

EVALUATION OF QUALITY PARAMETERS IN GLUTEN-FREE BREAD FORMULATED
WITH BREADFRUIT (*Artocarpus altilis*) FLOUR

by

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Abstract

Flour from the fruit of breadfruit trees (*Artocarpus altilis*) holds the potential to serve as an alternative to gluten-containing flour and may aid in alleviating food insecurity. This study assessed the effects breadfruit flour contributes to gluten-free bread quality. Breadfruit flour was included at a baker's percentage (0, 20, 35, 50%) of a gluten-free flour blend, and was treated with various leavening agents (yeast, 15% baking powder, 20% baking powder) to create varying gluten-free bread formulas. Density and pH of each batter was assessed along with loaf density, yield, specific volume, pH, water activity, crust color (L^* , a^* , b^*), crumb color (L^* , a^* , b^*), and texture. Additionally, a consumer sensory study was performed to ascertain degree of liking of appearance, color, flavor, texture, aftertaste, likelihood to purchase, and overall acceptability.

Significant differences ($p < 0.05$) were found in batter pH, loaf density, yield, specific volume, color (crust b^* , crumb L^* , a^* , b^*), pH, water activity, and texture among flour inclusion and leavening treatments. Consumer testing yielded significant differences ($p < 0.05$) between the control and a yeast leavened 20% breadfruit formula in appearance, color, flavor, aftertaste, likelihood to purchase, and overall acceptability. While most consumers rated the breadfruit treatment lower than the control, five celiac panelists rated it higher. Among all treatments, loaves produced from 20% breadfruit flour inclusion had significantly lower density, yield, hardness, adhesiveness, gumminess, chewiness, and crumb yellowness (b^*), as well as higher specific volume, springiness, crust yellowness (b^*) and darkness (L^*), crumb darkness (L^*), and magenta hue (a^*) compared to other breadfruit flour inclusion levels. Similarly, loaves leavened with yeast had significantly lower batter pH, loaf pH, density, yield, hardness, chewiness, crust yellowness (a^*), crumb darkness (L^*), magenta hue (a^*), and yellowness (b^*) as well as higher

loaf water activity, volume, springiness, and crust darkness (L^*) compared to other breadfruit flour inclusion levels. These results indicate breadfruit flour can be used at $\leq 20\%$ in gluten-free bread formulas to replace rice flour and has potential as a fiber supplement. Further research is needed to assess how breadfruit flour affects the quality of other gluten-free product formulas.

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Preface

A team from the Pacific Business Center Program (PBCP) at the University of Hawaii Manoa won the award for Research and Analysis from the University Economic Development Association (UEDA). This top award was given to the PBCP's Pacific Region Breadfruit Initiative, whose purpose is to promote breadfruit in the Pacific and to address the lack of food security in the Pacific. PBCP's Senior Business Development Manager, C. L. Cheshire, approached food scientists at Kansas State University seeking their expertise in gluten-free food product development. The scientists from Kansas State University were added to the Pacific Region Breadfruit Initiative team, and were tasked with researching breadfruit flour's potential as an ingredient for developing gluten-free value-added food products.

Chapter 1 - Literature Review

I. Celiac Disease

i. Definition

Celiac disease (CD) is a genetically inherited autoimmune enteropathy triggered by the ingestion of gluten containing grains such as wheat, rye, and barley (Leonard, Camhi, Huedo-Medina, & Fasano, 2015). CD is intolerance to gluten rather than an allergy, meaning that the body's immune response is directed against its own tissue instead of against a perceived foreign substance (Pongdee, 2011). In order for CD to manifest, a combination of celiac coding genes, exposure to gluten, and environmental factors must occur. In patients with CD, the underlying predisposing genes primarily consist of 2 HLA-class II genes: HLA-DQ2 and HLA-DQ8.

Though other non-HLA genes are suspected to contribute to the development of CD, HLA-DQ2 and/or HLA-DQ8 are present in virtually all patients diagnosed with CD (Schuppan, Junker, & Barisani, 2009). Exposure to dietary gluten is inevitable for Western populations since gluten containing grains, and their by-products, are used in virtually all facets of Westernized food industries (Steffen Husby, Olsson, & Ivarsson, 2014). Gluten is comprised of two proteins: glutenins, which are polymeric aggregated proteins, and gliadins, which contain monomeric proteins (Sapone et al., 2012). Once consumed, gluten is partially digested into gliadin fragments that gain entry through the epithelial barrier of the intestinal mucosa due to increased mucosal permeability. In the lamina propria, the immunopathogenesis of CD occurs as a result of the deamidation of gliadin by the enzyme TTG, rendering gliadin a more immunopathic molecule that affects the adaptive immune system. The adaptive response to gliadin involves antigen-producing cells, which express the HLA class II DQ2 and/or DQ8 molecules on their surfaces and uptake and display gliadin peptides. These antigen-producing cells interact with gliadin-

specific CD4⁺ T_H1 cells, which produce inflammatory cytokines. The resulting damage to the intestinal mucosa from the onslaught of inflammatory mediators presents as villous atrophy and crypt hyperplasia, which are characteristic histologic signs of CD (Schuppan et al., 2009). While predisposing genes and exposure to gluten are both crucial factors in developing CD, most HLA-DQ2/DQ8 carriers (about 30% of the population) who are exposed to gluten (>99% of the population) see no manifestation of CD. Factors such as vitamin D intake, season of birth, early life factors that impact intestinal environment (i.e. breast-feeding, infection, and alterations to intestinal microbiota) have been implicated in the pathogenesis of CD (Hörnell, Lagström, Lande, & Thorsdottir, 2013; Lebwohl, Green, Murray, & Ludvigsson, 2013; Pozo-Rubio et al., 2012; Riddle, Murray, Cash, Pimentel, & Porter, 2013; Tanpowpong & Camargo, 2014).

