (2010) reported milk allergies include sheep, goat and cow milk. Milk allergy can be a cross reaction between the homologous allergens in milk from these animals. According to a report by Schanchez-Valverde (2009) potential contributing factors to infants having allergy can be attributed post caesarean section, the ingestion of formula during the first few days of life. Concern of consumption of milk could be a contributing fact in children with type I diabetes (Michalski, 2007). A report from the National Institute of Diabetes and Digestive and Kidney Diseases (2009) stated that primary lactose deficiency develops over time and normally begins at about the age of 2. The report further stated that lactose intolerance is linked to genetics and that Hispanics Americans, African Americans, and Asian Americans are the ethnic populations at high risks for the problem. The report also stated that secondary lactose deficiency is caused by small intestine injury from severe diarrhea, celiac disease, Crohn's disease and chemotherapy. According to the German Institute and Efficiency in Health Care (2012) as many as 1 in 5 adults, teenagers and children in northern Europe suffer from lactose intolerance. Shrier and others (2008) reported that individual with osteoporosis, obesity, diaetes, hypertension, cataracts, cardiovascular disease, multiple sclerosis, prostate cancer, ovarian cancer, breast cancer, colorectal cancer as well as other medical problems were linked to consumption of milk and other dairy products.

Celiac disease affects 1% of individuals in the US (Fasano and others, 2003) and such individuals are highly recommended to be on a gluten-free and milk-free diet. Autism is estimated to affect 1 in 88 children (Velasquez-Manoff, 2012). It is recommended that individuals with autism be on a combined gluten-free milk-free diet (Johnson, 2009).

2.1.3 Problems with Gluten-Free Diet

Identified problems with compliance of a gluten-free diet include the lack of available gluten-free alternatives in social settings (Olsson, 2008). The cost of gluten-free products is considerable higher then gluten containing similar products. Another reason for the lack of compliance is the limited education on what is gluten-free and the fact that even the smallest amount of consumption of gluten containing product continues to damage the intestinal villi. Falini (2009) reported that 50% of the pediatric celiac population does not comply with the dietary requirements. This report also stated that the parents' attitude is major influence the child's compliance.

Individuals on a gluten-free diet find it difficult to dine out. In a survey of catering outlets by McIntosh and others (2011) it was found that even the staff of such companies lacked confidence that the food could be guaranteed as gluten-free. Menu choices labeled gluten-free when tested were found to contain 18 to 1820 mg kg1 of gluten. It was also reported that very little formal training was provided to restaurant staff. Training was provided informally by the head chef or manager with no record that the managers had received any formal training. Another survey by Zarkadas and others (2006) included individuals reporting avoiding eating out and traveling because of being a Celiac. Zarkadas and others (2006) reported that there were problems finding stores that sold gluten-free products and there was a need for better food labeling to identify gluten-free foods. Concerns of even being hospitalized were reported because it was difficult to maintain a gluten-free diet in a hospital.

Celiac disease not only affects the individual with the problem but extends beyond to family and friends. A study collecting information from family members and friends of individuals diagnosed with Celiac disease (Sverker and others, 2007), focused on the dilemmas experienced by close relatives of individuals with Celiac disease. Disease-related worries, management of daily life, social life, relationship with friends and interaction with service professionals in restaurants and shops were concerns. Close relatives reported feeling bad and concerned when the gluten intolerant person could not eat food served at parties or restaurants. The report stated that they experience less pleasure at social events because of the anxiety related to the risk of their family member or friend not being able to fully participate in the event. The report stated there were concerns even in their own homes of possibly contaminating the food of the Celiac. A major concern was how the restricted freedom contributed to daily life. Some subjects reported that serving only gluten-free food was the only logical option. In some cases relatives of Celiac individuals were afraid that even casual conversation or upsetting news could make the Celiac individual ill. The study pointed out the need for education to families and society of not only the diet but the need to explore communications, emotions, and relationships in such situations.

Nutritional concerns of a gluten-free diet were studied by Lee and others (2009). In their study a review of the Celiac's diet was done by a dietitian who is a specialist in Celiac disease. The study reported that substituting the alternative grains increased the nutrient profile significantly. Tables 1 and 2 (USDA, 2010) list the calories, carbohydrates, fat, fiber and proteins of both gluten flours and gluten free flours.

In a study to investigate the accuracy of labeling gluten-free, Cawthorn and others (2010) reported that ten of seventeen labeled gluten free products contained gluten. This clearly indicates the misleading problems faced by individuals attempting to maintain a gluten-free diet.

Table 1 Calories, Carbs, Fat, Fiber and Protein in Flours (USDA, 2010)

Flour Type 1 CUP	Calcium mg	Calories	Carbohydrate grams	Fat Total grams	Fiber, Total grams	Protein grams
Peanut Flour, Defatted	84	196	20382	03.33	9.5	31.32
Sunflower Seed Flour	73	209	22.93	1.03	3.3	30.76
Carob Flour	358	229	91.55	0.67	41.0	4.76
Cottonseed Flour	449	337	38.11	5.83	2.8	38.50
Chickpea Flour (bean)	41	356	43.19	6.15	9.9	20.60
Soy Flour	173	366	29.56	17.35	8.1	29.01
Rye Flour, Light	21	374	81.83	1.39	14.9	8.56
Buckwheat Flour, Whole Grain	49	402	84.71	3.72	12.0	15.14
Wheat Flour , Whole Grain	41	407	87.08	2.24	14.6	16.44
Rye Flour, Dark	72	415	87.99	3.44	28.9	17.96
Corn Flour, Whole Grain Yellow	8	422	89.91	4.52	8.5	8.11
Corn Flour, Whole Grain White	8	422	89.91	4.52	8.5	8.11
Tricale Whole Grain Flour	46	439	95.08	2.35	19.0	17.3
Wheat Flour, White All Purpose	19	455	95.39	1.23	3.4	12.91
Arrowroot Flour	51	457	112.83	0.13	4.4	0.38
Barley Flour	47	511	110.29	2.37	14.9	15.54
Potato Flour	104	571	132.93	0.54	94	11.04
Brown Rice Flour	17	574	120.84	4.39	7.3	11.42
White Rice Flour	16	578	126.61	2.24	3.8	9.40
Acorn Flour	96	1136	123.92	68.4	0.0	0.0
Sesame Seed Flour	360	1192	60.4	84.16	0.0	69.84

Table 2 Calcium, Iron, Niacin, Sodium, Zinc Content in Types of Four (USDA, 2010)

Flour Type 1 CUP	Calcium mg	Iron mg	Niacin mg	Sodium mg	Zinc mg
Acorn Flour	96	2.72	5.4	0.0	1.44
Arrowroot Flour	51	0.42	0.0	3	0.09
Barley Flour	47	3.97	9.278	6	2.96
Brown Rice Flour	17	3.13	10.017	13	3.87
Buckwheat Flour, Whole Grain	49	4.87	7.380	13	3.74
Carob Flour	358	3.03	1.954	36	0.95
Chickpea Flour (bean)	41	4.47	1.621	59	2.59
Corn Flour, Whole Grain White	8	2.78	2.223	6	2.02
Corn Flour, Whole Grain Yellow	8	2.78	2.223	6	2.02
Cottonseed Flour	449	11.90	3.821	33	10.99
Peaut Flour, Defatted	84	1.26	16.200	108	3.06
Potato Flour	104	2.21	5.611	88	0.84
Rye Flour, Dark	72	8.26	5.466	1.0	7.19
Rye Flour, Light	21	1.84	0.816	2	1.78
Sesame Seed Flour	360	34.4	30.32	96	24.16
Soy Flour	173	5.35	3.629	11	3.29
Sunflower Seed Flour	73	4.24	4.680	2	3.18
Tricale Whole Grain Flour	46	3.37	3.718	3	3.46
Wheat Flour , Whole Grain	41	4.66	7.638	6	3.52
Wheat Flour, White All Purpose	19	5.80	7.380	2	0.88
White Rice Flour	16	0.55	4.092	0.0	1.26

2.1.4 Assays for Gluten-Free

The protein in wheat grain consists of approximately 80 to 90% gluten. In the rye grain the gluten protein consist of 70 to 80 %. The barley grain contains approximately 60% of the glutn protein. The properties of the gliadin and glutenin are the key factors in the elasticity of dough. However, for individuals with Celiac disease these proteins are not digestible and cause damage to the intestinal tract. There are several immunoassays to measure the antibodies Skerritt and Hill (1990) and R5 quantization (Allred and Ritter, 2010). In this research four commercial ELISA assays were used. The results of this study showed discrepancies of gluten detection and quantification between Skerritt (1985) and R5 base test. Two other studies show discrepancies in the assays results (Thompson and Mendez, 2008 and Gélinas and others, 2008) indicating that using different assays does not reveal the accurate content of gluten in barley.

An investigation into the efficiency and limitations of the immunochemical assays for testing of gluten-free food was performed by Denery-Papine and others (1999). In this study the research compares as many as fifteen different assays. For the assay formulated by Ciclitira and Lennox (1983), which is a competitive RIA, it was noted that no rye, barley and oats prolamins could be detected. The immunochemical test the simple ELISA with nitrocellulose support developed by Skerritt (1985) had a sensitivity of 200,000 ng/ml gliadins. Skerritt and Hill (1990) developed formulations of sandwich ELISA indicating sensitivity of 100 ng of gliadins/mL. He then developed a formula sandwich ELISA with a sensitivity of 16 mg gluten/100 g. Skerritt and Hill (1990) revised formulations are the assays currently being accepted by AOAC. The major problem is that this is the only detection system being used. In reviewing a formulation by Troncone and others (1986), sandwich ELISA had a sensitivity of 5 and had a cross-reaction with rice and maize prolamins. Freedman's and others (1987) formulation of a sandwich ELISA had a sensitivity of 15 ng/ml gliadins and was able to detect the prolamines in wheat, oats, barley, and rye. Comments were made on the results of Friis' (1988) competitive ELISA which showed sensitivity at 10 ng/ml gliadins. Ayob's and others(1988) competitive ELISA had a sensitivity of 30 ng/ml gliadin and Mills' and others (1989) sandwich ELISA had sensitivity 30 ng/ml gliadins. Ellis and others (1994,1998) produced two versions of a sandwich ELISA which provided sensitivities 4ng and 15 ng gliadin as well as 0-6 mg gluten/100 g and 0-016 mg gluten/100 g. Detection of barley, rye, and oats was possible with no cross-reaction to maize, rice, millet, and sorghum. The method of Chirdo and others (1995, 1998) sensitivity with 1 ng gliadin/ml and 0-1 mg gluten/100 g detected tricale, barley, rye, and oats prolamins showed no cross-reactions. Further developed competitive and sandwich methods which ranged from 1 to 20 ng gliadins/ml and 0-5mg gluten/100g (Sorell's and others, 1998) sandwich method showed a sensitivity of 3ng gliadins and 0-3 gluten/100g. Andresh and other's (1995) sandwich showed a sensitivity of 25 ng. There was detection of barley prolamines but cross-reaction with maize.

Such problems with the recognized tests for identifying the content of gluten in products justifies concerns that even using such test does not provide a safety net for individuals searching for accurate labeling. A case report by Biagi and others (2004) reported that an individual consuming as little as 1 milligram of gluten a day was found to have continued damage of the intestinal mucosa. He further reported that despite the fact that even with the strict European standard of labeling gluten-free only if the products had <200 parts per million of gluten that 6 % of these products tested contained more than 300 mg of gliadin/kg. A study by Catassi and others (2007) was done in which an attempt was made to determine a safe gluten threshold. The justification for his study was that the researcher felt it was almost impossible to maintain a zero gluten free diet. Certainly, this is a problem when companies such as mentioned by Biagi and others (2004) label products gluten-free and in fact know they contain gluten. Catassi and others (2007) found that Celiacs, despite resolution of symptoms, continued to have persistent inflammatory damage to the intestinal mucosa. This he stated was related to intentionally or inadvertently consuming gluten. Serious consideration of revising such standards that continue to falsely claim a product is safe for a Celiac individual to consume must be considered.

2.1.5 Availability and Cost of Specialty Items

A study was done by Lee and others (2007) comparing the food prices of foods that would be needed for a gluten-free substitute such as bread, pasta, crackers, cereal, waffles, cookies, pretzels, pizza and macaroni with cheese. The results of the study demonstrated a difference in the availability of gluten-free foods varied between stores and regions. The largest selection of gluten free, 94% of products, could be found in health food stores. However, in the upcale groceries the availability of gluten-free products averaged at 42%. In regular groceries the average was 36% of gluten-free specialty items. A cost comparison was also performed during this study. The gluten-free products were reported to be 240% more expensive than the comparable food item. In a study performed by Sverker and others (2007) on Celiac disease, it was reported there were difficulties in finding and identifying gluten-free food items. A study done by Stevens and Rashid (2008) and Zarkadas and others (2006) investigated the availability of labeled gluten-free products. This study reported that the price of gluten-free products were significantly more expensive than comparable products that were not gluten-free. A study by Laureati and others (2012) which investigated the sensory factors of gluten free foods reported that many studies conducted on gluten-free products gave poor sensory attributes to gluten-free foods and that in examples such as bread, products were more dense and staled quickly.

2.1.6 Shelf Life

A study by Krupa and others (2010) reported that gluten-free breads are of low quality and subject to rapid staling. This study reported that the addition of native starch increased the tendency of amylopectin to retrograde during storage while the presence of modified starch decreased retrograde enthalpy. A report of Miñarro and others (2010) stated that gluten-free breads showed no difference in shelf life because of the formulation. He further reported that ovalbumin addition in the formulation added to a delay in staling.

2.2 GLUTEN-CONTAINING GRAINS

2.2.1 Introduction

In a study by diCagno and others (2005) it is reported that the ingestion of the prolamins of wheat, namely α-, β-,γ-, and ω gliadin subgroups which cause the damage to the intestinal villi to individual with Celiac disease. Note the gluten grains of barley, kamut, oats, rye, spelt, and wheat are from the same plant family of *Poaceae.* In the case of rye was secalin and barley contain hordein. These released Pro and Gln-rich polypeptides. These were responsible for T-cell mediated immune response. The identified peptides in wheat, rye and prevented the agglutination of K 562(S) cells by the fragment 31-43 of A-gliadin. These factors were contributing to the digestive problems for individuals with gluten intolerance.

2.2.2 Barley

Barley is classified as *Hordeum vulgare*. The waxy barley starch consists of 97-100% amylopectin and regular barley starch consists of 15-25% amylose and 75-85% amylopectin. It serves as a grain that is often used in soups, stews, and bread and used for malt which is used in distilling beverages and beer. The nutritional value of barley includes the proteins, thiamine (viamin.B1, riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), vitamin B6, folate (vitamin B9), vitamin C, calcium, iron magnesium, phosphorus, potassium and zinc. Barley also contains carbohydrates, sugars, dietary fiber and fat (Griffey and others, 2010).

2.2.3 Kamut

This grain is considered ancient wheat classified as *Triticum tugidam var. polonicum* or *Triticum var turanicum*. It is has a nutty flavor. Kamut is higher in protein and many minerals, especially selenium, zinc, and magnesium than other wheat products (Gauthier and others, 2006).

2.2.4 Oats

Oats are of the *Avena stiva* species of the plant family of *Poaceae*. They are derived from a grass of the primary cereal domesticates wheat and barley. Oats are rich in antioxidants α-tocotrienol, α-tocopherol, and avenanthramides, and total dietary fiber including β-gluan (Oliver and others, 2010). Oats are commonly used by crushing into an oatmeal or ground into a fine flour. Oats are often used as a bakery product to make cookies, cakes, bread and breakfast cereals.

The components identified in oats include protein, pantothenic acid (vitamin B5), folate (vitamin B9), iron, magnesium, and β-glucan. Oats also included carbohydrates, dietary fiber, and fat. The *Avenin* is a prolamine in oats that is toxic to the intestinal mucosa and can trigger the reaction in Celiacs (CSA, 2006).

2.2.5 Rye

The tribe classification of rye on the scientific grain chart is *Triticeae*. It is of the *Secale cereale species.* The nutritional components of rye include micronutrients such as vitamin B, vitamin E and other minerals. It is considered to have an increased bioavailability of nutrients in grain cereals. Martinez-Villaluenga and others (2009) reported that rye provides higher antioxidant capacity then wheat. That is that rye has *Trolox* equivalent antioxidant capacity (TEAC), peroxyl radical scavenging capacity (PRSC), diphenyl-1picryldydrazl (DPPH) ad

Folin-Clocalteu reagent (FCR). Rye is use to produce bread, beer, whiskey, and vodka as well as being use as boiled or rolled similar to rolled oats.

In addition to presenting gastrointestinal problems for individuals with Celiac disease, rye is suspected to cause physical and mental problems. It is believe that rye is highly susceptible to the ergot fungus which is the suspect cause of these problems.

2.2.6 Spelt

Spelt is classified in the genus as *triticum*. The binomial name is *Tricum aestivum spelta*. It is a hexaploid species of wheat. This grain was mainly used from the fifth century until the beginning of the twentieth century. It was gradually replaced by a higher producing yield of free-threshing wheat. Today it is produced mostly by organic growers. It is used in the production of bread and pastry products, pasta, beer and vodka. The nutritional benefits include carbohydrate, protein, fat dietary minerals and vitamins (Abddel-Aal, 2008).

2.2.7 Wheat

The wheat grain comes from the *Plantae Kingdom* of Genus *Triticum. T. aestivum*, which is a hexaploid species most commonly cultivated (Bonjean and Angus, 2001). Other common cultivated species of wheat are Durum wheat (*T. Durum, Einnkorn (T. monococcum), Emmer (T. Dicoccum),* and Spelt (*T. spleta*). Wheat starch and gliadin protein are connected with the endosperm of the grain. Therefore, freeing the wheat starch from the gliadin is very difficult. Wheat is considered a stable food used to make flour for leavened, flat and various types of breads, cookies cakes, breakfast cereals, pastas, and as fillers in several food products. The nutritional content of wheat includes proteins, thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B2), pantothenic acid (vitamin B5), vitamin B6, folate (vitamin B9), clcium, and iron. Also fat, carbohydrates, and dietary fiber are present in wheat.

The price of wheat has increased drastically due to freezes, floods, and droughts. In 2007, a bushel of wheat rose to $9.00 a bushel. This was highest price noted. The cost of transporting wheat also increased in 2008, which has driven the price higher.

2.3 GLUTEN-FREE INGREDIENTS

2.3.1 Introduction

The nutritional profile of a gluten-free diet was investigated by Lee and others (2009). Findings reported that the alternate grain products increased the nutrient profile of the gluten-free diet at P=0.002. The nutrients found in such a diet included proteins at 20.6 g. versus 11 g., iron 18.4 mg versus 1.4 mg, calcium 182 mg versus 0 mg, and fiber 12.7 mg versus 5 mg. This report put in better prospective the need for individuals with a gluten intolerance to maintain a gluten-free diet despite the alternate reports of nutritional deficiencies.

2.3.2 Acorns

Acorns were used as a food source over 2,000 years ago by the Greeks. Acorns played an important role in the American Indian food source. The Cherokee, Pima, Comanches, Apache, and Caddoan tribes harvested acorns. They were used as flour, pounded into meats, fat and berries (Sanaturalareas, 2009). Meyers and others (2006) reported that acorns have become a part of the diets in other parts of the world including Spain, Italy, Korea, China, and Japan. The acorns need to be leached before consuming to removing the tannin. Meyers and others (2006) reported excessive consumption of tannin has been found to cause kidney problems. The nuts are gathered during the fall months and allowed to dry in the sun. In their shells, the dried acorns can be stored for several years. After dried the acorns can be crushed and then leached for different uses.

The nutrients of the acorn include magnesium, calcium, potassium, nitrogen, phosphorus and sulfur. It is a reliable source of carbohydrates, proteins, 6 vitamins, 8 minerals, and 18 amino acids (Atkins, 2006). This offers a great opportunity for an untapped food source in the increasing demand for food. The amount of protein in 1 oz. of raw acorns is 1.68 g, calories 109.71, calories from protein 6.71, percent of calories from protein 6.1% (high protein in foods) (Charef and others, 2009). Acorns are lower in fat than most nuts and rich in complex carbohydrates, vitamins and minerals (www.sanaturalareas.org, 2009).

A search for acorn flour indicated that the only means of obtaining such flour is to collect the acorn and process the acorn. No companies have available acorn products in local supermarkets, specialty stores or advertise the sale of acorn flour or acorn products on internet search. There are several recipes posted on the use of acorn flour.

2.3.3 Almond

Almond flour is heavier and coarser than most flours. Almond flour is used in making breads, pastas and baked goods, usually with other products. However, quick bread can be made with almond flour and no time is needed for rising. The nutritional content includes carbohydrates, dietary fiber, protein, sugars, vitamin E, folate, calcium, iron, magnesium, phosphorus, and potassium.

It has been reported that almonds significantly lower LDL and increase HDL with participants that were fed almond-flour muffins (Bager and Lass, 2005). These investigators developed a collection of recipes using almond flour. The recipes included beginning and side recips (sauces, dips and dressings), appetizers and salads, soups and side dishes, eggs, beef, poultry, fish and seafood, savory baked goods, muffins, cookies and bars, cakes and pies, candies, sweet condiments, and yogurt and ice cream. Almond flour can be purchased in specialty stores or on the internet.

2.3.4 Amaranth

Amaranth (*Amaranthus caudatus, A. cruentus and A hypochondriacus*) is a dicotyledonous plant and cosmopolitan genus of herbs (Tapia-Blácido and others, 2010). There are about 60 species. The plants are inflorescent with foliage ranging from purple and red to gold. Members of this genus share many characteristics. Several species are often considered weeds. Around the world amaranth is used as leaf vegetables, cereals, and ornamentals.

Amaranth is a food of the Aztecs. It has a corn like aroma with a woody flavor and is used as flour for breads, pasta and also in porridge type dishes. The nutritional content of amaranth includes high protein, dietary fiber, iron, magnesium, zinc, calcium and vitamin B (Green and Jones, 2009, and Tapia-Blácido and others, 2010).

2.3.5 Arrowroot

Arrowroot is tuber that contains about 23% starch. Arrowroot starch has been often adulterated with potato starch and other similar substances. Pure arrowroot is a light, white powder, odorless when dry, but emitting a faint, peculiar odor when mixed with boiling water, and swelling on cooking into a perfect jelly. It is an edible starch from the rhizomes (rootstock) of West Indian arrowroot (Jyothi and others, 2009). It is chiefly cultivated in the West Indies (Jamaica and St. Vincent), Australia, Southeast Asia, and South and East Africa. It often use in place o corn starch (Hagman, 1990). Arrowroot flour can be purchased in specialty stores or on the internet.

2.3.6 Bean flour

Garbanzo and other bean flours are usually combined with rice flour for baking. Small quantities can be added in preparing meat dishes such as hamburgers, meatballs and meat loaf (Hagman, 1990). Hagman (1990) developed multiple recipes using gluten-free grains and provided suggestion for using milk free substitutes in recipes. These recipes can be found in her collection of books: The Gluten-Free Gorumet, More From the Gluten-Free Gourmet, The Gluten-Free Gourmet Bakes Bread, Living Well Without Wheat, The Gluten Free Gourmet, and The Gluten-Free Gourmet Cooks Fast and Healthy. The point made by Hagman (1990) is that the gluen-free diet is a diet for life. This is what prompted her to continue to develop new recipes.

2.3.7 Buckwheat

Buckwheat is not a grass but a member of the rhubarb family. Botanically speaking is classified as a fruit. It is use in making pancakes and soba noodles. In addition, it is used as a cereal, side dish and added to soups and stews. The nutritional benefits are identified as high protein content, magnesium, vitamin B6, dietary fiber, iron, niacin, thiamin and zinc (Green and Jones, 2009).

2.3.8 Coconut

Coconut flour is produced as an extraction from coconut milk. It has been reported that the dietary fiber from coconut has preventive measures in chronic diseases such as cancer, cardiovascular diseases, and diabetes mellitus (Trinided and others, 2006). This is accomplished according to Trinided and others (2006) by fermentation to produce short chain fatty acids with butyrate acetate propionate which increases amounts of dietary fiber in coconut. The coconut flour is roduced by extracting the coconut oil from a cold press to be milled into flour. It can be used as fillers and bulking agents as well as mixed with other flours to create products. Coconut flour is reported to contain 3.6% moisture, 3.1% ash, 10.9% fat, 12.1% protein and 60.9% dietary fiber (Gunathilake and Abeyraathne, 2008).

2.3.9 Corn

Corn (maize) is considered the *genus Zea mays*. It is a grass domesticated by indigenous peoples in Mesoamerica in prehistoric times. Native Americans cultivated it in numerous varieties throughout the Americas. Maize is the most widely grown crop in the Americas (332 million metric tons annually in the US alone). Sweet corn is usually shorter than field-corn varieties

Corn starch is refined from corn and is used for thickening for pudding and sauces. It can also be used for baking in combination with other flours. Corn flour is milled from corn and used in baking breads and pastry. Cornmeal is ground from corn. It can be used alone or combined with other flours in baking (Hagman, 1990). These types of flour are readily available in neighborhood grocery stores.

The nutritional content of corn are protein, starch fiber, fat, ash, vitamin B1, vitamin B4, vitamin C, foliate, phosphorus, manganese, beta-cryptoxanthin. The protein structure is rich in alipatic AA (Alanine, Glycine, Leucine, Isoleucine, etc.) (af.ndsu.edu, 2009),

2.3.10 Guar Gum

Guar gum (guaran) is a galactomannan. It is the ground endosperm of guar beans. The guar seeds are dehusked, milled, and screened to obtain the guar gum. It is typically produced as a free flowing, pale, off-white colored, coarse to fine ground powder.

Guar gum is economical because it has almost 8 times the water-thickening potency of cornstarch. nly a very small quantity is needed for producing sufficient viscosity. It can be used in various multi-phase formulations: as an emulsifier because it helps to prevent oil droplets from coalescing, and/or as a stabilizer because it helps to prevent solid particles from settling. It helps prevent ice crystals. It is high in fiber.

2.3.11 Quinoa

Quinoa is a native South American grain that has a soft crunchy texture. It has a high nutritional content which includes a high quality protein, high iron, magnesium, vitamin B, calcium and fiber content.

2.3.12 Palm

Palm flour is classified as *Archontohoenix alexandrae* is reported to contain high levels of dietary fiber and minerals. It is suggested to be used with other flours, such as rice in gluten-free baking. Results of a study show that it increases firmness and decreased adhesiveness of dough in baked products (de Simas and others, 2009).

2.3.13 Poi

Poi is a staple food, which comes from the taro plant (*Colocasia esculenta*). Fresh poi is sweet and edible by itself. Poi is used as a pudding, in bakery products and also can be used as a milk substitute (Hermandez, 2004).

2.3.14 Potato

The potato is a tuberous crop from the perennial *Solanum tuberosum* of the *Solanaceae* family (also known as the nightshades). The word potato may refer to the plant itself as well. In the region of the Andes, there are some other closely related cultivated potato species. Potato starch is very fine white powder used as a thickening agent. Potato flour is used in baking (Fenster, 206).

2.3.15 Rice

Rice is a seed of a monocot plant *Oryza sativa.* In the southern United States, rice has been grown in southern Arkansas, Louisiana and eastern Texas since the mid 1800s. Many Cajun farms grew rice in the wet marshes and low lying prairies where they could also farm crayfish when the fields were flooded. In recent years, rice production has continued to increase in North America, especially in the Mississippi River Delta areas in the states of Arkansas and Mississippi (mith, 1998). White rice flour has several textures. It can be fine to regular. It is milled from polished white rice. It is used in products such as pasta. However, it is used for baking either alone or in combination with other flours.

Brown rice flour is milled from unpolished rice and contains bran. It is higher in nutritional value than white rice flour. It has a shorter shelf life then white rice flour. Research shows that rice flour and rice starch increase the elasticity in the early stages of bread baking and the volume of the resulting bread (Yang, 2010).

2.3.16 Sorghum

Sorghum is classified as *Sorghum sudanense Stapf,* from *poaceae family.* Technically it is a grass. High pressure-treated sorghum flour was found by Vallons and others (2010) to be a replacement for gluten gliadin in the production of gluten-free breads.

2.3.17 Soy

Soy is a bean from the *Fagordeoe family*. The flour is commonly used in combination with other flours. It is high in protein and fat content and has a nutty flavor.

2.3.18 Sweet Rice

Sweet rice or sticky rice is classified as *Oryza sativa var. glutinosa or Oryza glutinosa* on the grain chart. It is a type of short-grained Asian rice that is especially sticky when cooked. It should not be confused with the other varieties of Asian rice that become sticky to one degree or another when cooked. It can be purchased in specialty stores or on-line.

2.3.19 Sweet Potato

The sweet potato (*Ipomoea batatas*) is of the family *Convolvulaceae* is a dicotyledonous plant. Its large, starchy, sweet tasting tuberous roots are an important root vegetable Purseglove, 1991; Woolfe, 1992). The young leaves and shoots are sometimes eaten as greens. Of the approximately 5 general and more than 1,000 species of *Convolvulaceae,* it should be noted that some of the *I. batatas* plants are actually poisonous.

In North America the sweet potato is often marked as yam which is the softer, orange variety and is distantly related to the potato (*Solanum tuberosum*). The sweet potato is very distinct from the actual yam, which is native to Africa and Asia and belongs to the monocot family Dioscoreaceae. To prevent confusion, the United States Department of Agriculture requires that sweet potatoes labeled as "yams" also be labeled as "sweet potatoes."

The genus Ipomoea that contains the sweet potato also includes several garden flowers called morning glories, though that term is not usually extended to *Ipomoea batatas*. Some cultivars of *Ipomoea batatas* are grown as ornamental plants; the slightly ambiguous name "tuberous morning glory" may be used in a horticultural context.

This plant is a herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose color ranges between red, purple, brown and white. Its flesh ranges from whit through yellow, orange, and purple.

On checking the availability and use of sweet potato flour, it was noted that it is mostly used for animal food. Sweet potato flour was located for human consumption through

Amazon.com. A specialty company, Ener-G Food, did develop bread using sweet potato flour, but pulled it from the market because of problems with mold (Wylde, 2009).

2.3.20 Tapioca

Tapioca flour is made from the Cassava root. It is often used in combination with other flours for baking. Tapioca flour can be purchased in specialty stores or on the internet.

2.3.21 Teff

Teff (*Eragrostis tef) is* found in Ethopia. This grain is used as a hot cereal, in a side dish, and in casseroles. It is used as a thickener for sauce and also used in pasta. Teff is reported to work similar to gluten grains in baking (Mohammed and others, 2009). The nutrition content includes high protein, calcium, iron and vitamin B (Green and Jones, 2009).

2.3.22 Xanthan Gum

Xanthan gum is produced using the bacteria, *Xanthomonas Competris*, to ferment corn sugar. It is used as a binder, thickener, and stabilizer. It is used in making salad dressings as a suspension, in pie fillings, in canned gravies and sauces and in ice cream to provide a smoother texture.

Xanthan consist of glucose molecules connected by β-1,4 glyosidic links which are similar to cellulose. Every second glucose unit carries a side chain which is composed of β-o-mannose, β-1,4 glucoronic acid and α-1,2-o-mannose together with a pyruvic acid unit (Salah and others,2010). Xanthan gum is frequently mixed with guar gum because the viscosity of the combination is greater than when either one is used alone (scientificpsychic, 2010).

2.4 MILK SUBSITUTES FOR GLUTEN-FREE PRODUCTS

2.4.1 Almond Milk

Almond milk is a beverage made from ground almonds. It contains no cholesterol or lactose. Commercial brands may be plain or flavored. A commercial brand of almond milk is Almond Silk produced by Silksoy Company, White Waves Food (www.silksoymilkco, 2011).

2.4.2 Coconut Milk

Coconut milk contains 54% moisture, 35 % fat and 11% solid non-fat. The fat content plays an important role in coconut milk flow. Coconut milk stabilizes natural proteins as an emulsion (Peamprasart and Chiewchan, 2006 and Jiraeangtong and others, 2008).

2.4.3 Rice Milk

Rice milk available produced by Whole Foods Market contains organic whole grain brown rice, organic brown rice syrup solids, organic expeller preserved, safflower and/or sunflower seed oil, tricalcium phosphate, sea salt, organic vanilla extract, carrageen, natural flavor, vitamin A palmitate, ergocalciferol (vitamin D2), and vitamin B 12. A rice dairy whip is produced by the Soyatoo Co, San Francisco, Ca.

2.4.4 Soy Milk

Ingredients found in soy milk substitute can included filter water, organic whole soybeans, organic evaporated cane juice, natural flavors, calcium, sea salt, sodium citrate, potassium citrate, carrageenan, vitamin A palmitate, ergocalciferol (vitamin D2), Dl-alpha tocophenol acetate (vitamin E), riboflavin (vitamin B1), cyanocobalamin (vitamin B12), and zinc sulfate. Such a product has been developed by Whole Food Market. The company states that this soy milk produt is gluten free and non-dairy is an alternative to milk. It can be used as an excellent alternative for milk in food preparation including baking (Whole Food Market, 2008).

A soy whip is available, which is a great alternative to a dairy whip. Such a product is produced in Germany by Soyatoo (www.soyatoo.com, 2011). The product ingredients include soy water, soybean, organic coconut oil, organic fractionated palm kernel oil, organic sugar-beet syrup, organic maltodextrin-dried corn derived, tartaric acid, carrageenan, sea salt, natural vanilla extra, and propellant: nitrous oxide.

An alternative for butter is available produced by Earth Balance (2010). This product contains a natural blend of palm fruit, canola, soybean, flax and olive, filtered water, less than 2% of sea salt, natural flavor (plants derived from corn, no MSG, no alcohol, no gluten) pea protein, sunflower lecithin, lactic acid (non-dairy, derived from sugar beets) and naturally extracted annatto for color.

2.5 BAKING GLUTEN-FREE BREAD

2.5.1 Baking with Gluten-Free Flours

An identified problem with baking bread with gluten-free flours is the fact none of the gluten-free flours contain the components of gluten, namely glutenin and gliadin. The glutenin provides the higher molecular weight and contributes to the elasticity. The gliadin provides the lower molecular weight component which provides extensibility (Hazen, 2011). The report of Hazen (2011) stated that gluten is needed to produce the volume in bread. He further stated that one cannot just convert a regular recipe to gluten free. He stated that gum, emulsifiers, and egg whites are needed for stability to produce the desired texture of bread products. He further stated use of egg whites replaces the protein missing in gluten free flours. Hazen (2011) suggested that each bakery product requires a different method which makes conversions more difficult.