ii. Prevalence

Initially, CD was only reported in young children and for many years, was exclusively considered a pediatric affliction. This association as a pediatric entity led to the long-standing assumption that CD develops during childhood. However, CD is worldwide disorder affecting people of various ages as well as ethnicities. Numerous serologic screenings indicate the prevalence of CD is 1% among Western nations. While CD was once believed to primarily occur in people of European descent, significant prevalence has been identified in Middle East, Asia, South America, and North Africa. The globalization of the world market brings wheat-based foods to nations that traditionally relied on gluten-free grains such as rice or maize, which is one proposed reason for increased CD prevalence in these areas (Kearney, 2010). Sex differences also exist with respect to the rate of CD diagnosis, with one study finding a female/male ratio of 2 to 3:1. Because iron deficiency anemia was a significant presenting manifestation in women,

this sex difference was only true in CD diagnoses made during adulthood (Bardella et al., 2005). Several factors reflect the differential rate of diagnosis among sexes including greater health care interacting in female compared to male subjects, higher rates of autoimmune diseases among women in general, and a higher likelihood of symptomatic disease among females compared to males (Dixit et al., 2014). In the pediatric population, the age of diagnosis has increased over time as the diagnosis of CD in adulthood increases (Green et al., 2001; Laurin, Stenhammar, & Fálth-Magnusson, 2004; Whyte & Jenkins, 2013). Adults present less with the intestinal manifestations common in children, and instead present with abnormalities such as iron deficiency anemia or osteoporosis (Rampertab, Pooran, Brar, Singh, & Green, 2006; Ravikumara, Tuthill, & Jenkins, 2006; Roma et al., 2009). Globally, as more mass screening studies are performed in a variety of populations, greater numbers of previously undiagnosed CD cases are identified (Ravikumara, Nootigattu, & Sandhu, 2007).

iii. Detection

Detecting and diagnosing CD is initiated when patients clinically present a spectrum of intestinal and non-intestinal symptoms including diarrhea, abdominal pain, osteoporosis, anemia, arthritis, skin disorders, increased liver enzyme levels, and neurologic abnormalities. With less than 50% of adults presenting with primary gastrointestinal symptoms, a high index of suspicion is important in making a correct CD diagnosis (Fasano et al., 2003; Green et al., 2001). Overall, CD is under diagnosed given the majority of presentations are not overt gastrointestinal symptoms. It is advised that symptomatic subjects as well as those at risk of developing CD received targeted screening in order to correctly diagnose the disease (Tonutti & Bizzaro, 2014). In addition to serologic testing, biopsy and diagnostic evaluation of the intestinal mucosa can

provide histologic evidence of CD and lead to a proper diagnosis (S. Husby et al., 2012). Various assays exist that have the ability to detect specific antibodies associated with CD including antibodies against deamidated gliadin peptides, the TTG enzyme, and the endomysium (Table 1.1) (Rashid & Lee, 2016). CD diagnosis relies on serologic and histologic studies in addition to a response to a gluten-free diet (GFD).

Table 1-1 Serologic Tests for Celiac Disease

ANTIGEN	ANTIBODY TYPE	TEST	SENSITIVITY, % (RANGE)	SPECIFICITY, % (RANGE)
Gliadin	IgA	ELISA	85 (57–100)	90 (47–94)
	IgG	ELISA	80 (42–100)	80 (50–94)
Endomysium	IgA	IFA	95 (86–100)	99 (97–100)
	IgG	IFA	80 (70–90)	97 (95–100)
Tissue transglutaminase	IgA	ELISA	98 (78–100)	98 (90–100)
	IgG	ELISA	70 (45–95)	95 (94–100)
Deamidated gliadin peptide	IgA	ELISA	88 (74–100)	90 (80–95)
	IgG	ELISA	80 (70–95)	98 (95–100)

ELISA—enzyme-linked immunosorbent assay, IgA—immunoglobulin A, IgG—immunoglobulin G, IFA—immunofluorescence assay. Rashid & Lee, 2016

iv. Long Term Effects

If left untreated, active CD can cause many long-term complications to arise such as adenocarcinoma of the small intestine, enteropathy-associated T-Cell lymphomas, development of other autoimmune conditions, and extraintestinal lymphoproliferative disorders such as T- and B-cell non-Hodgkin lymphomas (Cosnes et al., 2008; Green et al., 2003). The overall possibility for CD patients developing cancers is twice that of the general population (Green et al., 2003). Additionally, 5% of CD patients may also experience refractory CD. Refractory CD is the

persistent, or recurrent, clinical symptoms along with histologic changes, despite adherence to a strict GFD. The most common cause of refractory CD is the unintentional microingestion of gluten-containing foods. Two types of refractory CD exist: Type II and I. Patients with Type I refractory CD exhibit normal intraepithelial lymphocytes as opposed to Type II, in which there is a clonal expansion abnormal intraepithelial lymphocytes. Type II refractory CD is associated with enteropathy-associated T-cell lymphoma as well as an increased risk for ulcerative jejunitis (Bagdi, Diss, Munson, & Isaacson, 1999; Green & Cellier, 2007).

A. Treatment

The most common treatment for CD is strict adherence to a GFD, which entails avoidance of gluten-containing grains including wheat, rye, and barley. Patients diagnosed with CD can benefit from consultations with a nutritionist who can assist with appropriate food selection and avoidance (Green, Lebwohl, & Greywoode, 2015). A variety of non-dietary therapies under investigation target various aspects of CD pathogenesis. These non-dietary therapies include intraluminal agents, immunomodulators, and vaccination (Crespo Pérez, Castillejo de Villasante, Cano Ruiz, & León, 2012; Crowe, 2014).

v. Gluten Free Diet

A GFD is a diet devoid of gluten-containing grains like barley, rye, and wheat. Alternative gluten-free grains one may consume on a GFD include but are not limited to rice, oats, quinoa, buckwheat, corn, and millet. Until recent years, very few value-added, processed, or packaged gluten-free foods existed at the retail level. Of those products that did exist, many exhibited negative attributes including impaired dietary palatability, high monetary cost, and nutritional

inadequacy (Staudacher & Gibson, 2015). Removing gluten from baked goods reduces a products elasticity, extensibility, and water binding capacity. When compared to gluten-containing products, gluten-free foods are perceived as having lower quality as well as lower palatability (Pietzak, 2005). Gluten-free foods are reported to cost up to five times more than their gluten-containing counterparts, making GFDs more of a monetary burden than standard diets (Lee, Ng, Zivin, & Green, 2007; Singh & Whelan, 2011). GFDs, like any other exclusion diet, may put individuals at risk for nutritional deficiencies. Reduced intake or complete exclusion of cereal grains may lead to reduced intake of dietary fiber, vitamins and minerals inherent to cereal grains (i.e. calcium, zinc, iron, copper, phosphorous) as well as those commonly used to fortify wheat flour such as vitamins A, B1, B2, B3, B5, B6, B9, B12, D, calcium, iron, and zinc (Shepherd & Gibson, 2013; Wild, Robins, Burley, & Howdle, 2010).