A study by Miñarro and others (2010) investigated the influence of the unicellular protein upon creating gluten-free breads. His conclusion was that while it was possible to make starch-based breads for good characteristics, this produced low specific volume and hard crumb. However, inclusion of unicellular protein to the gluten-free starch produced less bake loss and the unicellular protein was less preferred by consumers. Mezaize and others (2009) performed a study to formulate gluten-free French bread that would be suitable for individuals with Celiac disease. He focused on hydrocolloids, guar gum, hydroxypropyl methyl cellulose, and xanthan gum. Additionally, he sed buckwheat flour, egg powder and whey protein. He reported having characteristics that would be potentially acceptable to the Celiac population. However, sensory studies were not performed and the use of whey would be a problem in developing a gluten-free/milk-free product.

Stathopolous and Kennedy (2008) attempted to use concentrated casein to produce an acceptable gluten-free bread product. He reported that casein had been used in gluten-free baking in order to provide more nutrition and produce a better product. The conclusion of his research was that even with the casein in the product there was a significant difference in casein samples and wheat samples. He suggested that if one was to use the casein there would still have to be modifications in the formula to produce a product of similar quality to wheat. He does not cover potential problems casein would produce for individuals on a gluten-free diet that also required a milk-free diet.

An investigation using in baking with teff by Yigzaw and others (2004) suggested that teff provides an ideal amount of protein especially for children ages 2 to 5. Results of Yigzaw's study suggest that it canbe a cheaper, simpler and faster process in baking. However, he suggested more investigation in toxicity and sensory evaluation. The investigator stated using teff in baking provides a crispy crust.

As noted by Hazen (2011) gluten-free flours do not have the elasticity that the gluten flours do. They are much denser and a combination of flours, starches and emulsifiers are necessary to achieve the texture and taste of gluten breads. Different starches, emulsifiers and proteins are needed to maximize the baking properties, especially in breads.

The use of coconut flour in a study by Trindad and others (2006) increased dietary fiber and there was no difference on the mineral availability. On the basis of Trindad's and others (2006) study justification for further use of coconut flour is indicated. Exploring the use of amaranth flour was the focus of research by Schoenlechner and others (2010). The conclusions of their study were the water content affected the pore size and number and albumen affected the firmness of this particular bread. Nieburg (2011) reported that natural starch can boast dough hydration and improve functionality of gluten free baked products. According to his report, natural starch would reduce undesirable taste and enhance shelf life.

2.5.2 Problems with Gluten-Free Baked Products

Gallagher and others (2003) reported that many gluten free breads available today are of low quality, having dry crumbling crumb and having poor mouth feel and poor flavor. The objective of their study was to use dairy in creating bread to enhance flavor and texture. Gallagher and others (2003) was using the standard of including milk in the gluten-free bread because it had been established in baking gluten products. He reported that adding dairy protein produced increased crumb and volume but only when additional water was added did volume increase and a softer crust and texture resulted, so the dairy alone did not reach the volume and texture desired. The additional problem with this formulation and acknowledged by the researcher, is that consumption was a problem for Celiacs because of the milk in the product.

Gluten-free bread was reported to be of poor technical quality and showed low specific volume, high crumb hardness and high staling rate (Sciarini and, others 2010). He used several formulas to produced gluten-free bread using hydrocolloids including carrageenan, alginate, xanthan gum, carboxymetyl cellulose and gelatin. Xanthan gum produced the highest bread volume. However, it was a lighter crust color. He reported that xanthan gum may also have interfered with the Maillard reaction producing the lighter bread crust color. His conclusions were that the xanthan gum led to a higher volume with lower firmness and staling than the other hydrocolloids. Also, in the case of gluten-free bread high water content was strongly association with the volume of the bread.

The reported problem in baked gluten-free products according to Schoenlechner (2010) is that replacement products that are gluten free are of low nutritional value. He states that "gluten plays a major role in food structure and it is involved in the formation of the three-dimensional network, which finally influences the textural and sensorial properties of the final product." As with the authors Sciarini and others (2010) and Gallagher and others (2003) he states the water content in the gluten-free bread determines the final structure. Schoenlecher and others (2010) used amaranth and albumen in his studies exploring the production of gluten-free bread. Noted in his conclusion was the statement that while pore size was reported to be more uniform, texture softer and mouth-feel favorable, there were apparently problems with attributes like bitter and moldy taste.

Onyango and others (2009) reported that in order to obtain quality gluten-free bread inclusion of selected proteins are necessary. He recommends examples such as skim milk, egg whites, soy protein isolate and soy protein. When he compared the different proteins he used he reported that the egg white differed significantly from the other proteins as it gave a lower crumb firmness and staling rate. He concluded by adding diglycerides an improved batter was produced. No sensory studies done on this product, therefore product acceptability cannot be measured with this formula.

CHAPTER 3 MATERIALS AND METHODS

This study was done in several stages. An internet search of the availability and cost of existing gluten-free products was performed. Next a survey was performed in local stores to ascertain how many of these products were available for the consumer that they could easily obtain during regular grocery shopping. The third portion of this study was developing a survey of the products located in the stores surveyed to see if Celiac consumers in other areas of the United States had local access to these products and to establish if there were sensory problems with any of the specialty products. The fourth step of this research was to conduct product development of gluten free French brea products, with sensory evaluation of the products developed. Finally, statistical analyses of all portions of the study were performed.

3.1 STORE SURVEY

3.1.1 Store Survey Experimental Design

Two large specialty stores as well as twelve local grocery stores were visited in Jefferson Parish and Baton Rouge of Louisiana to see how many of the labeled gluten-free items were available in each store. The second part of this survey was to identify how many gluten-free products were also milk-free. An attempt was made to visit health food stores in these two parishes but all listed health food stores found in the local telephone directories either had moved, closed or did not carry food items. The stores only had vitamins and supplements.

The data was collected by the same experimenter over a period of three months. A total of fourteen local stores were visited to collect data on where a consumer may purchase specialty gluten free products. Once a store was identified, a survey of all the specialty gluten- free items available was examined. Data was recored as to whether the product was gluten-free and also if it was milk-free. The type of product, the vendor, the labeling of ingredients, cost and any apparent problems with the product were also recorded.

3.1.2 Store Statistical Analyses

An Excel database was created. A listing of local stores, specialty products, and information on the products were listed in the database. Additionally, statistical analysis was performed including of frequency as well as the difference between stores. SPSS software by SPSS Inc, Chicago, IL., USA was also used in the data analysis.

3.2 CELIAC CONSUMER SURVE

3.2.1 Survey Design

A survey was taken to ascertain the Celiac consumers' sensory acceptability of gluten-free specialty items identified in the store survey. A total of 84 Celiac consumers were asked to complete a survey of 54 products previously identified in the store survey. Consumers ranged from 18 years to over the age of 56 and consisted of males and females from different areas of the United States. The survey asked participants to identify if they had purchased and consumed various gluten-free products. Consumers were asked if they had problems with any of the products concerned with taste, texture, color, and smell.

3.2.2 Survey Statistical Analysis

An Excel database was created to record the response of the consumers and perform a statistical analysis. Statistical analyses were performed including of frequency using SAS and SPSS software by SPSS Inc, Chicago, IL, USA was also used in the data analysis. The statistical program is attached in Appendix A.

3.3 PRODUCT DEVELOPMENT

3.3.1 Equipment

Equipment used for development of French bread included a Kitchen Aide mixer, rolling pin, measuring cups, measuring spoons, pastry brush, Mainstain bake ware set, oven, bread knife, and spatula, Glad Press'N Seal, aluminum wrap. Aluminum pans and parchment paper were secured from a local grocery story. Ingredients included rice flour, white bean flour, potato flour, guar gum and xanthan gum which were obtained from Bob Red Mills Natural Foods, Inc., Milwaukee, OR. Red Star Yeast was purchased online. Tapioca flour was obtained from Ener-G Foods. Seattle, WA. Sugar, corn oil, egg whites, rice vinegar and salt were obtained from a local grocery. Baking powder was secured from Rumford Clabber Girl, Terre Mute, IN 47808, and salt from Wal-Mart Stores, Inc, Bentonville, AR 72716.

3.3.2 French Bread Procedure

Breads were prepared using a Kitchen Aide Professional Mixer 600. Several different gluten-free flour combinations were explored in developing a workable recipe to substitute for the wheat flour in a French bread recipe.

3.3.2.1 Rice, Potato, and Bean French Bread

The ingredients of yeast, sugar and warm water were combined and fermented for 5 minutes. The flours and dry ingredients were then added. The ingredients were mixed for 1 minute. Oil, egg whites, vinegar and vanilla ingredients were added to the mixer and mixed for 5 minutes. The knead hook was then placed on the mixer and the ingredients kneaded for 5 minutes. The dough was placed in the shape of a bll and placed in an aluminum pan, let rise for 60 min @35°C in the convection oven. The dough was then shaped as a loaf of French bread and placed in a French bread pan and wrapped with plastic wrap, then placed in the convection oven 60 min @35°C. The bread was then removed from the oven. The bread was left in the pan and wrapped with Press ‘N Seal and placed in the refrigerator at 40°F for 12 hours. The bread was removed from the refrigerator and the Press ‘N Seal removed. It was placed in the convection oven to let rise for 60 min @ 35°C. The bread was then brushed with egg whites for browning and then placed in a preheated oven and baked at 400°F for 40 minutes. (See Appendix C for formulation ingredient levels used)

3.3.2.2 Rice and Bean French Bread

The same procedure was done as with the other gluten-free bread. Flours were mixed, kneaded, placed in the aluminum pan, allowed to rise in the convection oven, shaped as a loaf of French bread and placed in the French bread pan. The dough was return to the convection oven allowed to rise again, then removed from the oven, wrapped in Press ‘N Seal, then placed in the refrigerator at 40°F for 12 hours. Once removed from the refrigerator, the Press N Seal Wrap was removed. The breads were then returned for 60 min @ 35°C to the convection oven. The bread was then brushed with egg whites and then placed in a preheated oven and baked at 400°F for 40 minutes. (See Appendix C for formulation ingredient levels used).

3.3.2.3 Wheat French Bread

A control (wheat) French bread was prepared using the same procedures and ingredients except for using 2 cups of wheat flour in place of the non-wheat flours without the special flavors of garlic and vanilla. Preparation was done using a Kitchen Aide mixer, the convection oven, and standard oven. The ingredients of the French bread are listed in Appendix C.

3.4 CHEMICAL ANALYSES

3.4.1 Equipment

The equipment used for analysis included a spatula and glassware, a Denver Instrument M-220D balance, aluminum pans secured from VWR International, VWR convection oven, Pyrex desiccators, RVA-4 machine (Newport Scientific Pty. Ltd., Warriewood NSW, Australia.

3.4.2 Flour Moisture Analyses

Each drying pan was labeled for the corresponding sample and weighed in Denver Instrument M-220D balance and recorded. Each flour sample (2.5 g) was placed in a previously weighed aluminum pan (VWR International). Three samples of each of the fourteen flours and flour combinations were weighed and recorded. After weighing, the samples were placed on an aluminum pan and put in a VWR convection oven at 130°C for 1 hour. The sample was then placed in a Pyrex dessicator for one hour. Each sample was then removed from the dessicator and weighed in the Denver Instrument M-22D balance. Calculation of moisture content (MC) on a wet-weigh basis (wb) was performed as follows: %MC wb = [{(W2-W1)-(W3-W1)/(W2-W1)} * 100]. Note that W1 represents the weight of aluminum pan, W2 represents weight of the flour sample plus the aluminum pan, and W3 represents the weight of the aluminum pan and flour after drying.

3.4.3 Rapid Visco Analyses (RVA)

In order to ascertain the behavior of the different gluten-free flours alone and when combined with other gluten free flours Rapid Visco Analysis was performed on white rice flour, tapioca flour, potato flour, white bean flour, and wheat flour. Three replicates were done of each of the fours and flour combinations. Pasting characteristics of each flour were evaluated with a

RVA-4 machine (Newport Scientific Pty. Ltd., Warriewood NSW, Australia) using the AACC Method 61-02 (Newport Scientific, 1998).

Prior to analysis, the volume of water and weight of starch sample were determined based on the following formula using 10 g of the flour and flour combinations:

$S = 88 \times 3.00 / (100 - M)$; $W = 28.0 - S$ where S is the corrected sample mass (g), M is the actual moisture content of the sample (% as is) determined based on AOAC Method 925.10,and W is the corrected water volume (mL). Briefly, distilled water (~25.4 g) was measured into an RVA canister. Then, an appropriate weight (~2.60 g) of sample was weighed into a pan and transferred into the canister with water. The paddle was placed into the canister and the sample was thoroughly dispersed into the liquid by vigorously jogging the blade up and down at least 10 times through the sample. The canister, with the paddle, was inserted into the instrument and the measurement cycle was started by carefully pressing the motor tower. Each sample was first held at 50^{o}C at a spindle speed of 960 rpm. After 10 sec, the rotating speed was reduced to 160 rpm. The temperature was increased at 12^{o}C /min to 95^{o}C and held at the temperature for 2.5 min. It was finally cooled to 50^{o}C. The speed was kept at 160 rpm until the end of the test. The pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and peak time (PTime) were measured by the RVA with the RVA-4 machine (Newport Scientific Pty. Ltd., Warriewood NSW, Australia TCW3) software. Total setback (TSB) and Breakdown (BD) were calculated as the difference between FV and MV, and PV and MV, respectively. Analysis was done in duplicate. SAS statistical analysis is attached in Appendix D.

3.4.4 Proximate Analyses

Proximate analyses of the white rice flour, white bean flour and potato flour were performed by Bob's Red Mill Natural Food Company. Note that this company supplied the flours for this study. The results of these analyses are detailed in Appendix E, F and G.

3.4.5 Texture Analyses

Texture analysis was performed using the TA-XT Plus Analyzer (Texture Technology, Scarsdale, NY). The load cell was 5 kg with 9.8 g of force. Height setting was 27 mm, speed 10 lb. sec, and contact force set at 30 grams. Test compression speed was 2.0 mm/s, and trigger force s. The cylindrical aluminum shaped probe was 30 mm in diameter. The temperature was set at 25°C. Graph preparation "x" axis was set equal to strain and "y" axis equal to stress. The strain used was 40 g. The texture parameters were set to determine hardness, cohesiveness, adhesiveness, chewiness, gumminess, springiness, and fracturability.

Three loaves of each formulation of bread were made. Samples were taken of each of the loaves of bread. Samples were sliced from the different areas of the bread including an area approximately an inch from each end of the loaves and a sample from the center. Each sample was 1 cm. in width.

Hardness is the force necessary to obtain a given deformity. Fracture is the force of a significant break in the first bite. Cohesiveness is the strength of the internal bonds making up the body of the sample. Adhesiveness is the necessary force to overcome the attractive forces between the surface of the food and the surface of the materials which the food comes in contact with. Gumminess is the energy needed to disintegrate a semisolid food until swallowing. Chewiness is the energy needed to chew a solid food until ready to swallow. Springiness is the distane recovered by the sample during the time between the end of the first bite and the start of a second bite. Resilience is the measure of how well a product "fights to regain its original position" (Gunasekaran and Mehmet, 2002). The texture profile analysis (TPA) instrument, which imitates the grinding of the jaw, was operated to perform in two bites. The statistical analysis was performed using SAS. The program is attached in Appendix H.

3.4.6 Gluten Testing Procedure

The GlutenTox Home kit was supplied by Biomedical Diagnostics, www.glutentox.com. Each of the ingredients to be used in the gluten-free breads developed was tested before baking to identify if they had gluten content. The procedure was performed according to the instructions of the kit. The sample of flour and each subsequent sample were ground to the smallest particle using a blender. The provided spoon was used to measure 1 gram of the sample and for the liquid ingredients 1 ml was measured. The sample was then placed into the extraction bottle. The extraction bottle was closed and placed on a shaker for 2 minutes. This solution was set aside for 5 minutes. Using a new disposable plastic pipette 10 drops of the sample solution were taken from the bottle and placed in the dilution bottle. The instructions suggested using 2 drops to for 20 ppm and to use 8 drops to test for 5 ppm. Therefore, 10 drops was used to obtain a higher percentage of assurance. Samples were gently shaken for 15 seconds. Six drops of the dilution were placed on the S zone stick which was set aside for 10 minutes, after which it was checked for a reaction. The gluten testing procedure was also performed following baking of the two gluten-free breads by grinding up 1 gram of sample of the baked bread in a blender. The sample was then placed into the extraction bottle. The extraction bottle was closed and placed on a shaker for 2 minutes. This was placed aside for 5 minutes. Using a new disposable plastic pipette 10 drops of the sample solution were extraction from the bottle and placed in the dilution bottle. As done previously, six drops of the dilution were placed on the S zone stick and set aside for 10 minutes, then read for the results. Appendix I contains specific details of the gluten testing kit. Specific instructions and information on the kit is attached in Appendix I.

3.4.7 Color

The color spectrum is the combination of different parameters which are visualized where L* is lightness, a* is redness and b* is yellowness. The parameters of L*, a*, b* are expressed on the basis of luminance which cannot be determined by the human eye. L* may have values ranging from 0 to 100 and a* and b* may range between -80 to +80 (Berger-Schunn, 1994), but are usually between -60 and +60. Negative values of a* and b* are denoted greenness and bluenes of samples, respectively.

The Minolta CR-200 Meter (Minolta Camera Co., Osaka, Japan) is a hand-held machine that is a vision system. The Minolta colorimeter was calibrated using the standard white plate (D65, Y =94.4, x =0.1358, y =0.334) before each time it was used. L*, a*, and b* values were measured under D65 illumination.

The computer software was connected by using the vision system. This was done by connecting the vision system using a light box and a Nikon D200 digital colour camera (Nikon Corp, Tokyo, Japan). The camera settings were at 36mm focal length, ISO 100 sensitivity,1/3 s F/11 shutter speed, -1 eV exposure compensation, and direct sunlight white balance (Yagiz and others, 2009).

The samples slices were taken from approximately 1 cm. from each end and the center sections of the breads. Two loaves of each of the gluten-free breads were baked. Five samples were taken from each of the four loaves. Seven samples were taken from one loaf of the wheat bread. Samples were place directly in front of the camera lens which was covered with clear plastic t protect the lens. The Minolta CR-200 Meter took three readings of each sample and averaged the measurements. SAS statistical analysis was performed and the program is attached in Appendix J.

3.5 MICROBIOLOGICAL ANALYSES

Microbiological analyses were performed on each of the bread samples developed including the wheat sample (control), rice and bean flour combination, and rice/bean/potato flour combination. This was done to assure that yeast, mold, and aerobic colony counts were safe for human consumption. Samples were taken from multiple loaves prepared for future sensory studies. Microbiological analyses was performed on the samples from three loaves of each of the breads, the day after bread were baked.

The basis for microbial plate count is to dilute the bacteria to a certain level and then trap them in or on a solid medium where the individual cells will divide and produce macroscopic colonies which are counted through the transparent plate and medium (Goff and others, 2003).

A total 25 g of sample was added to 100 ml of PBS in a Whirl-Pak Filter Bag and then placed in a Tekmar Co-Unique Scientific Apparatus (P.O. Box 317856, Cincinnati, OH 4522-1856. The procedure was performed under guidelines of International Standards Organization, ISO 5541-1:1986, International Standards Organization, ISO 6887-1:1999, FDA, 1998.

3.5.1 Yeast and Mold Count

Under a Fume Hood, A/B3 Biological Safety Cabinet (2880 Berger Rd, Suite X, Hatfield, PA 19440, 1 ml of the sample was taken using a pipette and diluted to 10^{-5}. From each dilution 1 ml was removed with a pipette and placed on the center of 3M Strip Petri film which was gently laid down. This was done up to 10^{-5} dilutions for yeast and mold count. The Petri films were then laced on the counter at room temperature to be read in 5 days.

3.5.2 Aerobic Count

In a similar manner 1 ml of dilutions up to 10^{-3} was analyzed by placing 1 ml on a 3M Petri film for aerobic count. The Petri film was placed in the VWR incubator at 37°C to be read in 24 hours and subsequent days.

3.6 SENSORY STUDIES

3.6.1 Sensory Study General (Non-Celiac) Population

The sensory study was conducted using a randomized complete block design. A total of 105 consumers (non-Celiac) were presented with two coded gluten-free bread samples and one wheat bread. Each sample was cut 1 cm in size. Water was provided for consumers to use during the test to minimize any residual between samples. Consumers were instructed to sniff the sample for smell and odor acceptability. Then they were asked to evaluate the product for overall appearance, crumb color, overall aroma, crumb moistness and crumb softness, overall flavor and overall liking on a nine-point hedonic scale. The sale ranged from 9 for ‘extremely like’ to dislike extremely ‘1’. They were instructed to take one or two bites and slowly masticate the product before rating the sample. Consumers were asked to rate softness using a 3 point rating for softness, 1 = not enough, 2 = just about right (JAR), and 3 = too strong. Consumers were asked to rate overall acceptance and intent to purchase using binomial (yes/no) scale. Consumers were asked to rate overall acceptance and their intent to buy this product if it were wheat-free and milk-free.

3.6.2 Sensory Study Target (Celiac) Population

A total of 84 Celiac consumers required to maintain a gluten-free and/or milk-free diet were presented with a randomized complete block design. Each consumer was presented with two coded gluten-free/milk-free bread samples. The study was conducted in the manner as with the general population. The consent sheets and sensory evaluation forms for the study are shown in Appendices K and L.

3.6.3 Statistical Analyses

Statistical analyses using SAS, Cary, North Carolina were performed including frequency, ANOVA, Tukey with significance set at Alpha = 0.05. The program is attached in Appendix M.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 STORE SURVEY

The fourteen stores visited included those in the Baton Rouge and Jefferson Parishes of Louisiana to determine the availability of gluten-free products in the stores and to identify the products that were also milk-free. Both chain stores and small health food stores were visited. The classified “health food store” was not easy to find. In one case, the store had no gluten-free products and this store was dropped from the list of stores. Two other stores listed in the telephone directory had closed and in both cases their relocation could not be determined. It was foud that in general the large supermarkets had a variety of gluten-free products and availability differed from store to store. The largest selection of special products was available in the larger specialty stores and one store provided the researcher a list of gluten free products in the store. One store surveyed specialized in a large selection of Indian and Spanish food products. Many of these products are gluten free but not marketed to individuals on a gluten free diet and not labeled as gluten free. A Jewish deli had many labeled gluten-free products and “parva” (milk free), during Passover, that were not readily available beyond the Passover period.

Results of this survey reveal that the available gluten free pastas and flours as well as many of the mixes are milk free. A total of 555 products surveyed that were gluten-free included baking ingredients or mixes, bread mixes, premade breads, candies, cereals, substitute cheeses, cookies, desserts, margarines, frozen meals, shelf meals, substitute milk, pasta, pastry mixes, prebaked pastry, pizza, pie, pie and pizza shells, rice, snack bars, and yogurt (Figure 1). In some cases products clearly labeled dairy-free, actually contained milk components such as lactose, casein, and whey. Of the 555 gluten-free products found in this survey, only 165 of these products were also milk-free. It was noted that some products were present in more than one store, while for the same chain store some products differed. Table 3 illustrates the availability of the different gluten-free products and the portion of the available products which are also milk-free. The difficulty in finding gluten-free and milk-free products even in the same chain stores are comparable the findings of Lee and others (2007), Olsson's and others (2008), Zarkadas' and others (2006), and Alvarez-Jubete and others (2010). Alvarez-Jubete and others (2010) reported concerns on the limited supply of gluten-free specialty products as well as the nutritional value of existing products. Investigations done via a survey of 2,618 Celiacs by Zarkaas and others (2006) reported that gluten-free products were hard to find and costly. They reported that 71% of the participants had difficulty in finding gluten-free products. Quality issues of the gluten-free products were reported by 83% of participants. Reported by 85% of those surveyed were problems determining if products were gluten-free. Olsson and others (2008) concluded that the lack of available gluten-free products contributed to the lack of compliance to the diet. Neither of these investigators studied how many gluten-free products were also dairy-free.

Problems were found with the packing of products, especially with those found in the freezer. It was noted, that several items contained ice crystals or had freezer burn. In several cases, the product was not appropriately packaged and was place in a simple plastic bag with just a tie to close the package. Another problem noted with packaging of food intended for more than one serving was that it was not packaged in a resealable container. This could present a problem for the consumer to store products for further use. This study as in Zarkadas' and others (2006) and Cawthorn's and others (2010) studies found problems with labeling which also contribtes to problems with finding safe gluten-free products.

Stevens and Rashid (2008) surveyed the availability of gluten-free products in two large-chain groceries and found 56 gluten-free products. They compared the price of these products with similar products in the store. The prices of many gluten free products ranged from four to five times more than equivalent products that were not gluten-free. Products found in the local stores visited had prices ranging examples: a package of cinnamon raisin rolls (6 in a package) cost $5.98($.99 each) and an English muffin pack (4 in a package) cost $4.98 ($1.25 each). Eight fresh baked cinnamon rolls at a large grocery were $1.99 ($.24 each) and nine fresh cupcakes were priced $3.99 ($.44 each). The local store prices are similar to the findings of Stevens nd Rashid (2008).

Olsson and others (2008) indicating that gluten-free products were more costly when compared to gluten products. Due to the high price of gluten-free products, Lee and others (2007) and Olsson and others (2008) reported individuals that required a gluten-free diet, often did not purchase the specialty item. A study by Ylimaki and others (1989) reported similar findings to the present study in that celiac individuals surveyed reported challenges in following their diet because of the limited amount of gluten-free products available and in the case of bread products they were undesirable to taste. The limited shelf space could be a potential reason for such a limited supply on store shelves.

4.2 CELIAC CONSUMER SURVEY

A total of 84 Celiac consumers evaluated 54 gluten-free products previously indentified in the store survey. Table 4 list the specific product types without brand name listed that were included in the consumer survey and the reported problems with each product. The consumer survey revealed problems with taste, texture, color, and smell. The total number of problems across all panelists and products reported for texture was 227. The highest incidence of problems was found with bread products. The number of taste problems reported totaled 233, smell problems totaled 58, and color problems totaled 37. The majority of the problems were reported with prebaked products. This may be compared with Krupa's and others (2010) research which reported that gluten-free breads were subject to low quality and rapid staling. These finding confirm those findings of Gallagher (2003) and Schoenlecher and others (2010) of the poor quality of bread products and poor flavor and texture. This further documents the need to improve gluten-free bread products so that they are more acceptable to the taste of the consumer.

Table 3. Store Survey

Stores	**% gluten-free available in stores**	**%gluten-free contain milk**
Store #1, Baton Rouge, LA	3/555= 0.5%	0
Store #2, Baton Rouge, LA	3/555 = 0.5 %	0
Store #3, Baton Rouge, LA	3/555 = 0.5%	0
Store #4, Baton Rouge, LA	4/555 = 0.7%	0
Store #5, Baton Rouge, LA	5/555 = 0.9%	0
Store #6, Jefferson Parish, LA	30/555= 5%	14/30= 46.6%
Store #7, Jefferson Parish, LA	6/555= 1%	0
Store #8 Jefferson Parish, LA	9 /555= 1.6%	1/9=1.1%
Store #9, Baton Rouge, LA	4/555= 0.7%	0
Store #10, Jefferson Parish, LA	58/555=10.4%	2/58= 3.4%
Store #11, Baton Rouge, LA	7/555= 1.2%	0
Store #12, Baton Rouge, LA	16/555 = 2.87%	2/16 = 1.25%
Store #13, Jefferson Parish, LA	472/555 = 85%	143/472= 30.29%
Store #14, Baton Rouge, LA	358/555 = 65%	108/358 = 30.17%

Note* 555 products available 1) The percentage of gluten-free items available in each store calculated by dividing the number of gluten-free items available in each store by total number of gluten free items available in all of the stores surveyed. 2)The percentage of gluten-free products in each store that contained milk was calculated by dividing the number of gluten-free products in that store.

4.3 PRODUCT DEVELOPMENT

After a survey of various stores in the local area a gluten-free/milk free French bread could not be found even in any stores. The process to develop such a product to fill the void was done. Several gluten free flours were analyzed to determine which flours had similar attributes to wheat flour to develop a French bread. Two alternative French breads were selected based on RVA analysis and trial runs of breads including white rice flour with white bean flour and second combining white rice flour, potato flour and white bean flour. A study by Krupa and others (2010) investigated the use of bean starch in combination with rice flour to bake gluten-free bread. Krupa and others (2010) reported that the gluten-free bread produced with bean starch and rice flour was similar in texture, crumb and freshness to that of regular wheat bread. It is not clear what the exact composition of ingredients was in the bread developed in their study. No mention of milk was indicated in the study. The main focus of the study was to investigate the results of using bean starch. The conclusion of the study was that the addition of bean starch improved the chemical composition and quality of fresh gluten- free bread. It should be noted that this was not French bread.

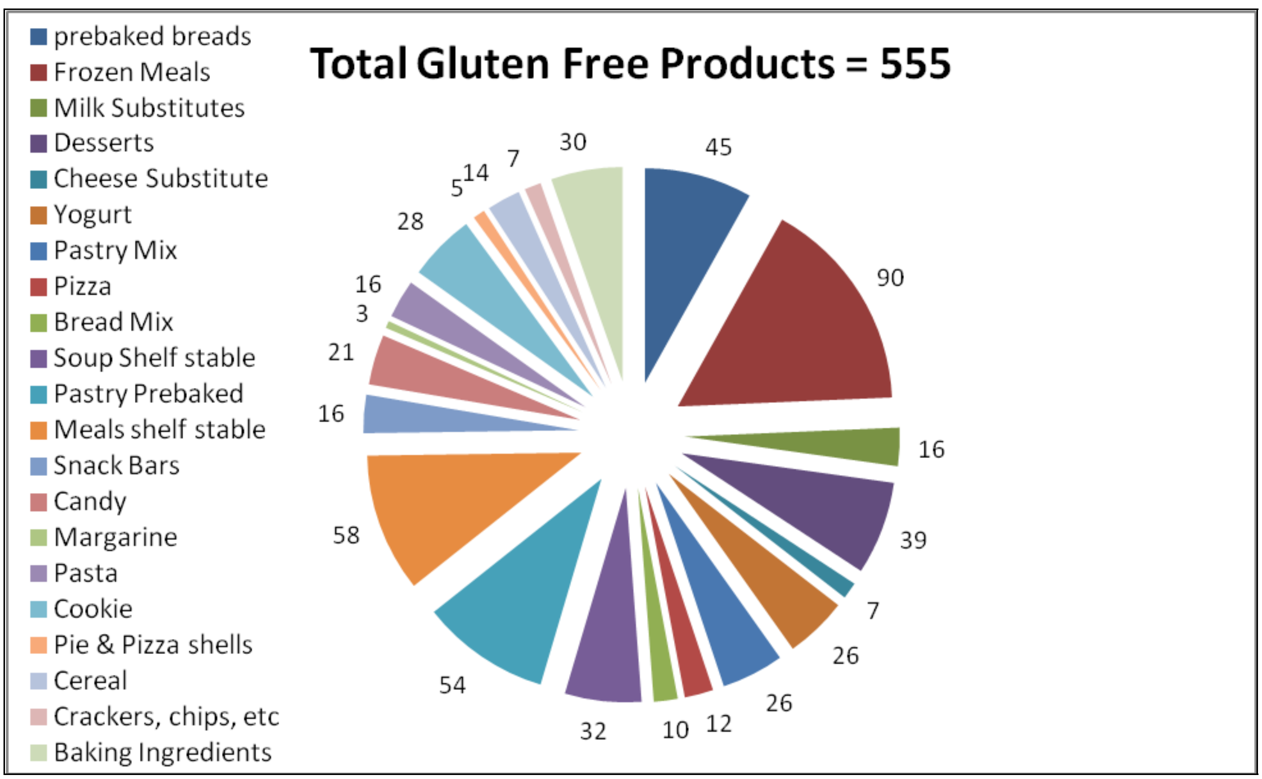


Figure 1. Percent of Gluten-Free Product Types Available in Local Stores

4.4 CHEMICAL ANALYSES

4.4.1 Flour Moisture Analyses

For each of the flours and flour combinations three replicates were done. These results were done to determine the moisture content demonstrated in Table 5. These findings were used in performing the RVA texture analyses.

4.4.2 Rapid Visco Analyses

Triplicate analyses of white rice flour, tapioca flour, potato flour, bean flour and wheat flour were preformed. Each of the flour and flour combinations were analyzed to determine the viscoelastic behavior comparing the curves of gluten-free flours to wheat flour. The use of the RVA analysis can provide a useful tool in determining the quality of the flour (Cozzolino and others, 2012). Final viscosity of rice bean flour was 1895±4.24, for rice potato bean flour was 1629.5±248.19, and the wheat flour was 1816±50.91. These three flours were closest in viscosity and would potential produce similar bread quality when compared to the research of Williams and others (2013). These analyses were performed to determine which gluten-free flours that would best respond to baking procedures similar to wheat flour. The same type of comparisons were performed by Lazaridou and others (2007) using rice flour as one of the mixtures in the gluten-free flour. Their findings were that rice flour resulted in creep-recovery curves which shifted to higher values compared to the wheat flour. In the present study the rice values were also higher than wheat. Comparisons of the different mixtures with 50%/50% combinations to 100% wheat are illustrated in Figure 2.