A. Foods Containing Gluten

Though some food products (i.e. bread) inherently contain gluten, a vast majority of the Western food industry utilizes ingredients and additives that are derived from and/or contain gluten. Ingredients like “wheat flour” blatantly indicate that they contain gluten, while other gluten-containing ingredients such as barley malt, soy sauce, and Worcestershire sauce are not as obvious and thus require labeling to indicate the presence of an allergen. Ingredient legends on food products may also list gluten-containing grains by their Latin names like *Triticum vulgare* (wheat), *Hordeum vulgare* (barley), *Secale cereal* (rye), Triticale (cross between wheat and rye), and *Triticum spelta* (spelt, a wheat variety) which can be misleading as to whether the product indeed contains a gluten allergen. Various additional sources of gluten in food products may come from starches (i.e. dextrin, maltodextrin, and modified starch/modified food starch), which

are commonly used to thicken soups, gravies, or other creamy roux-based foods. Natural and artificial flavors may also harbor hidden gluten if their flavor-carrying agent is a gluten-containing starch (Loucks, 2013).

II. Gluten-Free Trends

i. Market Trends

Gluten-free foods continue to maintain their status as a growing niche-market within the food industry. In the retail sector, gluten-free food sales flaunted a compound annual growth rate (CAGR) of 34% over a five-year period ending in 2014. By the end of this period, market sales reached \$973 million. The sustained growth of the gluten-free market is perpetuated by both the increased interest and perceived value consumers place in gluten-free diets. In a July/August 2014 survey, data revealed that more than one-third of consumers allege that gluten-free is a significant factor they consider when shopping for food (Packaged Facts, 2015). The population of gluten-free consumers is not solely limited to the 1% who suffer from CD, but additionally includes consumers who suffer from a wheat allergy, non-celiac gluten sensitivity, or seek to pursue a gluten-free diet due to perceived health benefits. As previously discussed, CD is an autoimmune condition where a gluten-triggered immune response is directed against one's own small intestine rather than against a foreign invader such as viruses or bacteria (Pongdee, 2011). Those who suffer from CD must strictly adhere to a diet devoid of gluten, and thus make up the core of gluten-free consumers. Consumer may also suffer from a wheat allergy, where the body's immune system overreacts to wheat causing symptoms including hives, lightheadedness, shortness of breath, vomiting, and may cause anaphylaxis. It is crucial that wheat-allergy sufferers not only maintain a strict GFD, but also take care to avoid foods produced on/in

machinery or a facility that produces wheat-containing products since cross-contamination may occur. Consumers suffering from wheat allergies additionally make up the core of the gluten-free population. Non-celiac gluten sensitivity (NCGS) patients further extend the gluten-free consumer population. NCGS patients present clinical symptoms of CD such as abdominal pain, gas, bloating, foggy-mind, lethargy diarrhea, and fatigue, but their endoscopies are negative or normal. Eliminating gluten from NCGS patients' diets alleviates symptoms while reintroducing it causes symptoms to return (Catassi et al., 2013). Because NCGS patients find symptom relief while on a GFD, they have added to the expansion of the gluten-free consumer population. Consumers who believe GFDs are "healthy" further the augmentation of the gluten-free consumer population (Staudacher & Gibson, 2015). Looking ahead, the gluten-free market is expected to experience continued growth; sales are projected to exceed \$2 billion in the year 2019. Several key factors identified for perpetuating market growth include consumer interest and use of gluten-free foods, growth in demographic groups showing a propensity to purchase gluten free foods, a Food and Drug Administration (FDA) ruling intended to clarify on the definition of "gluten-free", a higher volume of better quality gluten-free products available in the retail sector, as well as the escalating prevalence of diet-associated health issues (Packaged Facts, 2015).

ii. Gluten Free Synonymous with "Healthy"

GFDs are gaining enthusiasm from individuals who, though not formally diagnosed with CD, perceive GFDs as healthful. The perceived benefits associated with GFDs include weight loss, treating and/or minimizing risk of future disease, as well as various other health benefits.

Marketing information from the US indicates that, of the 30% of consumers who were

considering a GFD, the reasoning for following a GFD was for “good health” (NPD Group, 2013). Among consumers, there exists an invalidated assumption that gluten-free foods are healthier than their gluten-containing alternatives. This assumption is perpetuated by the notion that gluten causes abhorrent gastrointestinal and non-gastrointestinal maladies. The correlation of GFDs and health is further bolstered by a host of other factors including a vast array of accessible (and often misinterpreted) web-based dietary information, zeal from celebrity endorsements of elimination diets, and the increasing availability of gluten-free foods in retail stores as well as restaurants (Levinovitz, 2015). Though GFDs are praised for being “healthful”, unsavory and even perilous repercussions are likewise associated. Gluten-devoid products have an altered flavor, texture, and overall appeal compared to their gluten-containing counterparts. A decline in quality attributes such as these results in a lack of fulfillment/enjoyment obtained from GFD diets (Pietzak, 2005). Similar to other exclusion diets, GFDs may not provide adequate amounts of nutrients compared to gluten-containing diets and may promote various vitamin and/or mineral deficiencies (Shepherd & Gibson, 2013). GFDs have also been implicated in the development of psychological issues, specifically eating disorders. Patients at risk of developing eating disorders may use supposed food intolerances to gain control over their food intake without suspicion (Musolino, Warin, Wade, & Gilchrist, 2015). Likewise, adhering to a GFD may cause and/or be the result of orthorexia nervosa, which is a phenomenon characterized by healthy, natural, and clean eating to the extent social and psychological health becomes compromised (Donini, Marsili, Graziani, Imbriale, & Cannella, 2004). Like many exclusion diets, GFDs have inherently inevitable social consequences such as difficulties while dining at restaurants or in the households of those who do not follow a GFD (Staudacher & Gibson, 2015). Compared to a non-exclusion diet, a GFD has a higher financial cost with reports of gluten-free

products costing five-times that of their counterparts (Lee et al., 2007; Singh & Whelan, 2011).

Due to the potential risks, it is advised that those interested in a GFD consult with nutritionists as well as health care providers prior to embarking on one.

III. Alternative Gluten-Free Flours

i. Amaranth Flour

Amaranth (*Amaranthus* spp.) is a grain praised for its ability to adapt to diverse growing conditions such as low nutrient soils, a wide range of temperature as well as irradiation levels, and its tolerance to drought stress (Janick, 1996). Amaranth boasts a protein score, which is defined by the World Health Organization (WHO) as a measure of protein quality, of 74; by comparison, wheat is scored 47, soy beans 68-89, rice 69, and maize 35 (O'Brien & Price, 1983). The amino acid composition of amaranth seed is comparative to the levels recommended by WHO/Food and Agriculture Organization of the United Nations (FAO) to maintain a healthy human diet (Comino et al., 2013; Gambus, Gambus, & Sabat, 2002). Amaranth seeds contain a high content of lysine, arginine, and histadine (Gorinstein et al., 2002). Amaranth seeds additionally support the intake of recommended daily levels of calcium, iron, sodium, and vitamins due to the high levels inherently present in amaranth (Becker et al., 1981). In addition to the total fat and protein content per dry matter of amaranth is significantly higher than that of wheat, maize, and sorghum, the overall nutritional value of amaranth is regarded as significantly higher than milk, soybean, wheat, and maize (Brenner et al., 2000; Cheeke & Bronson, 1979; Hamer, 2005; Pond & Lehmann, 1989; Yue, 1987). When used in gluten-free bread formulations, bread formulations containing amaranth flour presented similar values for specific volume, water activity, and firmness compared to a control gluten-free bread formulations.

Additionally, bread containing amaranth flour presented greater amounts of proteins, lipids, and ash improving their nutritional profile compared to non-amaranth-containing gluten-free bread (Machado Alencar, Steel, Alvim, de Morais, & Andre Bolini, 2015).

ii. Arrowroot Flour

Arrowroot (*Maranta arundinacea*) is a starchy tuber crop similar to potatoes and cassava.

Containing 10-25% extractable starch, arrowroot is known to be the richest (unenriched) natural starch on Earth (Spennemann, 1992). Arrowroot's starch granules have a round, polygonal shape, are white in color, and contain an amylose content ranging between 16-20%. The starch has long been praised for its high digestibility and medicinal properties ("The Wealth of India: Raw Materials," 1962). Arrowroot starch possesses demulcent properties and is often used to treat complaints of bowel irritation and/or inflammation (Matthew, 2007). Arrowroot lacks gluten, making it an ideal candidate for replacing wheat flour in gluten-containing formulas. Common food uses of arrowroot starch include using it as flour in gluten-free bakery products, a bulking agent for gluten-free powdered flavorings, and a thickening agent in gluten-free dressings, soups, and sauces (Jyothi, Sheriff, & Sajejev, 2009).

iii. Legume Flour

Legumes are the edible dicotyledonous seeds of plants belonging to the Leguminosae family (Naivikul & D'Appolonia, 1978). They are an important source of food protein containing high levels of lysine, leucine, aspartic acid, glutamic acid, and arginine, providing well-balanced essential amino acid profiles when consumed with foods rich with sulfur-containing amino acids and tryptophan. Legumes also possess functional properties that hold an influential role in food

formulations and processing (Boye, Zare, & Pletch, 2010; Dakia, Wathelet, & Paquot, 2007; Roy, Boye, & Simpson, 2010). Legume proteins such as chickpea flour (*Cicer arietinum* L.), pea-protein isolate (*Pisum sativum* L.), and carob germ flour (*Ceratonia siliqua* L.) have been used in the development of soups, extruded products, ready-to-eat snacks, and bakery products. Due to the nutritional as well as functional properties of legumes, they are used as an alternative to common flours in gluten-free formulations (Bengoechea et al., 2008; Bienenstock, Csaski, Sagi, & Sagi, 1935; Feillet & Roulland, 1998; Plaut, Zelcbuch, & Guggebheim, 1953; Rice & Ramstad, 1950; Smith et al., 2010; Y. Wang et al., 2001). Gluten-free breads made with legume flours show good physio-chemical characteristics and adequate sensory profiles. Carob germ flour generates batters with poor characteristics, but good rheological properties whereas chickpea and pea isolate flours yield breads with good results in all parameters including texture, bake loss, specific volume, and water activity (Miñarro, Albanell, Aguilar, Guamis, & Capellas, 2012).

iv. Buckwheat Flour

Buckwheat (*Fagopyrum* spp.) is botanically classified as a fruit, but is typically consumed as grain or flour. Two species of buckwheat are cultivated for food: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tartaricum*) (Ikeda, 2002; Mazza & Oomah, 2003; Skrabanja et al., 2004). Buckwheat is a dietary source of protein containing a favorable amino acid profile, starch, dietary fiber, essential vitamins and minerals, as well as trace elements and rutin (Bonafaccia, Marocchini, & Kreft, 2003; Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001; Vojtišková, Kmentová, Kubáň, & Kráčmar, 2012). The phytochemicals and dietary fiber found in buckwheat are known to control blood sugar, lower

cholesterol, reduce high blood pressure, and prevent cancer (Skrabanja et al., 2004; Wijngaard & Arendt, 2006). Research has shown that when buckwheat flour is substituted for wheat flour at 10%, successful bread can be produced. However when levels of buckwheat were used at 20% or greater, bread became less acceptable in physical properties and sensory characteristics. Because buckwheat flour contains higher levels of nutrients compared to other cereals, incorporation of it into gluten-free breads may improve the diets' of consumers (Bilgiçli & İbanoğlu, 2015).

v. Coconut Flour

Coconut is the seed harvested from the coconut tree (*Cocos nucifera*), which is botanically classified as a fruit. Coconut flour is obtained by extracting the oil from the coconut fruit pulp, then the pulp is dried, and finally milled (Hagenmaier, Quintio, & Clark, 1975). Coconut flour reportedly contains 3.6% moisture, 3.1% ash, 12.1% protein, 10.9% lipids, and 60.9% dietary fiber (Gunathilake & Abeyrathne, 2008; Trinidad et al., 2001). When coconut flour is used in gluten-free baking applications, finish bread loaves are slightly smaller than gluten-containing loaves, which is likely due to the high fiber content of coconut flour. Despite smaller size, these loaves have highly acceptable crumb structure and flavor (Pejcz, Mularczyk, & Gil, 2015).