Table 4. Reported Problems from Consumer Survey

Products	Taste	Texture	Smell	Color
Bread #1	34	34	9	4
Bread #2	6	9	1	1
Bread #3	2	3	0	0
Bread #4	6	5	2	1
Bread #5	6	8	3	0
Cereal#1	7	5	0	0
Cereal#2	3	3	2	1
Cereal#3	11	5	0	1
Cheese-Imitation #1	0	0	0	0
Cheese-Imitation #2	0	0	0	0
Cheese-Imitation #3	1	1	0	0
Cheese-Imitation #4	2	1	0	0
Cookie #1	7	8	1	1
Cookie #2	1		0	1
Cookie #3	10	11	1	2
Cookie #4	9	11	3	1
Ice Cream Sub.#1	2	1	0	0
Ice Cream Sub.#2	0	0	0	0
Ice Cream Sub.#3	1	1	1	1
Ice Cream Sub.#4	0	0	0	0
Ice Cream Sub.#5	1	1	1	1
Meal Frozen #1	2	1	0	0
Meal Frozen #2	2	1	0	0
Meal Frozen #3	2	2	1	1
Meal Frozen #4	1	0	0	0
Meal Frozen #5	3	2	1	0
Meal Frozen #6	0	0	0	0
Meal Frozen #7	10	8	5	2
Meal Frozen #8	0	0	0	0
Meal Frozen #9	4	3	0	0
Meals Shelf Stable #1	0	0	0	0
Meals Shelf Stable #2	2	0	1	0
Pastry #1	8	11	2	1
Pastry #2	3	4	3	2
Pastry #3	3	5	1	0
Pasta #1	8	11	2	2
Pasta #2	6	5	0	1
Pasta #3	12	19	2	3
Pasta #4	2	1	0	0
Pizza #1	10	9	2	2
Pizza #2	2	2	0	1
Pizza #3	0	0	0	0
Pudding #1	0	0	0	0
Pudding #2	1	1	1	1
Pudding #3	1	0	0	0
Snack Bar #1	13	11	5	1
Snack Bar #2	8	1	2	0
Snack Bar#3	3	2	1	2
Snack Bar #4	6	5	1	0
Waffles #1	6	1	1	2
Waffle #2	4	4	3	1
Yogurt #1	2	0	0	0
Yogurt #2	0	0	0	0
Yogurt #3	0	0	0	0
Total Products	233	227	58	37

Celiac consumer survey participants = 84

Table 5. Moisture Content of Flours

Flour		% of Moisture	Stand. Deviation
Tapioca	=	10.67	0.22
Rice	=	11.06	0.07
Potato	=	6.51	1.19
White Bean	=	11.12	1.54
Tapioca 75%/Potato 25%	=	9.99	0.02
Tapioca 75%/White Bean 25%	=	11.92	1.53
Rice 75%/Potato 25%	=	9.52	0.05
Rice 75%/White Bean 25%	=	10.11	1.19
Tapioca 50%/Rice 50%		10.50	0.02
Tapioca 50 %/Potato 50%	=	7.63	0.19
Tapioca 50%/White Bean 50%	=	9.96	0.40
Rice 50%/Potato 50%	=	8.37	0.36
Rice 50%/White Bean 50%	=	9.81	0.34
Potato 50%/White Bean 50%	=	9.62	2.05
Wheat	=	10.75	0.55

In Figure 3 the combinations of 75/25% are compared to100% wheat. The combination of 50% rice flour, 25% white bean flour and 25% potato flour is compared to 50% rice flour/50% bean flour and 100% wheat flour in Figure 4. SAS statistically program is located in Appendix D.

It should be noted that it has been reported by Matos and Rosell (2012) that peak viscosity, pasting temp and setback during cooling can be predictors of the dough level of bread firming properties during storage.

4.4.3 Texture Analyses

Bread is spongy in nature and when stale tends to harden and crumble. The force to compress bread crumb determines the firmness. These factors play an important role in the quality of bread including hardness, adhesiveness, resilience, cohesion, springiness, gumminess and chewiness. Analyses of the textural properties are necessary in order to assess the necessary acceptable qualities of the consumer. Krupta and others (2010) reported that analysis of hardness of bread was best done at the center part of the bread. In his study, as in the present study, the samples were sliced so that the top crust, center, and bottom crust were included. Table 7 shows the average, standard deviation and coefficient variation for texture analyses of each of the breads. The hardness, adhesiveness, resilience, cohesion, springiness, gumminess and chewiness of freshly baked bread results are shown

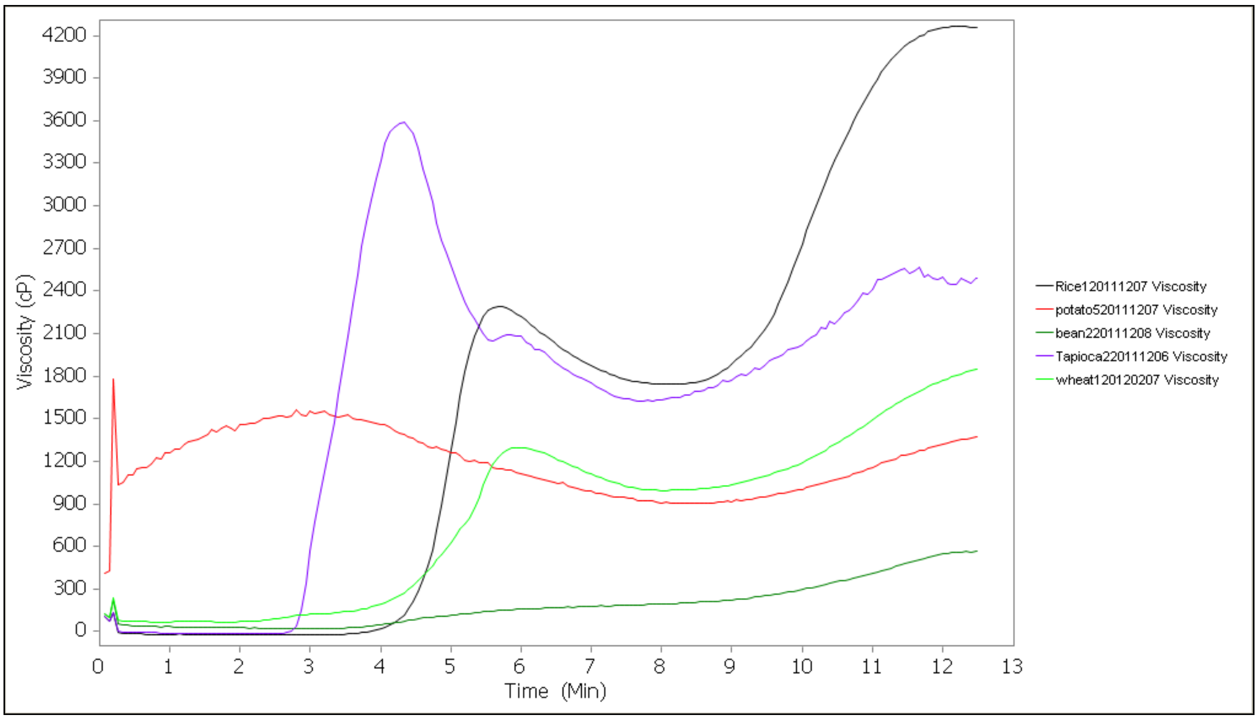
Table 6. RVA Comparison of Flours

Flour	PV	MV	BKD	FV	TSB
Potato50%/Bean50%	270±24.04^{I}	258.5±14.85^{J}	11±9.90^{J}	509±41.01^{I}	250.5±26.16^{H}
Rice50%/Potato50%	979.5±64.35^{GF}	840±42.43^{HG}	139.5±21.92^{HI}	1536.5±0.70^{G}	696.5±43.13^{F}
Tapioca50%/Rice50%	2246.5±0.71^{C}	1517.5±0.71^{CB}	729.5±0.71^{D}	2412.5±0.71^{DC}	895±0.00^{DE}
Tapioca50%/Bean50%	1533±48.08^{D}	1100±25.46^{EF}	433±22.63^{F}	1626.5±40.31^{FG}	526.5±14.84^{G}
Rice50%/Bean50%	**566.5±2.12^{H}**	**569.5±0.71^{F}**	**-3±1.41^{J}**	**1895±4.24 ^{FE}**	**1323±1.41^{C}**
Rice75%/Bean25%	1050.5±0.71^{EF}	1042.5±0.71^{EF}	8.5±0.71^{J}	2990.5±0.71^{B}	1948±0.00^{B}
Rice75%/Potato25%	1361.5±0.71^{D}	1198.5±0.71^{DE}	163.5±0.71^{H}	2442.5±0.71^{DC}	1243±0.00^{C}
Tapioca75%/Potato25%	3283.5±0.71^{B}	1446.5±0.71^{C}	1837.5±0.71^{B}	2257.5±0.71 ^{D}	811±1.41^{DEF}
Tapioca75%/Bean25%	2516.±0.71^{C}	1391.5±0.71^{CD}	1124.5±0.71^{C}	2169±0.00^{ED}	777.5±0.71^{EF}
Rice50% Potato25% Bean25%	**714.5±157.68^{GH}**	**691±162.63^{HI}**	**13.5±9.19^{JI}**	**1629.5±248.19^{GF}**	**928.5±99.70^{D}**
Rice100%	2343±73.54^{C}	1771±36.77^{A}	572±36.77^{E}	4310±80.61^{A}	2554±65.05^{A}
Bean 100%	185.5±10.60^{I}	187±9.90^{J}	-1.50±0.71^{J}	577±15.56 ^{I}	390±5.66^{HG}
Potato100%	924.5±146.37^{GF}	710±45.25^{HI}	214.5±101.12^{GH}	1083.5±50.21^{H}	373.50±4.95^{H}
Tapioca 100%	3676±115.97^{A}	1669.5±65.76^{AB}	2006.5±50.21^{A}	2574±120.21 ^{C}	904.5±54.45^{DE}
Wheat 100%	**1268.5±41.72^{DE}**	**940±5.66^{FG}**	**303.5±0.71^{G}**	**1816±50.91^{FG}**	**851±9.90^{DE}**

PV = Peak Viscosity, MV =Minimum Viscosity, BKD =Breakdown, FV =Final Viscosity, TBS = Total Setback. Means with same letters have no significant difference between flours for each RVA parameter.

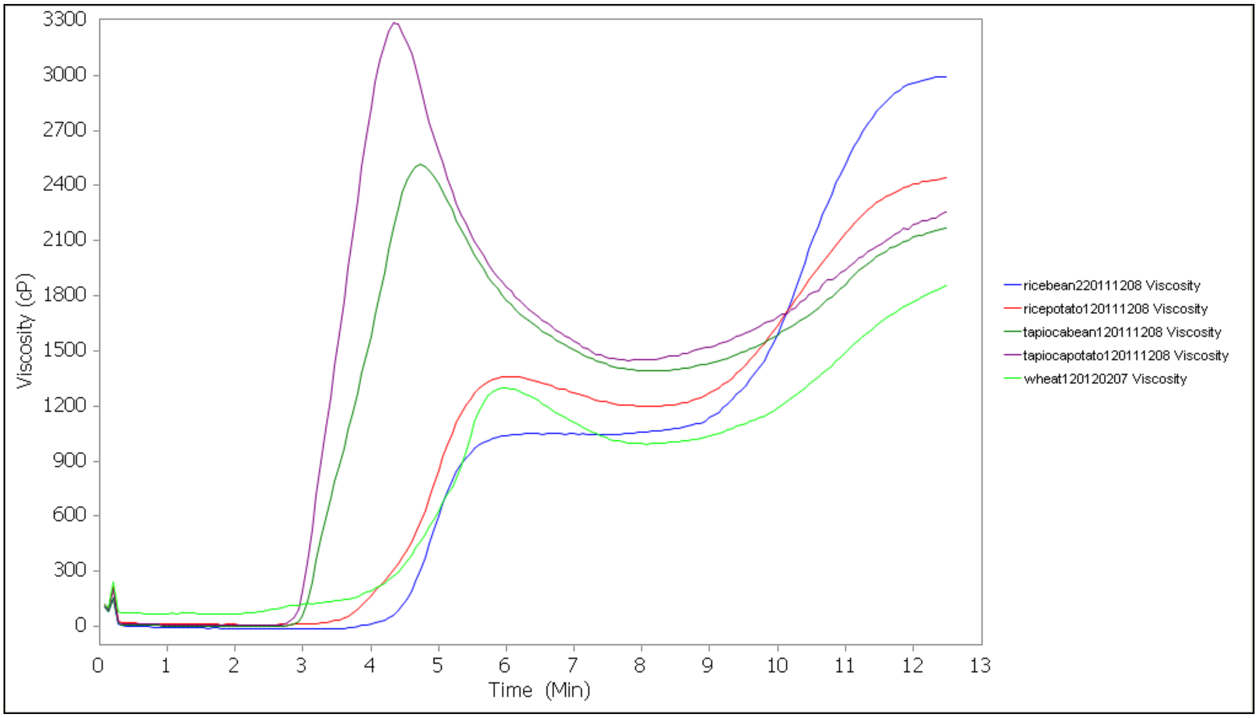
The important qualities of bread are dependent on the uniformity of the grains which affect the texture. The higher the fracture dimensions ranges the finer the bread (Gonzales-Barron and Butler, 2008). Statistical analysis shows that there was no significant difference in the hardness, adhesiveness, resilience, cohesion, springiness, gumminess and chewiness between the three samples at Alpha 0.05. According to Lazaridou and others (2007) the se of xanthan may decrease the overall distribution of uniform gas cells in the bread which may have contributed to the springiness in the bread. He reported further states that with the addition of hydrocolloids in gluten-free flours have generally reduced the bread's elasticity, but in some case polysaccharides increase it. Thus far no exact science of the formulation is available. Findings of the current study are similar to studies by Lazaridou and others (2007). Lazaridou and others (2007) reported no significant difference in the texture when pectin and agarose were used compared to xanthan.

These findings appear to be in normal range for bread when compared to similar studies of bread done by Baron and Butler (2008) and Krupta (2010). Note should be made of the similarity of the textures of the two gluten-free breads to the wheat bread.



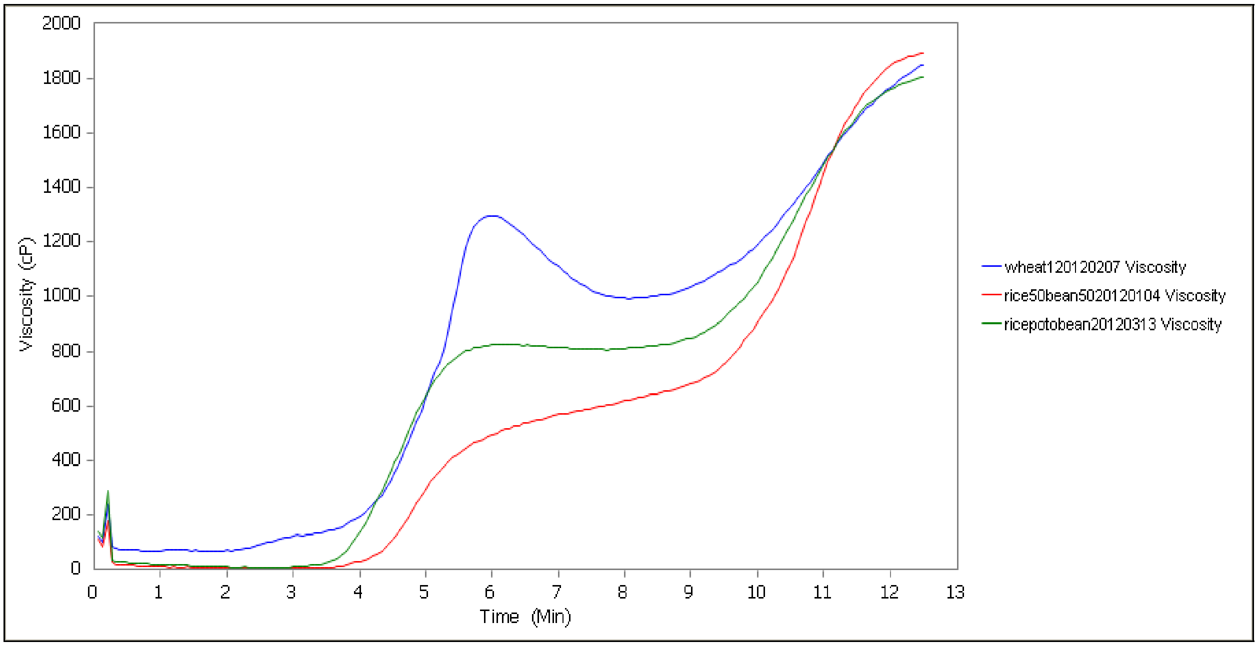
Note: Color Black = Rice, Red = Potato, Dark Green = Bean, Light Green= Wheat, Purple = Tapioca

Figure 2 RVA Flour Analyses of 100% Gluten-Free Flours and 100%Wheat



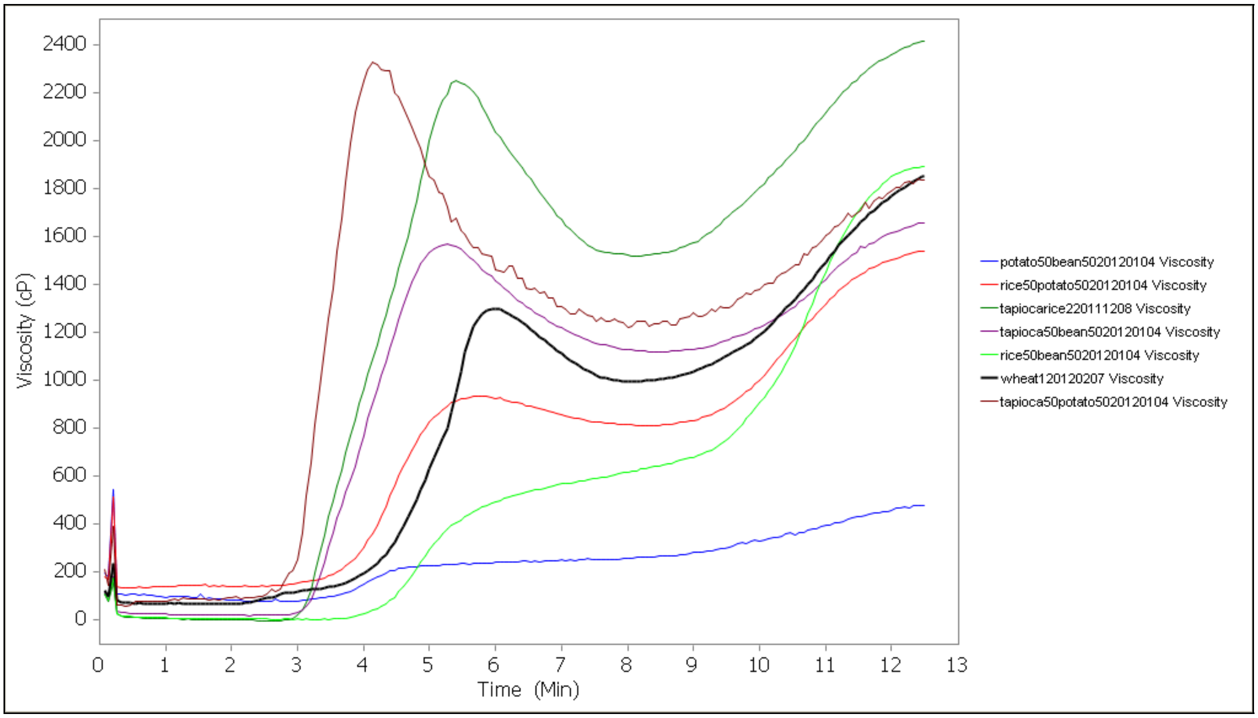
Note: Color Blue = RiceBean, Red = RicePotato, Dark Green = TapiocaBean, Purple = TapiocaPotato, Light Gren = Wheat