Coconut flour has also had success when combined with wheat flour in noodle formulations. Formulations using 20% coconut flour produce quality noodles, showing potential for future use in gluten-free noodle formulations (Gunathilake & Abeyrathne, 2008).

vi. Corn Flour

Corn flour is the starch derived from the endosperm of maize kernels (*Zea mays* L.). Corn is used in many food and non-food applications and was the most produced cereal in the world in 2013

(Food and Agriculture Organization (FAO), 2014). Corn flour can come in a variety of colors (white, yellow, purple, black, etc.) dependent upon the type of maize kernel, but chemical composition across varieties is considered homogeneous (Moreira, Chenlo, Arufe, & Rubinos, 2015). The main components of maize are (% w/w weight basis): carbohydrates (≈ 77), water (≈ 11), total fiber (≈ 7), proteins (≈ 7), lipids (≈ 4), and ash (≈ 1.8) (Gwirtz & Garcia-Casal, 2014). Milling of corn flour is critical in determining flour-dough properties, as smaller average particle size increases the damaged starch content thus increasing water absorption capabilities (Moreira et al., 2015). Corn flour has long been used to successfully make gluten-free bread, and has shown promise as a constituent of gluten-free noodle formulations. Corn flour can be substituted up to 50%, along with other ingredients, to produce good quality noodles. Corn-flour noodles have also been shown to higher cook yield than wheat-flour noodles (Shobha, Vijayalakshmi, Puttaramnaik, & Asha, 2015).

vii. Flaxseed Flour

Flaxseed (*Linum usitatissimum*) is an oilseed crop esteemed for containing nutrients including lipids, protein, and dietary fiber, which are associated with a healthy diet (Jenkins et al., 1999). On a moisture-free basis, flaxseed is comprised of 21% protein, 28% dietary fiber, 41% lipids, and the remaining percentage of carbohydrates, vitamins, and minerals. Flaxseed has a unique fatty acid profile consisting of (total percentage of all fatty acids) 73% polyunsaturated fatty acids, 18% monounsaturated fatty acids, and 9% saturated fatty acids. Flaxseed is a rich source of ALA, a component of omega-3 fatty acids, which constitutes 57% of total fatty acid composition; linoleic and omega-6 fatty acids constitute 16% (Morris, 2001). The plant lignin precursor SDG presides in plentiful amounts within flaxseed. Since plant lignin are phenolic

compounds, which show anticancer activities, flaxseed may very well help prevent cancer (Sung, Lautens, & Thompson, 1998). Due to its functional properties, flaxseed flour is commercially used to produce numerous bakery products (Carter, 1993). Research performed on the effects of flaxseed flour on quality of gluten-free bread formulations shows promise. Increasing the flaxseed content decreases crumb hardness and yields softer gluten-free bread compared to formulations lacking flaxseed flour. The firming rate of gluten-free breads is likewise decreased when flaxseed flour is used, which implicates that flaxseed hold potential as an anti-staling agent (Ozkoc & Seyhun, 2015).

viii. Hemp Flour

Hemp (*Cannabis sativa* L.) has been cultivated for thousands of years due to its wide variety of uses as a bast fiber, food, and medicine. Prior to the 1900's, hemp fiber was used to produce paper, textiles, and was even used in construction and industrial applications. Hemp contains Δ -9-tetrahydrocannabinol (THC), which is known for its hallucinogenic properties. In an effort to prevent the abuse of hemp as a drug, cultivars containing less than 0.3% THC have been cultivated and their use is regulated by nation-specific governmental agencies (Novak, Zitterl-Eglseer, Deans, & Franz, 2001). Hempseeds are characterized as being highly nutritional due to their contents including phytochemicals, Vitamins (A,C, and E), minerals, dietary oil, fiber, and protein (Leizer, Ribnicky, Poulev, Dushenkov, & Raskin, 2000; Oomah, Busson, Godfrey, & Drover, 2002) Hempseed oil is rich in linoleic and linolenic polyunsaturated fatty acids, which have been proven to reduce human cholesterol and blood pressure levels in addition to providing immune support (K. Jones, 1995). Two main proteins compose hempseed: albumin (33%) and edistin (65%), both of which are known for their ease of digestibility (Callaway, 2004). Research

on the effects of hemp flour on gluten-free bread show that, though hemp flour helped improve dough properties, it negatively affects final loaf crumb structure and flavor (Pejcz et al., 2015).

ix. Millet Flour

Millet refers to any number of different species belonging to the order Poales. Many varieties of millet exist, but the four major types are proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), and pearl millet (*Pennisetum glaucum*) (Issoufou, Mahamadou, & Guo-Wei, 2013). Millet has been implicated to provide several health benefits including lowering blood pressure, reducing tumor incidence, preventing cancer, and preventing cardiovascular diseases (Chandrasekara & Shahidi, 2011, 2012; Issoufou et al., 2013). Millet is recognized as a good source of magnesium, manganese, and phosphorus. In gluten-free formulas, millet flour has been reported to cause a “crumbly” texture in breads and muffins (Taylor & Emmambux, 2008).

x. Nut Flour

Nut flours/meals are made from grinding the cake that remains after oils are pressed from nuts. A variety of nut flours exist including acorn, almond, cashew, chestnut, hazelnut, peanut, and walnut. Acorn flour is comprised of 31-55% starch, 2.75-8.44% protein (containing a high content of essential amino acids), and 0.7-9% lipids though some species may exceed 31% (Korus, Witczak, Ziobro, & Juszczak, 2015). Almond flour has a composition of about 50% lipids, 7% starch, 10% fiber, and 21% protein (“Almond Meal/Flour,” 2015). The composition of cashew flour is reported to contain 42.9% fat, 26.1% protein, 19% carbohydrates, and 3.11% fiber (Ogunbenle & Afolayan, 2015). Chestnut flour contains high-quality proteins (4-7%),

sugar (20-32%), starch (50-60%), dietary fiber (4-10%), lipids (2-4%), vitamins E and B group, minerals potassium, phosphorus, and magnesium (Chenlo, Moreira, Pereira, & Silva, 2007; Sacchetti, Pinnavaia, Guidolin, & Rosa, 2004). Hazelnut flour is additionally high in protein (35-41%), fiber (10%), and other nutritional constituents (Yağcı & Göğüş, 2008). Peanut flour is comprised of 26-27% protein, 43-45% fat, 2-3% fiber, and 18-20% nitrogen free extract (Sibt-e-Abbas et al., 2015). The composition of walnut flour contains 14.18% protein (of which, glutamic and aspartic amino acids are most prevalent), 58.42% carbohydrates, 3.03% fiber, 10.22% fat, and 3.14% ash (Ogungbenle, 2009). When used in gluten-free baking applications, nut flours can enhance the nutritional profiles of formulas otherwise lacking in nutrients such as protein, vitamins, minerals, and dietary fiber due to the removal of wheat flour (Korus et al., 2015). Nut flours, such as acorn, have even proven to strengthen gluten-free dough properties and increase volume up to a certain level. Additionally, peanut and almond flours used to produce gluten-free cookies increased the sensory acceptability compared to cookies lacking nut flours (Granato & Ellenderson, 2009).

xi. Oat Flour

Oat flour is derived from plant *Avena sativa*. It is processed by grinding oats into a fine powder, sifting the powder through screens to separate fine and coarse fractions, then collecting the fine fractions and regrinding the coarse ones until they are fine enough to be sifted through the appropriate screens (Kick, 2011). Gluten-free muffin formulas see a significant increase in protein, fat, fiber, and minerals when oat flour is used instead of a mixture of rice and corn flour (Ziobro, Litwinek, & Mickowska, 2015). However, research on current kilning and milling methods used to process oats indicate that the oat flour produced is not suitable for bread making

due to the beta-glucans present in oat bran which makes dough lack elasticity as well as extensibility (Londono, Smulders, Visser, Gilissen, & Hamer, 2015).

xii. Potato Flour

The potato (*Solanum tuberosum*) is an important staple crop in many nations around the world. Potato starch/flour is commonly used for its ability to form starch gels, which have a variety of use in food applications. Potato starch granules are quite large, compared to other starch granules like rice, and range in size from 5-100 μ m (Noda et al., 2005). Compared to wheat starch, potato starch is relatively large and contains more phosphorus in the amylopectin. Potato starch has been shown to successfully replace wheat flour up to 80% while still maintaining acceptable physical, chemical, and sensorial properties compared to 100% wheat flour bread. While potato starch hold promise for replacing wheat in gluten-free applications, it should be noted that it contains lower levels of protein, fat, fiber, vitamins, and minerals compared to wheat flour (Bouras, Dilmi Koiche, Asal, & Mezaini, 2015).

xiii. Quinoa Flour

Quinoa (*Chenopodium quinoa*) is considered a pseudo cereal due to the fact its seeds can be ground into flour and otherwise used as a cereal (Kozioł, 1992; Schlick & Bubenheim, 1996). The oil content of quinoa is reported to range from 1.8-9.5%, with a reported unsaturated fatty acid content of 70% containing linoleic and oleic acid percentages of 38.9 and 27.7% respectively (Dini, Rastrelli, Saturnino, & Schettino, 1992; Kozioł, 1992). Additionally, quinoa boasts high levels of magnesium, copper, zinc, iron, and calcium. Antinutritional substances such as saponins, phytic acid, protease inhibitors, and tannins are also found in quinoa (Vega-Gálvez

et al., 2010). Antinutrients are defined as substances which decrease the bioavailability of nutrients by changing protein functionality, solubility, digestibility, or absorption (Harland & Harland, 1980; Rickard & Thompson, 1997). Research has shown that when quinoa flour is substituted for wheat flour at 10%, successful bread can be produced. However when levels of quinoa were used at 20% or greater, bread became less acceptable in physical properties and sensory characteristics. Because quinoa flour contains higher levels of nutrients compared to other cereals, despite the presence of antinutrients, incorporation of it into gluten-free breads may improve the diets' of consumers (Bilgiçli & İbanoğlu, 2015).

xiv. Rice Flour

Due to its widespread cultivation and accounting for 29% of the world's total cereal production, rice (*Oryza* spp.) is one of the most important foods in the human diet. Rice starch/flour granules typically range from 2-7µm in size, making them one of the smallest starch granules (Wani et al., 2012). Rice starch is one of the most common alternative flours to wheat flour when formulating gluten-free products. The high amylose content of rice starch aids in increasing dough consistency and springiness of gluten-free breads (Sivaramakrishnan, Senge, & Chattopadhyay, 2004). Research on the use of rice flour in gluten-free breads shows that it greatly improves the specific volume as well as the uniformity of pore distribution in the breadcrumb (Kang, Sohn, Yoon, Lee, & Ko, 2015). In products containing rice flour, it is necessary to use hydrocolloids, emulsifiers, enzymes, or proteins in order to confer viscoelastic properties (Rosell & Marco, 2008). It should also be noted that rice flour lacks the protein, fat, fiber, vitamin, and mineral content of wheat flour and thus products substituting rice for wheat flour may need to be enriched (Kennedy & Luo, 2015).

xv. Sorghum Flour

Sorghum is a heat-tolerant, drought resistant plant that has traditionally been used as animal feed in Western countries. Since sorghum is considered safe for celiac patients, due to the fact it is more closely related to maize than wheat rye or barley, it has grown in popularity and use in the food industry as a wheat replacement (Ciacci et al., 2007). Research performed on sorghum flour performance in gluten-free breads shows that flours with lower amount of fiber and smaller particle size yield bread with more acceptable volume, crumb, color, and texture. However, it should be noted that these characteristics do not exert their influences independently of one another, indicating that damaged starch plays an important role in the functionality of sorghum flour (Trappey, Khouryieh, Aramouni, & Herald, 2015). Similarly, sorghum flour with smaller particle size and greater starch damage yielded better quality gluten-free tortillas (Winger, Khouryieh, Aramouni, & Herald, 2014).

xvi. Soy Flour

Soy flour is made from the beans of the oilseed plant *Glycine max*. Soybeans are a good source of protein (up to 50%), fiber, saturated fat, and calcium. Soy flour has long been used in the bakery products to improve protein quality, mechanical behavior, and shelf life. Because soy is a leguminous plant, its proteins do not contain gluten-making soy flour an alternative to wheat flour (Curic, Novotni, Tusak, Bauman, & Gabric, 2007). Soy flour (used at 3-12%) has been shown to improve the quality of bread by increasing dough water absorption, improving loaf elasticity, extensibility, and crust/crumb color (Xhabiri, Seferi, & Sinani, 2012). Soy flour has

also been successful in increasing the nutritional profile and improving overall quality of gluten-free noodles as well as cookies (Mariani, Vogt, & Venzke, 2013; Sereewat et al., 2015).