Figure 3 RVA Flour Analyses 75%/25% Combination Gluten-Free Flours and 100% Wheat Flour



Note: Color Blue = Wheat, Red = Rice/Bean, Green = Rice/Potato/Bean

Figure 4 RVA Flour Analyses Rice50%/Bean50% Combination Flours; Rice50%/Potato25%/Bean25% Combination Flours; and 100%Wheat Flour



ote: Color Black = Wheat, Blue =Potato/Bean, Red = Rice/Potato, Dark Green = Tapioca/Rice, Purple = Tapioca/Bean, Light Green = Rice/Bean, Brown = Tapioca/Potato

Figure 5 RVA Flour Analyses Combination 50%/50% Gluten-Free Flours and 100%Wheat Flour

Table 7. Texture Analyses of Breads

Bread	Hardness	Adhesiveness	Resilience	Cohesiveness	Springiness	Gumminess	Chewiness
Rice/Bean	10.25±0.49[a]	0.12±0.62[a]	51.05±0.28[a]	0.88±0.01[a]	43.56±7.55[a]	9.01±0.59[a]	3.93±0.75[a]
Rice/Bean/ Potato	10.45±1.68[a]	0.10±0.47[a]	47.26±9.38[a]	0.81±0.11[a]	40.18±12.02[a]	8.58±2.14[a]	3.33±3.33[a]
Wheat	10.00±0.10[a]	-0.42±0.43[a]	45.75±5.99[a]	0.82±0.02[a]	63.86±5.43[a]	8.18±0.162[a]	5.22±0.46[a]

a) This table represents 50% rice flour and 50% bean flour; 50% rice flour, 25% potato flour and 25% bean flour; 100% wheat flour. b) Average (3 samples), St. Dev (Standard deviation of 3 samples); c) Means are not significantly different between breads when letters are the same for each texture parameter.

4.4.4. Color Analyses

The crumb colors of the rice/bean bread combination, rice/bean/potato combinations and control (wheat) are shown in Table 8. The results reveal no significant difference in the color cumb readings of the two gluten-free breads and the wheat (control) bread. Findings are similar to those reported by Clerici and others (2009). In their study they used rice flour so we can easily compare the crumb color. Crumb color was reported with L* value that ranged from 60.9

to 66.7, a* value ranged from -124 to 4.04 and b* value ranged from 3.90 to 4.65. Kiskini and others (2012) used a mix of gluten-free flours which also produced similar crumb color to this research. Kiskini's and others findings were L* ranged from 50.7 to 66.9, a* ranged from 2.0 to 6.1, and b* ranging from 19.4 to 27.6. Matos and Rosell (2012) study on gluten-free bread color analyses L* values ranged from 72.2 to 81.5, a* values ranged from -0.80 to -2.59 and b* values ranged from 5.06 to 21.9. The current study L*, A*, and B* results are similar to the findings of Mastos and Rosell (2012). Computer SAS analyses was run on the data obtained from the Minolta readings are on Table 8. The SAS color analysis program is located in appendix J.

Table 8 Color Analysis of Breads

Breads	**L***	**A***	**B***
Rice50%/Bean50%	73.6 ± 5.78^{a}	70.8 ± 2.85^{a}	73.1 ± 2.02^{a}
Rice50%/Potato25%/Bean25%	-0.89 ± 0.99^{a}	-0.71 ± 0.27^{a}	-0.96 ± 0.43^{a}
Wheat 100%	16.9 ± 1.82^{a}	17.3 ± 1.85^{a}	16.6 ± 0.95^{a}

Number of samples of Rice Bean Bread = 10; number of samples for Rice Potato Bean Bread = 10; Number of samples of wheat bread = 7. Significant alpha 0.05. Means with the same letter are not significantly difference between breads.

4.4.5. Gluten Testing Procedure

Upon using the GlutenTox Home gluten testing kit all of the ingredients used in the production of all the breads developed were tested prior to baking. All ingredients both dry and liquid for both the rice/bean bread and rice/bean/potato tested negative for gluten. Samples from the baked gluten-free breads also tested negative. To confirm the accuracy of the test the wheat flour and the sample of the wheat bread were also tested and both tested positive for gluten.

4.5 MICROBIOLOGICAL ANALYSES

Microbiology analyses were done on samples of the French bread developed including the wheat bread (control) and two gluten free breads, the first consisting of 50 percent of white rice flour, 25 percent potato flour and 25 percent white bean flour and the second consisting of 50 percent white rice flour and 50 percent white bean flour. All samples were tested 24 hours after baking and the yeast, mold and aerobic plates were checked for counts initially a day later. No evidence of yeast, mold or aerobic counts present on any of the plates after 3 days. All of the breads were found to have 'zero' counts of CFU before sensory studies were performed.

A study done by Pascall and others (2008) reported microorganisms on soy bread ranging from 1.3 ± 0.3 to 6.7 ± 1.7. In a study by Lainez and other (2008), mold growth was lower than 1 CFU/g. All Petri film samples of rice bean bread, rice potato bean bread and wheat bread in this study were to be within acceptable standards for human consumption at less than 0 CFU.

4.6 SENSORY EVALUATION

An initial sensory evaluation of a gluten-free bread sample with white rice combined with white bean flour, a sample of rice flour combined with white bean flour and potato flour, and a sample using wheat French bread sample were performed. Initially, the three samples were provided to the 105 individuals from the general (non-Celiac) population. Sensory results of individuals not on a gluten-free and/or milk-free diet indicated a borderline acceptability. At an alpha level of 0.05 the overall appearance, overall aroma, crumb moistness, crumb softness, overall flavor and overall likeness showed a statistical difference from the wheat bread and both gluten-free breads. However, there was no statistical difference in the two gluten-free breads. The evaluation of crumb color with an alpha level of 0.05 there is no significant difference between the wheat bread and the rice bean bread. There was a statistical difference between the wheat bread and the rice potato bean bread. However, there was no significant difference beween the two gluten-free breads. The mean and standard deviation of each of the sensory evaluations are listed in Tables 9 and 10.

The statistical analyses indicate that general (non-Celiac) population sensory evaluation of the combination of rice, bean, and potato flour indicate marginal acceptability of these breads.

Statistical analysis revealed no significant difference in the overall appearance of the combined flours rice bean bread and the other gluten-free bread with rice, potato and bean flour. There was a significant difference in the overall appearance of the two gluten-free breads compared to the wheat bread with an F Value of 18.35 and Alpha level of <0.0001. Finding of crumb color indcate no significant difference between the two gluten-free breads and also no significant difference between the wheat bread and the rice bean bread. There was a significant difference in crumb color between the rice potato bean combination compared to the wheat with F value of 7.66 and Alpha level of 0.0006. Overall aroma indicates no significant difference between the gluten-free breads. Comparing them to the wheat bread noted a significance F level of 19.74 with an Alpha level of <0.0001. Crumb moistness show there is no significant difference between the two gluten free breads but a significance with F level of 18.85 and an Alpha level of <0.0001. Crumb softness shows the gluten-free breads to have no significant difference, but when compared to the wheat at F level of 21.03 and Alpha of <0.0001 there is a significant difference. Overall flavor shows no significant difference between the gluten-free breads but a significant difference when compared to the wheat breads with a F value of 54.76 and an Alpha level of <0.0001. Overall liking shows no significant difference between the gluten-free breads but a significance when compared to the wheat bread at a F level of 50.67 with an Alpha of <0.0001.

A second study was performed with a target (Celiac) population that included individuals on agluten-free and milk-free diet participated in a sensory study which included a sample consisting of white rice, white bean, and potato flours and a second sample consisting of rice and bean flour. The Celiac population sensory results are illustrated in Tables 11 and 12. The statistical analyses at alpha 0.05 revealed no significant difference in overall appearance, crumb color, overall aroma, crumb moisture, crumb softness, overall flavor, and overall likeness between the two gluten-free breads.

Table 9. Non-Celiac Population Sensory Results

Brea	Overall Appearance	Crumb Color	Overall Aroma	Crumb Moistness	Crumb Softness	Overall Flavor	Overall Liking
Wheat	6.81±1.28[a]	6.70±1.36[a]	6.72±1.58[a]	6.71±1.62[a]	6.66±1.46[a]	6.51±1.49[a]	6.59±1.52[a]
Rice/Potato/Bean	5.53±1.81[b]	5.93±1.59[ba]	5.59±1.88[b]	5.46±1.90[b]	5.45±1.80[b]	4.09±1.96[b]	4.38±1.66[b]
Rice/Bean	5.88±1.66[b]	6.29±1.44[b]	5.31±1.79[b]	5.42±1.76[b]	5.30±1.78[b]	4.44±2.02[b]	4.52±1.90[b]

1)Wheat 100%; Rice 50%/Potato 25%/Bean 25%; Rice 50%/Bean 50%. 2) Statistical significance set at Alpha 0.05; 3) Means for each attribute with same letters have no significant difference between breads; 4) Hedonic scale ranged from 9 for 'extremely like' to dislike extremely '1'.

Table 10 Non-Celiac Population Sensory Frequency Results

Frequency Procedure	Bread	1	Percent	2	Percent	3	Percent
Just About Right Moistness	Wheat	8	7.62	77	73.33	20	19.05
Just About Right Crumb Softness	Wheat	7	6.67	88	83.81	10	9.52
Intent to Purchase	Wheat	65	61.90	40	38.10	N/A	N/A
Intent to Purchase if DW Free	Wheat	66	62.86	39	37.14	N/A	N/A
Just About Right Moistness	Rice/Potato/Bean	18	9.52	90	47.62	81	42.86
Just About Right Crumb Softness	Rice/Potato/Bean	27	14.29	100	52.91	62	32.80
Intent to Purchase	Rice/Potato/Bean	62	32.80	127	67.20	N/A	N/A
Intent to Purchase if DW Free	Rice/Potato/Bean	79	41.80	110	58.20	N/A	N/A
Just About Right Moistness	Rice/Bean	27	23.08	47	40.17	43	36.75
Just About Right Crumb Softness	Rice/Bean	34	29.06	58	49.57	25	21.37
Intent to Purchase	Rice/Bean	28	23.93	89	76.07	N/A	N/A
Intent to Purchase if DW Free	Rice/Bean	37	31.62	80	68.38	N/A	N/A

a)Wheat 100%; Rice 50%/Potato 25%/Bean 25%; Rice 50%/50%.; b) Moistness: 1 = Not moist enough, 2 = Just about right, 3 = Too Moist; c) Softness: 1 = Not soft enough, 2= Just about right, 3 = Too soft; d) Intent to Purchase: 1 = yes; 2 = no. e) Intent to Purchase Dairy Wheat: 1 = yes, 2 = no.

Table 11 Celiac Population Sensory Results

Bread Measure	Overall Appearance	Crumb Color	Overall Aroma	Crumb Moistness	Crumb Softness	Overall Flavor	Overall Liking
Rice/Potato/Bean	6.52±1.32[a]	6.71±1.25[a]	6.51±1.52[a]	6.26±1.66[a]	6.25±1.54[a]	5.38±2.15[a]	5.57±2.04[a]
Rice/Bean Mean	6.55±1.52[a]	6.64±1.46[a]	6.35±1.46[a]	6.31±1.76[a]	6.27±1.56[a]	5.44±2.07[a]	5.52±2.05[a]

1) Wheat 100%; Rice 50%/Potato 25%/Bean 25%; Rice 50%/50%. 2) Statistical difference set at Alpha 0.05. 3) Means for each attribute with same letters have no significant difference between breads; 4) Hedonic scale ranged from 9 for 'extremely like' to dislike extremely '1'.

The previous sensory studies of Carr and others (2006) and Torbica and others (2010) considered the breads in their studies acceptable because of scores of 5 and above. The results of the present study indicate that Celiac consumers found the gluten-free milk-free breads more acceptable then the population of non celiac consumers. In the present study the celiacpopulation rated the gluten-free breads more acceptable than the non-celiac population but there was not a significant difference at alpha 0.05. Laureati and others (2010) compared evaluations of a non-celiac population with a celiac population of gluten-free product. He reported there was no significant difference of the two population evaluations. This is comparable to the present study in which both the non-celiac population and celiac population evaluated the two gluten-free breads.

Table 12 Celiac Population Sensory Frequency Results

Frequency Procedure	Bread	1	Percent	2	Percent	3	Percent
Just About Right Moistness	Rice/Potato/Bean	9	10.71	49	58.33	26	30.95
Just About Right Crumb Softness	Rice/Potato/Bean	10	10.90	54	64.29	20	23.81
Intent to Purchase	Rice/Potato/Bean	45	53.57	39	46.43	N/A	N/A
Intent to Purchase if DW Free	Rice/Potato/Bean	52	61.90	32	38.10	N/A	N/A
Just About Right Moistness	Rice/Bean	11	13.10	43	51.19	30	35.71
Just About Right Crumb Softness	Rice/Bean	15	17.86	51	60.71	18	21.43
Intent to Purchase	Rice/Bean	46	54.76	38	45.24	N/A	N/A
Intent to Purchase if DW Free	Rice/Bean	49	58.33	35	41.67	N/A	N/A

Moistness: 1 = Not moist enough, 2 = Just about right, 3 = Too Moist; Softness: 1 = Not soft enough, 2= Just about right, 3 = Too soft; Intent to Purchase: 1 = yes; 2 = no; Intent to Purchase Dairy Wheat: 1 = yes, 2 = no.

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

As a result of literature search there was clear evidence of a lack of sufficient gluten-free milk-free food products. With further investigation by surveying local specialty stores and groceries this evidence was confirmed. A survey of celiac subjects' evaluation of problems included texture, taste, color, and smell of gluten-free milk free food products. As a result of this study it was concluded that bread products were the products that presented the most problems to the consumer, especially in taste and texture. At this point, it was decided to develop a gluten-free and milk-free French bread which was not available as a gluten-free milk-free product. Since bread is a vital source of dietary intake it was concluded this would provide an important addition to the gluten-free milk-free market.

Analyses of several gluten-free flours was performed and it was concluded that the combination of rice flour with bean and/or potato flour were the best potential combinations for making a gluten-free milk-free French bread. Sensory studies were performed on two flour combinations of gluten-free milk-free bread and were found to be marginally acceptable by the general population. The target (Celiac) population rated both gluten free breads acceptable and gave a higher rating of acceptability compared to the general population.. Texture and color analyses as well as SAS statistical analyses of the consumer studies confirmed these conclusions.

From the result of this study, it can be concluded that the development of a larger variety of tasty gluten-free milk-free breads and other food products should be considered for development. The two gluten-free milk-free breads were acceptable by the Celiac population in this study and marginally acceptable by the non-celiac population. There are 17 gluten-free ingredients which can be used either individually or in combination with other gluten-free ingredients to provide a variety of products, which will be creating and providing a variety of nutritious tasty food products. The potential for creating gluten-free milk-free products are great and it is possible that the products can be developed to be acceptable by the non-celiac population as well.

It is recommended that further studies be done using other gluten-free ingredients to potentially enhance texture, flavor, and overall appeal of a French bread. It is also suggested to potentially use egg substitutes or develop a formula without eggs to provide the available of products for individuals with egg allergies. It is also recommended a formula with the use of milk substitutes. There is a need to investigate shelf life of the French bread product. A suggestion to develop the ready-made bread dough which can be frozen and consumers can potential defrost and place in a pre-heated oven and bake for 30 minutes, providing them with fresh baked bread. Finally, a larger target population of individuals on a gluten-free and milk-free diet is strongly recommended.