xvii. Tapioca Flour

Tapioca flour is the granulated form of manioc starch (Montes, Rodrigues, Cardoso, Camilloto, & Cruz, 2015). Tapioca is a naturally gluten-free ingredient, making it a substitute for wheat flour in food formulations. When used in gluten-free formulas, polymeric substances like proteins or hydrocolloids are needed to reproduce the viscoelasticity of gluten and provide structure to retain gas. Research has shown that the addition of guar gum is a suitable hydrocolloid to add to tapioca flour since it increases the volume of gluten-free breads and additionally increases dough viscosity and decreases gluten-free dough stickiness (Rodriguez-Sandoval, Cortes-Rodriguez, & Manjarres-Pinzon, 2015). Tapioca flour has also been successfully used, in conjunction with other gluten free flours, to produce acceptable gluten-free baked goods such as cookies (Montes et al., 2015).

xviii. Teff Flour

Teff (*Eragrostis tef*) is the smallest of all cereal grains in the world. Teff, a grain devoid of gluten, has a similar nutritional to wheat, thus substitution of teff for wheat flour yields gluten-free products with higher vitamin, mineral (calcium, iron, magnesium, and zinc), and fiber content than other gluten-free flour alternatives (Hopman et al., 2008). Since beer is typically made from fermenting barley, a gluten-containing grain, gluten-free grains like teff have potential use in the production of gluten-free beer (Gebremariam, Zarnkow, & Becker, 2014). In research on the use of teff in gluten-free baking applications, teff has been shown successfully

replace rice flour up to 50% in muffins. Replacing rice flour with teff increased iron and fiber to levels considered “a good source”, indicating that substituting other gluten-free flours with teff flour can increase a product nutritional profile (Bhaduri S & Navder KP, 2015).

IV. Additives Used for Improvement of Gluten-Free Products

i. Gluten-Free Product Quality Issues

Though a vast variety of gluten-free products are becoming more widely available, an issue still exists with the quality of these foods compared to gluten-containing products. Gluten is the main structure-forming protein in wheat flour, providing the viscoelastic characteristics of dough and likewise contributing to crumb structure and overall appearance of baked goods. Removal of this vital protein creates significant problems, which negatively affect quality attributes of gluten-free products (Rodriguez-Sandoval et al., 2015). Gluten-free products available on the market are associated with low quality, exhibiting poor appearance, volume, structural integrity, flavor, and mouth feel. The lack of gluten and its replacement with high starch alternative flours expedites the onset of staling due to starch retrogradation (Elke K. Arendt, Ryan, & Dal Bello, 2007). Many approaches exist to combat the problems that plague gluten-free products including the use of gums, hydrocolloids, phosphates, and acids to improve texture and structure. Alternative approaches to improve shelf life include freezing product and manipulating packaging parameters.

ii. Approaches to Improve Quality

A. Gums

Gums are essential ingredients in gluten-free products because their structure-forming properties improve the texture and final appearance of products. In gluten free-products, gums are used to simulate the viscoelastic properties of gluten (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). Gums also provide other functional properties such as increasing moisture retention and retarding staling (Rojas, Rosell, & Benedito de Barber, 1999). Various gums such as locust bean, guar, carrageenan, guar, and xanthan are commonly used to impart these functional properties in gluten-free formulations (Anton & Artfield, 2008). Research has shown that when used in gluten-free bread, guar gum yielded bread with the softest texture (fresh and over 72 hour storage), highest volume, and darkest color compared to other gums (Ozkoc & Seyhun, 2015).

B. HPMC

Hydroxypropylmethylcellulose (HPMC) is a hydrocolloid used to give viscoelastic properties back to gluten-free bread. HPMC improves gas retention and water absorbing characteristics, normally supplied by gluten, and has an affinity for both aqueous as well as nonaqueous phases of a dough system, thus maintaining its uniformity and stability. During baking, HPMC polymers lose their affinity for water and gel with one another instead, causing an increase in viscosity, strengthening gas cell walls, and preventing excess moisture loss. However this gel structure does not persist post cooling, and its loss causes no adverse effects in the texture of the final product (Bell, 1999). Rice bread formulations incorporating HPMC have comparable quality to wheat bread (Cato, Refael, Gan, & Small, 2001; Ylimaki, Hawrysh, Hardin, & Thomson, 1988). HPMC has additionally been used in wheat bread yielding product softer texture, better specific volume, and enhanced sensory characteristics (Collar, Conte, Fadda, & Piga, 2015; C.M Rosell, Rojas, & Benedito de Barber, 2001).

C. DATEM

Diacetyl tartaric acid ester of monoglycerides (DATEM) is an oil-in water-emulsifier often used in bread making (Ribotta, Pérez, León, & Añón, 2004; Sapirstein & Bushuk, 1995). It has a hydrophilic-lipophilic balance (HLB) value of 8-10, and the FAO/WHO has set the acceptable daily intake (ADI) at 0-50 mg kg⁻¹ and the lethal dose 50% (LD₅₀) at >10 g kg⁻¹ of body weight (Hao, Xia, Chen, & Liao, 2002). In research performed on hearth bread, DATEM had a significant effect on increasing the loaf volume, area of bread slice, form ratio, and height (Aamodt, Magnus, & Færgestad, 2005). The use of DATEM in gluten-free bread could yield similar results as hearth bread, so long as other factors that affect these characteristics (proofing time, flours used, protein content, etc.) are optimized.

D. Phosphates

Phosphates are derivatives of the element phosphorus. Phosphates serve many functional roles in food, and additionally can improve the nutritional value of foods since phosphorus is an essential mineral that is critical to maintain healthy teeth, bones, as well as blood chemistry. In baked goods, phosphate salts serve as a leavening agent. When combined with sodium bicarbonate, phosphates release carbon dioxide that causes leavening. Unlike the by-products produced during yeast fermentation, phosphates don't produce flavors, and can thus be used in a variety of baked goods. An additional function of phosphate is as a shelf-life extension agent. Calcium phosphate is used in bread to inhibit certain bacteria from growing that would make the product appear moldy or rancid ("Questions & Answers About Phosphates," 2015).