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APPENDIX A. SAS PROGRAM OF STORE GLUTEN-FREE MILK-FREE SURVEY

Store Survey of Products Gluten-Free and Milk-Free

```
dm 'log;clear;output;clear';
footnote "Store Survey of Products Gluten-Free and Milk-Free";
data one;input Sample Type $ Vender $ GlutenFree MilkFree Store1 Store2
Store3  Store4 Store5 Store6 Store7  Store8 Store9  Store9 Store10 Store11
Store12 Store13 Store13 Store14;
datalines;
1 PrairieBread WholeFoodBake 1.00 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
1 SandwwichBread WholeFoodBake 1.00 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
1 SundriedTomato&Garl WholeFoodBake 1.00 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
.....
;
proc sort;by Sample Type $ Vender $ GlutenFree MilkFree Store1 Store2 Store3
Store4 Store5 Store6 Store7  Store8 Store9  Store9 Store10 Store11 Store12
Store13 Store13 Store14;
proc freq; by Store1 Store2 Store3  Store4 Store5 Store6 Store7  Store8
Store9  Store9 Store10 Store11 Store12 Store13 Store13 Store14;
tables Type Vender GlutenFree MilkFree & Store1 Store2 Store3  Store4 Store5
Store6 Store7  Store8 Store9  Store9 Store10 Store11 Store12 Store13 Store13
Store14;;
* NORMAL DISTRIBUTION;
*****************************************************************************;
* PLOT NORMAL DISTRIBUTION WITH MEAN = 0.0 AND STANDARD DEVIATION = 1.0;
%LET MEAN=0.0;
%LET STDDEV=1.0;
proc means mean std n maxdec=2;by Sample Store1 Store2 Store3  Store4 Store5
Store6 Store7  Store8 Store9  Store9 Store10 Store11 Store12 Store13 Store13
Store14;
var Sample; by GlutenFree,by MilkFree;
proc anova;
      class Sample;
      sample = Sample Type $ Vender $ GlutenFree MilkFree Store1 Store2
Store3  Store4 Store5 Store6 Store7  Store8 Store9  Store9 Store10 Store11
Store12 Store13 Store13 Store14;
      proc means  mean std n cv; by glutenfree, by milkfree;
proc sort;by glutenfree; by milkfree;
proc freq; by glutenfree; by milkfree;
      table  = glutenfree milkfree/ chisq;
proc means mean std n maxdec=2;by GLUTENFREE; by MILKFREE;
var Sample Type $ Vender $ GlutenFree MilkFree Store1 Store2 Store3  Store4
Store5 Store6 Store7  Store8 Store9  Store9 Store10 Store11 Store12 Store13
Store13 Store14;
proc anova; by glutenfree; by milkfree;
Proc sort; by glutenfree;by milk free;
Proc means mean std cv n; by glutenfree; by milkfree;
run;

ods csv close;
```

APPENDIX B. CONSUMER SURVEY

PART 1

This survey is being done as part of a Master Program research project by Annette Bentley, a graduate student a Louisiana State University. The survey was approved by the Board of Governors of the Celiac Sprue Association of the United States of America.

If you are a celiac over the age of 18, your participation in this study is greatly appreciated.

Gender: Male ___ Female ____ Age: 18 to 25 ___ 26 to 35 ____ 36 to 45 ___ 46 to 55 ___ 56 and older ____

Currently geographical residence: North East___ North Central ____ North West_____ South East ___ South Central South West ____ .

This information is for research purposes only. The study will be a blind study, so that no information about the individuals who completed the form can or will be disclosed.

Product	*Have you purchased this product? Y=Yes, N = No. If no go to next product*	*Have you eaten this product? Y =Yes, N-No*	*Is there a problem with this product? Y =Yes, N =No. If no go to next product*	*Taste Y=Yes N=No*	*Texture Y=Yes N=No*	*Smell Y=Yes N=No*	*Color Y=Yes N=No*
BREAD PRODUCTS							
Ener-G Foods breads							
Whole Foods Bakehouse breads							
Gillian's Foods rolls							
Food for Life breads							
Whole Foods Bakehouse bagels							
Glutino bagels							
PREBAKED PASTRY ITEMS							
Van's (waffles, pancakes,etc)							
Ian's National Foods (waffles,etc)							
Kinnikinnick (donuts,etc)							
Udi's (muffins, etc)							
Crave Bakery pies							
Whole Foods Bakehouse (pastry)							
COOKIES							
Andrean Dream cookies							
Glutino Cookies							
Enjoy Like Cookies							
Pamela's Products Cookies							
PASTA							
Ener-G Foods (spaghetti, etc)							
Mrs. Leeper's(spaghetti,etc_)							
Lundenberg Farms (spaghetti,etc)							
DeBoles (spaghetti, etc)							

APPENDIX B. CONSUMER SURVEY

PART 2

Product	*Have you purchased this product? Y=Yes, N = No. If no go to next product*	*Have you eaten this product? Y =Yes, N-No*	*Is there a problem with this product? Y =Yes, N =No. If no go to next product*	*Taste Y=Yes N=No*	*Texture Y=Yes N=No*	*Smell Y=Yes N=No*	*Color Y=Yes N=No*
SUBSTITUTE ICE CREAM							
Haagen Dazs Sorbet							
Turtle Mountain So Delicious Products							
Turtle Mountain Purely Decadent Products							
Tofutti							
Julian Sorbet							
SUBSITUTE DAIRY DESERTS							
Zen Soy Pudding							
Kozy Shack Pudding							
Kozy Shack Flan							
DAIRY SUBSITUTE CHEESE							
Follow Your Heart (cheddar,etc)							
Vegan (mozzarella, etc)							
Tofutti(mozzarella, etc)							
Tofutti –It Better than Cream Cheese)							
PREMADE PIZZA							
Amy's Pizza							
Glutino Pizza							
Ian's Pizza							
CEREALS							
Barbara's Puffins							
Nature's Path							
Perky's							

Product	*Have you purchased this product? Y=Yes, N = No. If no go to next product*	*Have you eaten this product? Y =Yes, N-No*	*Is there a problem with this product? Y =Yes, N =No. If No go to next product*	*Taste Y=Yes N=No*	*Texture Y=Yes N=No*	*Smell Y=Yes N=No*	*Color Y=Yes N=No*
FROZEN MEALS/ENTRÉE'							
Amy's Rice Macaroni & cheese							
Amy's Brown Rice Vegetable Bowl							
Amy's Mexican tamale							
Vegan Plus (Vegan Shrimp,etc)							
Glutino(Chicken Alfredo, etc)							
Tendoor Chef (Chicken curry,etc)							
Ian's Natural (fish sticks, etc)							
Ethenic (Chicken Korma, etc)							
Gluten Fee Café (meals)							
SHELF STABLE MEALS/ENTRÉE'							
Vegan (meals)							
Priya (meals)							
Thia Kitchen (lemon grass, etc)							
YOGURT							
Stony Farm							
SoDelicious							
Whole Soy & Co							
SNACK BARS							
Enjoy Life							
Glutino							
Bumble Bar							
EnviroKidz							

APPENDIX C. INGREDIENT FORMULA FOR FRENCH BREAD

A1 Ingredient	A g or ml	A3 Percent	B1 Ingredient	B2 g or ml	B3 Percent	C1 Ingredient	C2 g/ml	C3 Percent
Rice Flour	129 g	17.07	Rice Four	129 g	17.07	Wheat flour	259 g	35.3
Bean Flour	129g	17.07	Potato Flour	64.5 g	8.54	Salt	4 g	0.55
Salt	4 g	0.5	Bean Flour	64.5 g	8.54	Yeast	24 g	3.27
Yeast	24 g	3.17	Salt	4 g	0.5	Sugar	24 g	3.27
Sugar	24 g	3.17	Yeast	24 g	3.17	X-Gum	7.5 g	1.02
x gum	7.5 g	1	Sugar	24 g	3.17	G-Gum	7.5 g	1.02
g gum	7.5 g	1	X Gum	7.5 g	1	Corn Starch	24 g	3.27
corn starch	24 g	3.17	G Gum	7.5 g	1	Baking Powder	24 g	3.27
baking flour	24 g	3.17	Corn Starch	24 g	3.17	Egg Whites	90 ml	12.27
egg whites	90 ml	12	Baking Flour	24 g	3.17	Corn Oil	17ml	2.32
corn oil	17 ml	2.25	Egg Whites	90 ml	12	Vinegar	2.5 ml	0.34
Vinegar	2.5 ml	0.33	Corn Oil	17 ml	2.25	Water	250 ml	34.1
Water	260 ml	34.4	vinegar	2.5 ml	0.33			
granlated garlic	13 g	1.7	Water	260 m;	34.4			
			Vanilla	13 ml	1.7			
total ingredients	755.5	100		755.5	100		733.5	100

A1 = Rice Bean Ingredients, A2 gram or milliliter of ingredient, A3 % of ingredient. B1 Ingredient of Rice Potato Bean Bread, B2, the gram or milliliter of ingredients, B3 the percentage of ingredients. C1 Wheat ingredients, C2 the grams or milliliter of ingredients,C3 Percent of ingredients.

APPENDIX D. RVA ANALYSES OF FLOURS SAS PROGAM

```
dm 'log;clear;output;clear';
Title "RVA ANALYSIS OF FLOURS";
data one;
input Sample$ Bread$ Peak Trough1 Breakdown FinalViscosity Setback
TotalSetback;
datalines;
Rice50Bean50 565 569 -4 1898 1333 1324
Rice50Bean50 568 570 -2 1892 1324 1322
…..
;
proc sort;by sample;
proc freq; by sample;
tables Trough1 Breakdown FinalViscosity Setback TotalSetback = sample;
proc means mean std n maxdec=2;by sample;
var Trough1 Breakdown FinalViscosity Setback TotalSetback;
proc anova;
class Sample;
model Trough1 Breakdown FinalViscosity Setback TotalSetback = sample;
means sample/tukey lines;
Proc sort;by sample;by bread;
Proc freq;by sample; by bread;
      table Trough1 Breakdown FinalViscosity Setback TotalSetback = sample;
Proc means mean std n maxdec=2;by sample; by bread;
      var Trough1 Breakdown FinalViscosity Setback TotalSetback;
proc anova; by sample;
      class bread;
      model Trough1 Breakdown FinalViscosity Setback TotalSetback;
      means sample/tukey lines;
Proc univariate;
      var Trough1 Breakdown FinalViscosity Setback TotalSetback;
run;ods csv close;
```

APPENDIX E. WHITE RICE FLOUR PROXIMATE ANALYSES

BOB'S RED MILL NATURAL FOODS, INC.,13521 SE Pheasant Ct. • Milwaukie, OR 97222 • (503) 654-3215 • FAX: (503) 653-1339),
www.bobsredmill.co

BRM White Rice Flour November 16, 2007

Total Weight: 40.00 g (1.41 oz-wt.)
Serving Size: 40.00 g (1.41 oz-wt.)
Serves: 1.00
Cost: --

Per Serving

Nutrient	Amount Per 100g	Amount Per Serving
Basic Components		
Calories	366.00	146.40
Calories from Fat	12.78	5.11
Calories from Saturated Fat	3.47	1.39
Protein	5.95 g	2.38 g
Carbohydrates	80.13 g	32.05 g
Dietary Fiber	2.40 g	0.96 g
Soluble Fiber	0.60 g	0.24 g
InSoluble Fiber	1.80 g	0.72 g
Sugar - Total	0.12 g	0.05 g
Other Carbs	77.61 g	31.04 g
Fat - Total	1.42 g	0.57 g
Saturated Fat	0.39 g	0.15 g
Mono Fat	0.44 g	0.18 g
Poly Fat	0.38 g	0.15 g
Trans Fatty Acids	0 g	0 g
Cholesterol	0 mg	0 mg
Vitamins		
Vitamin A IU	0 IU	0 IU
Thiamin-B1	0.14 mg	0.06 mg
Riboflavin-B2	0.02 mg	0.01 mg
Niacin-B3	2.59 mg	1.04 mg
Vitamin-B6	0.44 mg	0.17 mg
Vitamin-B12	0 mcg	0 mcg
Biotin	-- mcg	-- mcg
Vitamin C	0 mg	0 mg
Vit E Alpha-Tocopherol	0.11 mg	0.04 mg
Folate	4.00 mcg	1.60 mcg
Vitamin K	0 mcg	0 mcg
Pantothenic Acid	0.82 mg	0.33 mg
Minerals		
Calcium	10.00 mg	4.00 mg
Chloride	-- mg	-- mg
Chromium	-- mcg	-- mcg
Copper	0.13 mg	0.05 mg
Iodine	1.00 mcg	0.40 mcg
Iron	0.35 mg	0.14 mg
Magnesium	35.00 mg	14.00 mg
Manganese	1.20 mg	0.48 mg
Molybdenum	-- mcg	-- mcg
Phosphorus	98.00 mg	39.20 mg
Potassium	76.00 mg	30.40 mg
Selenium	15.10 mcg	6.04 mcg
Sodium	0 mg	0 mg
Zinc	0.80 mg	0.32 mg
Saturated Fats		
18:0-Stearic	0.03 g	0.01 g
Other		
Alcohol	0 g	0 g
Caffeine	0 mg	0 mg
Sugar Alcohol	-- g	-- g

APPENDIX F. WHITE BEAN PROXIMATE ANALYSES

BOB'S RED MILL NATURAL FOODS, INC.,13521 SE Pheasant Ct. • Milwaukie, OR 97222 • (503) 654-3215 • FAX: (503) 653-1339 • www.bobsredmill.com

BRM White Bean Flour November 16, 2007

Total Weight: 32.00 g (1.13 oz-wt.)
Serving Size: 32.00 g (1.13 oz-wt.)
Serves: 1.00
Cost: --

Per Serving

Nutrient	Amount Per 100g	Amount Per Serving
Basic Components		
Calories	336.00	107.52
Calories from Fat	10.62	3.40
Calories from Saturated Fat	2.74	0.88
Protein	21.11 g	6.76 g
Carbohydrates	62.25 g	19.92 g
Dietary Fiber	24.90 g	7.97 g
Soluble Fiber	7.22 g	2.31 g
InSoluble Fiber	17.68 g	5.66 g
Sugar - Total	6.00 g	1.92 g
Other Carbs	-- g	-- g
Fat - Total	1.18 g	0.38 g
Saturated Fat	0.30 g	0.10 g
Mono Fat	0.10 g	0.03 g
Poly Fat	0.51 g	0.16 g
Trans Fatty Acids	0 g	0 g
Cholesterol	0 mg	0 mg
Vitamins		
Vitamin A IU	0 IU	0 IU
Thiamin-B1	0.74 mg	0.24 mg
Riboflavin-B2	0.21 mg	0.07 mg
Niacin-B3	1.34 mg	0.43 mg
Vitamin-B6	0.44 mg	0.14 mg
Vitamin-B12	0 mcg	0 mcg
Biotin	-- mcg	-- mcg
Vitamin C	0 mg	0 mg
Vit E Alpha-Tocopherol	0.70 mg	0.22 mg
Folate	386.00 mcg	123.52 mcg
Vitamin K	-- mcg	-- mcg
Pantothenic Acid	0.73 mg	0.23 mg
Minerals		
Calcium	173.00 mg	55.36 mg
Chloride	-- mg	-- mg
Chromium	-- mcg	-- mcg
Copper	0.64 mg	0.20 mg
Iodine	-- mcg	-- mcg
Iron	7.73 mg	2.47 mg
Magnesium	183.00 mg	58.56 mg
Manganese	1.28 mg	0.41 mg
Molybdenum	-- mcg	-- mcg
Phosphorus	445.00 mg	142.40 mg
Potassium	1542.00 mg	493.44 mg
Selenium	12.80 mcg	4.10 mcg
Sodium	12.00 mg	3.84 mg
Zinc	2.81 mg	0.90 mg
Saturated Fats		
18:0-Stearic	0.02 g	0.01 g
Other		
Alcohol	0 g	0 g
Caffeine	0 mg	0 mg
Sugar Alcohol	-- g	-- g

APPENDIX G. POTATO FLOUR PROXIMATE ANALYSES

BOB'S RED MILL NATURAL FOODS, INC.,13521 SE Pheasant Ct. • Milwaukie, OR 97222 • (503) 654-3215 • FAX: (503) 653-1339 • www.bobsredmill.com

Bob's Red Mill Potato Flour July 19, 2006

Total Weight: 34.00 g (1.20 oz-wt.)
Serving Size: 34.00 g (1.20 oz-wt.)
Serves: 1.00
Cost: --

Per Serving

Nutrient	Amount Per 100g	Amount Per Serving
Basic Components		
Calories	351.00	119.34
Calories from Fat	7.20	2.45
Calories from Saturated Fat	1.89	0.64
Protein	8.01 g	2.72 g
Carbohydrates	79.90 g	27.17 g
Dietary Fiber	6.10 g	2.07 g
Soluble Fiber	-- g	-- g
InSoluble Fiber	-- g	-- g
Sugar - Total	0 g	0 g
Other Carbs	73.80 g	25.09 g
Fat - Total	0.80 g	0.27 g
Saturated Fat	0.21 g	0.07 g
Mono Fat	-- g	-- g
Poly Fat	-- g	-- g
Trans Fatty Acids	0 g	0 g
Cholesterol	0 mg	0 mg
Vitamins		
Vitamin A IU	0 IU	0 IU
Thiamin-B1	0.42 mg	0.14 mg
Riboflavin-B2	0.14 mg	0.05 mg
Niacin-B3	3.40 mg	1.16 mg
Vitamin-B6	-- mg	-- mg
Vitamin-B12	0 mcg	0 mcg
Biotin	0 mcg	0 mcg
Vitamin C	19.00 mg	6.46 mg
Vit E Alpha-Tocopherol	-- mg	-- mg
Folate	0 mcg	0 mcg
Vitamin K	-- mcg	-- mcg
Pantothenic Acid	1.50 mg	0.51 mg
Minerals		
Calcium	33.00 mg	11.22 mg
Chloride	-- mg	-- mg
Chromium	-- mcg	-- mcg
Copper	1.08 mg	0.37 mg
Iodine	-- mcg	-- mcg
Iron	17.20 mg	5.85 mg
Magnesium	88.00 mg	29.92 mg
Manganese	1.00 mg	0.34 mg
Molybdenum	-- mcg	-- mcg
Phosphorus	0.18 mg	0.06 mg
Potassium	1588.00 mg	539.92 mg
Selenium	-- mcg	-- mcg
Sodium	-- mg	-- mg
Zinc	1.54 mg	0.52 mg
Saturated Fats		
18:0-Stearic	-- g	-- g
Other		
Alcohol	0 g	0 g
Caffeine	0 mg	0 mg
Sugar Alcohol	-- g	-- g

APPENDIX H. TEXTURE ANALYSES SAS PROGRAM

```
dm 'log;clear;output;clear';
Title "Texture Analyses";
Footnote "Texture of Bean/Rice Flour Combination, Bean/Rice/Potato
Combination & Wheat only";
data one;
input TestID $ Bread $ Hardness Adhesiveness Resilience Cohesion Springiness
Gumminess Chewiness;
datalines;
TESTgf1 RiceBean 9.564  -0.651 51.268     0.854 64.356 8.171      5.258
TESTgf2 RiceBean 10.793 0.236 51.213      0.895 43.317 9.664      4.186
…………
;
proc freq;
      table bread;
proc sort data; by sample;
proc freq; by sample;
      tables Hardness Adhesiveness Resilience Cohesion Springiness Gumminess
Chewiness;
proc means mean std n maxdec=2; by Sample;
      var Bread Hardness Adhesiveness Resilience Cohesion Springiness
Gumminess Chewiness;
proc anova;
      class sample;
      model Bread Hardness Adhesiveness Resilience Cohesion Springiness
Gumminess Chewiness = sample;
      means sample/tukey lines;
Proc sort; by sample; by bread;
proc freq;by sample; by bread;
      tables Bread Hardness Adhesiveness Resilience Cohesion Springiness
Gumminess Chewiness= sample;
proc means mean std n maxdec=2;by sample; by bread;
      var Hardness Adhesiveness Resilience Cohesion Springiness Gumminess
Chewiness;
proc anova; by bread;
      class sample;
      model Hardness Adhesiveness Resilience Cohesion Springiness Gumminess
Chewiness;
      means sample/tukey lines;
proc sort; by sample; by bread;
proc freq; by sample; by bread;
      table Hardness Adhesiveness Resilience Cohesion Springiness Gumminess
Chewiness;
proc means mean std n maxdec=2;by sample; by bread;
      var Hardness Adhesiveness Resilience Cohesion Springiness Gumminess
Chewiness;
Proc anova; by bread;
      class sample;
      means sample/tukey lines;
Proc univariate;
var Bread Hardness Adhesiveness Resilience Cohesion Springiness Gumminess
Chewiness;
run;
ods csv close;
```

APPENDIX I. GLUTEN TOX TESTING KIT

7. Analysis of the results (GlutenTox Home follows the Codex Alimentarius norms):

Negative result: a single BLUE line (control line) appears in the central part of the test (C). The sample contains less than the specified (20 ppm or 5 ppm) amount of gluten and is suitable for celiacs.

Positive result: in addition to the control line (Blue), a PINK line also appears in the central part of the test (T). The sample contains more than the specified (20 ppm or 5 ppm) amount of gluten and is not suitable for celiacs.

NOTE: The intensity of the PINK line will depend on the quantity of gluten contained in the sample. ANY pink line is an indicator of unacceptable levels of gluten.

Invalid result: if the Blue control line does not appear, the test is considered invalid, even if a PINK line appears. An invalid result may be due to: an insufficient quantity of sample, a mistake made while following the steps of this manual, or a deterioration of the reagents. In case of an invalid result, it is necessary to repeat the test. If the problem persists, contact your supplier.

8. Quality control

The internal control is included in the test, in the form of the blue line that appears on the stick. This control checks that the sample volume is sufficient and that the procedure has been followed adequately. All kit components are manufactured under strict gluten-free conditions.

9. Specificity of the test

GlutenTox Home can detect the presence of the toxic fraction of gluten in wheat, rye, barley, and oat that could be harmful for celiacs. However, the test does not detect the presence of rice, corn or soy, as these ingredients are safe for people with celiac disease.

If the food to be tested is composed of several different elements, be sure the sample contains part of each element. If you test only one element, and gluten was distributed unevenly in the food, the result could be negative even if the food contains inappropriate amounts of gluten. For example, imagine testing cookie-dough ice cream: it is important to include both the cookie dough and the ice cream base in the sample for an accurate result.

10. Sensitivity of GlutenTox Home

The protocol of the kit is adjusted to detect a 20 ppm of gluten, taking into account international norms (Codex Alimentarius), however, it is possible to detect as low as 5 ppm for people that are extra-sensitive to gluten.

11. Reference

1. Shan L., et al.; "Structural basis for gluten intolerance in celiac sprue"; Science; 2002; 297: 2275-2279.

Biomedal DIAGNOSTICS

For more information, please visit our WEBSITE or contact us:

www.glutentoxhome.com
info@glutentoxhome.com

For information in the US:

www.glutentox.com
info@emportllc.com

GlutenTox Home

Biomedal DIAGNOSTICS

Quick test for the detection of gluten in foods

Ed.1-US - March 2011

1. Intended purpose

GlutenTox Home is a rapid and user-friendly test that detects gluten, which is harmful for celiac sufferers, in foods and drinks.

2. Introduction

Celiac disease is a disorder that damages the small intestine, causing the atrophy of the intestinal villi. It intervenes with the absorption of nutrients, including proteins, lipids, carbohydrates, mineral salts and vitamins. Celiac disease is caused by an inappropriate response of the immune system to gluten, a mix of proteins found in grains including wheat, barley, rye, and to a lesser extent, oat [ref.1].

Currently, the only treatment for celiac disease is a lifelong gluten-free diet. This is not always straightforward; in addition to being easily recognizable in many types of food, gluten can also be present in a less-obvious way, for example in food additives and preservatives.

According to the Codex Alimentarius Commission, food can be considered "gluten-free" if the amount of gluten does not exceed 20 parts per million (ppm*), and is considered "very low in gluten content" if the amount of gluten does not exceed 100 ppm.

GlutenTox Home gives you the option to test food or liquids for a gluten threshold of 20 ppm or 5 ppm.

* Milligrams of gluten per kilo of food.

3. Storage and disposal

GlutenTox Home must be stored at a temperature between 35ºF and 85ºF (2ºC - 35ºC). GlutenTox Home stick must be kept in its sealed metallic envelope until needed.

All components of the kit are fully disposable in ordinary trash.

4. Supplied materials for analysis

- GlutenTox Home stick and plastic pipette, contained in a sealed metallic envelope.
- Disposable plastic spoons, individually wrapped.
- Extraction bottles with yellow cap.
- Disposable plastic pipettes.
- Dilution bottles with blue cap.
- Instructions.

5. Useful materials, not supplied

- Watch (a stopwatch is preferable).
- Scale.
- Non-powdered disposable gloves.
- Mortar or any other utensil to grind the sample.
- Alcohol (ethanol).

6. Precautions

- Only for foodstuff analysis, **except for food containing chocolate.** (Visit our updated FAQ at www.glutentoxhome.com for additional special products or exceptions).
- To avoid contaminations that could interfere with the analysis, the use of non-powdered disposable gloves is recommended. If you do not have disposable gloves, be sure to wash your hands thoroughly before the test and dry them with a clean, non-contaminated towel.
- Once the GlutenTox Home stick has been removed from its metallic envelope, it must be kept under strictly clean conditions and used as soon as possible.
- Do not use any material from the kit after the expiration date printed on the box.
- Do not drink any solution or liquid from the kit (the extraction solution contains alcohol (ethanol)).
- **Keep out of reach of children. The test should only be performed by an adult.**

APPENDIX J. COLOR ANALYSES SAS PROGRAM

```
dm 'log;clear;output;clear';
Title "Color Analyses";
Footnote "Color of Bean/Rice Flour Combination, Bean/Rice/Potato
Combination";
data one;
input Sample $ Bread $ L A B;
datalines;
BR Bean/Rice  67.04 -2.01 18.12
BR Bean/Rice 75.86 -0.13 17.97
………..
;
proc sort data; by sample;
proc freq; by sample;
      tables L A B;
proc means mean std n maxdec=2; by Sample;
      var L A B;
proc anova;
      class sample;
      model L A B = sample;
      means sample/tukey lines;
Proc sort; by sample; by bread;
proc freq;by sample; by bread;
      tables L A B = sample;
proc means mean std n maxdec=2;by sample; by bread;
      var L A B;
proc anova; by sample;
      class bread;
      model L A B;
      means sample/tukey lines;
Proc univariate;
var Bread L A B;
run;
ods csv close;
```

APPENDIX K. CONSENT FORMS

PART 1

Research Consent Form for Consumer Research (for general population)

I,_______________________________ agree to participate in the research entitled "Consumer Acceptability of Non-Milk and Non Wheat French Breads" which is being conducted by Dr. Witoon Prinyawiwaatkul, Professor of the Department of Food Science of Louisiana State University, Agricultural Center, phone (225) 578-5188.

I understand that the participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records or destroyed. One hundred consumers will participate in this research. For this particular research, about 20 minutes participation will be required for each consumer.

The following has been explained to me:

1. In any case, it is my responsibility to report prior participation to the investigator any food allergies I may have.
2. The reason for the research is to gather information on consumer acceptability of bread. The benefit that I may expect from it is a satisfaction that I have contributed to development of more nutritious bread formulations.
3. The procedures are as follows: Three coded samples will be placed in front of me, and I will evaluate it by normal standard methods and indicate my evaluation on a score sheet. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participations entails minimal risk: The only risk which can be envisioned is that an allergic reaction to wheat, milk, rice flour, bean flour, potato flour, eggs, and commonly used baking ingredients. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.
5. The results of this study will not be released in any individual identifiable from without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand the additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be address to Dr. Michael Keenan (225)578-1708. I agree with the terms above and acknowledge.

I have been given a copy of the consent form.

________________________ ________________________________

Signature of Investigator Signature of Participant

Witness____________________ Date __________________________

APPENDIX K. CONSENT FORMS

PART 2

Research Consent Form for Consumer Research (Celiac Population)

I,_______________________________ agree to participate in the research entitled "Consumer Acceptability of Non-Milk and Non Wheat French Breads" which is being conducted by Dr. Witoon Prinyawiwaatkul, Professor of the Department of Food Science of Louisiana State University, Agricultural Center, phone (225) 578-5188.

I understand that the participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records or destroyed. One hundred consumers will participate in this research. For this particular research, about 20 minutes participation will be required for each consumer.

The following has been explained to me:

7. In any case, it is my responsibility to report prior participation to the investigator any food allergies I may have.
8. The reason for the research is to gather information on consumer acceptability of bread. The benefit that I may expect from it is a satisfaction that I have contributed to development of more nutritious bread formulations.
9. The procedures are as follows: Two coded samples will be placed in front of me, and I will evaluate it by normal standard methods and indicate my evaluation on a score sheet. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
10. Participations entails minimal risk: The only risk which can be envisioned is that an allergic reaction to rice flour, bean flour, potato flour, eggs, and commonly used baking ingredients. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.
11. The results of this study will not be released in any individual identifiable from without my prior consent unless required by law.
12. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand the additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be address to Dr. Michael Keenan (225)578-1708. I agree with the terms above and acknowledge.

I have been given a copy of the consent form.

________________________	_______________________________
Signature of Investigator	Signature of Participant
__________________________	_______________________________
Witness	Date

APPENDIX L. SENSORY EVALUATION FORM

Sample code____

1. What is your ***GENDER?*** ***( ) MALE*** ***( ) FEMALE***

2. How would you rate the ***OVERALL APPEARANCE*** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

3. How would you rate **CRUMB COLOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

4. How would you rate the **OVERALL AROMA** or **ODOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

5. How would you rate the **CRUMB MOISTNESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

6. How would you rate the **CRUMB MOISTNESS** of this based on your preference?

( ) **Not moist enough** ( ) **Just about right** ( ) **Too moist**

7. How would you rate the **CRUMB SOFTNESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

8. Please rate the CRUMB SOFTNESS of this product based on your preference?

( ) **Not soft enough** ( ) **Just about right** ( ) **Too soft**

9. How would you rate the **OVERALL FLAVOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

10. How would you rate the **OVERALL LIKING** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

11. Would you **PURCHASE** this product?

( ) **YES** ( ) **NO**

12. Would you **PURCHASE** this product if it were free of **DAIRY** or **WHEAT** ingredients?

( ) **YES** ( ) **NO**

13. Are you on a GLUTEN-FREE OR MILK-FREE DIET?

( ) YES **( ) NO**

APPENDIX M. SENSORY ANALYSES SAS PROGRAM

```
dm 'log;clear;output;clear';
Title "Results of Sensory Test of Samples  228(Rice/Bean), 184(Wheat), 212
(Rice,Bean,Potato)";
Footnote "diet 1= on gluten-free and/or milk-free diet; diet 2= not on diet";
data one;
input Sample $ Gender $ diet $  Oappearance Ccolor Oaroma Cmoist JARMoist
CrumSoft JARCsoft Oflavor Olike Purchase PurchasDW;
datalines;
228   1  2  3     4     4     3     3     3     1     2     3     2     2
228   1  2  8     8     4     8     2     8     2     6     7     1     1
…………
;
proc freq;
      tables Gender diet;
proc sort; by Sample;
proc freq; by sample;
      tables JARMoist JARCsoft Purchase   PurchasDW;
proc means mean std n maxdec=2;by Sample;
      var Oappearance Ccolor Oaroma Cmoist CrumSoft Oflavor Olike;
proc anova;
class Sample;
model Oappearance Ccolor Oaroma Cmoist CrumSoft Oflavor Olike = Sample;
means Sample/turkey lines;
proc sort; by Sample; by gender;
proc freq; by sample; by gender;
tables JARMoist JARCsoft Purchase   PurchasDW;
proc means mean std n maxdec=2;by Sample; by gender;
var Oappearance Ccolor Oaroma Cmoist CrumSoft Oflavor Olike;
proc anova; by gender;
class Sample;
model Oappearance Ccolor Oaroma Cmoist CrumSoft Oflavor Olike = Sample;
means Sample/turkey lines;
proc sort; by Sample; by diet;
proc freq; by sample; by diet;
tables JARMoist JARCsoft Purchase   PurchasDW;
proc means mean std n maxdec=2;by Sample; by diet;
var Oappearance   Ccolor Oaroma Cmoist CrumSoft Oflavor Olike;
proc anova; by diet;
class Sample;
model Oappearance Ccolor Oaroma Cmoist CrumSoft Oflavor Olike = Sample;
means Sample/turkey lines;
run;
ods csv close;
```

APPENDIX N. IRB APPROVAL FORM

LSU AgCenter Research & Extension

LSU AgCenter Institutional Review Board (IRB)
Dr. Michael J. Keenan, Chair
School of Human Ecology
209 Knapp Hall
225-578-1708
mkeenan@agctr.lsu.edu

Application for Exemption from Institutional Oversight

All research projects using living humans as subjects, or samples or data obtained from humans must be approved or exempted in advance by the LSU AgCenter IRB. This form helps the principal investigator determine if a project may be exempted, and is used to request an exemption.

- Applicant, please fill out the application in its entirety and include the completed application as well as parts A-E, listed below, when submitting to the LSU AgCenter IRB. Once the application is completed, please submit the original and one copy to the chair, Dr. Michael J. Keenan, in 209 Knapp Hall.

- A Complete Application Includes All of the Following:
  (A) The original and a copy of this completed form and a copy of parts B through E.
  (B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1 & 2)
  (C) Copies of all instruments and all recruitment material to be used.
    - If this proposal is part of a grant proposal, include a copy of the proposal.
  (D) The consent form you will use in the study (see part 3 for more information)
  (E) Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing and handling data, unless already on file with the LSU AgCenter IRB.
  Training link: (http://grants.nih.gov/grants/policy/hs/training.htm)

1) Principal Investigator: Witoon Prinyawiwatkul **Rank:** Professor **Student?** Y/(N)
Dept: Food Science **Ph:** 8-5188 **E-mail:** wprinya@lsu.edu
2) Co-Investigator(s): please include department, rank, phone and e-mail for each

- If student as principal or co-investigator(s), please identify and name supervising professor in this space

None

3) Project Title: Consumer Acceptance of New Food Products
4) Grant Proposal?(yes or (no)) **If Yes, Proposal Number and funding Agency**
Also, if Yes, either: **this application completely matches the scope of work in the grant Y/N**
OR
more IRB applications will be filed later Y/N
5) Subject pool (e.g. Nutrition Students) LSU Faculty, staff, students, and off-campus consumers
- Circle any **"vulnerable populations" to be used:** (children<18, the mentally impaired, pregnant women, the aged, other). Projects with incarcerated persons cannot be exempted.

6) PI signature ______ ****Date** 12/9/2011 (no per signatures)
****I certify that my responses are accurate and complete.** If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I leave the LSU AgCenter before that time the consent forms should be preserved in the Departmental Office.

Committee Action: Exempted ✓ Not Exempted ___ **IRB#** HE11-29
Reviewer Michael Keenan **Signature** Michael Keenan **Date** 12-14-2011

VITA

Annett Camnetar Bentley was born in New Orleans, Louisiana on May 6, 1939. She graduated from St. Joseph High in New Orleans, Louisiana in May of 1957. She worked from 1957 through 2003 in the medical community, starting out as a medical secretary working up to an administrator, developing educational programs.

Annette obtained a B.A.in Psychology Rutgers University, Newark, New Jersey, 1991. In 2003 she obtained a Masters Degree Medical Science, Medical Education at the University of Medicine and Dentistry - New Jersey Medical School – School of Health Related Professions.

Annette enrolled in the Graduate School at Louisiana State University Agricultural Center of Research and Extension in the Spring of 2007. She is pursuing a Masters in Food Science and is a candidate for her defense in the spring of 2013.