E. Acids

Acids serve a variety of functions in bread and can be incorporated into a formulation through several different means. Organic acid is also generated in bread as a by-product of biological fermentation by yeast and bacteria. Some organic acids serve mainly as flavor agents, imparting the familiar fermented flavor notes associated with biologically leavened breads. Additionally, breads like sour dough are characterized by the sour flavor notes imparted from acids produced by lactic acid bacteria (LAB). The most commonly used microorganisms for fermentation are *Saccharomyces cerevisiae*, *Lactococcus lactis*, and *Streptococcus thermophilus* (Hui, 2004). Another role of acids in bread is as a preservative. Acetic acid, sorbic acid, benzoic acid, and propionic acid are all common acids used as preservatives in baked goods. These weak acids preserve the shelf life of bread by inhibiting mold growth through oxidative stress, disruption of cell membrane homeostasis, and possible disruption of mitochondrial physiology (Hazan, Levine, & Abeliovich, 2004).

F. Freezing

Freezing gluten-free bread is one approach to delay staling and increase shelf life. Staling is the hardening of bread's crumb via a multiple mechanisms that cause starch in the product to retrograde (Chinachoti & Vodavotz, 2001; Zobel & Kulp, 1996). Water loss is another cause of staling, since water plays an important role in crumb firmness due to its plasticizing effect on the crumb network (Hug-Iten, Escher, & Conde-Petit, 2003). Gluten-free breads frozen at -28°C yielded a quality close to fresh bread, but when stored at -14°C, overall quality deteriorated and staling rate accelerated. For an extended shelf life, high quality retention, and decreased rate of

staling, gluten-free bread can be stored at -20°C without compromising quality (Ronda & Roos, 2011).

G. Packaging

Packaging materials and methods are important factors in determining the shelf life and overall quality of food products. A variety of packaging options exist including modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP), active packaging, and vacuum packaging. MAP works by altering gas levels inside the package to control enzymatic activity, microbial growth, and moisture migration. Active MAP consists of an atmosphere being constantly maintained by components in the packaging such as carbon dioxide or oxygen scavengers. Passive MAP occurs when a desired mixture of gases is sealed into the package. (Ooraikul & Stiles, 1991; Sandhya, 2015). CAP is used when a continuous change in environmental atmosphere is required for food respiration. Active packaging has the ability to constantly monitor attributes like moisture, oxygen, atmosphere, and temperature within the package and adapt to maintain ideal levels. Vacuum packaging involves removing all air and gasses from inside the package to prevent food deterioration (Barros-Velazquez, 2016). MAP, both active and passive, is most often used with gluten-free bread because of its ability to control the many factors that degrade quality over time. MAP consisting of carbon dioxide has shown to prevent the development of molds and extend the shelf life of gluten-free bread for more than 15 days at 20°C. In addition, MAP stabilizes the moisture content of gluten-free breads and prevents its loss and subsequently decreases staling rate (Vlášek, Langová, & Štencl, 2013).

V. Breadfruit

i. Description & History

The fruit of breadfruit trees (*Artocarpus altilis*), a plant belonging to the family Moraceae, has been a staple crop in the Pacific Islands for over 3000 years (Ragone & Raynor, 2009). The genus *Artocarpus* (Moraceae) is comprised of approximately 50 species, which are widely distributed among tropical and subtropical regions (Zerega, Ragone, & Motley, 2005). The name of the species is derived from the Greek words “atros” (bread) and “karpos” (fruit) referring to the fruit of this tree that smells/tastes like freshly baked bread when it is cooked (Ragone, Tavana, Bernotas, & Murch, 2001). Breadfruit was first cultivated in the western Pacific and was spread throughout the tropics by migrating Polynesians. Varieties of Polynesian breadfruit as well as bread nut from New Guinea were introduced into the Caribbean during the 1700s, and have since been distributed widely in Central and South America, Africa (Senegal, Ghana, and Liberia), India (costal regions of Karnataka and Kerala), Southeast Asia, Malaysia, Madagascar, Maldives, Seychelles, Indonesia, Sri Lanka, Northern Australia, and Southern Florida (Deivanai & Bhore, 2010). Breadfruit can be eaten at all stages of growth and can be prepared by a variety of methods including steaming, drying, frying, baking, and roasting. Breadfruit also holds the potential to help alleviate world hunger and increase food security (Liu, P. Jones, J. Murch, & Ragone, 2014).

Breadfruit holds a place in history for the role it played in the mutiny that occurred on the British Royal Navy vessel the HMS *Bounty* on April 28, 1789. The ship, captained by Lieutenant William Bligh, set out in 1787 for Tahiti, where they were to collect breadfruit plants and transport them to the West Indies. Three weeks into their return trip, relations between Bligh and

his crew were not favorable, since many wished to stay with the Tahitians and abandon their mission. The crew mutinied and set Bligh, as well as 18 loyalists, adrift at sea in a small rowboat while they returned to Tahiti. After an entire year of traveling, Bligh finally navigated his way back to England. The Royal Navy then dispatched the HMS Pandora to retrieve the mutineers and bring them to justice (Nordhoff & Hall, 1989). Charles Nordhoff and James Norman Hall published this harrowing tale in the 1939 book *Mutiny on the Bounty*. In 1962, Lewis Milestone and Carol Reed directed the film version of this breadfruit inspired tale.

ii. Botanical Identification

The taxonomical classification of breadfruit is as follows (Sushmita & Nayeem, 2013):

Kingdom: Plantae

Subkingdom: Mracheobionata

Division: Magnoliophyta

Class: Magnoliosida

Subclass: Hamamelididae

Order: Rosales

Family: Moraceae

Genus: Artocarpus

Species: altilis



<https://en.wikipedia.org/wiki/Breadfruit>



<http://farmsforestsfoods.blogspot.com/2014/01/edible-landscapes-and-trees-that-feed.html>

A. Growth Requirements

Artocarpus altilis is well adapted to tropical climates and fares especially well in the wet tropics where many staple grain crops do not (Ragone, 2011; Ragone & Raynor, 2009). It grows best in equatorial low lands, but has been found to grow in the highlands, though fruit production and quality decreases in the cooler highland conditions. Rain is also a crucial factor that affects the flowering and growth rate of fruit; fairly equal distribution of rainfall is required. For proper growth, *Artocarpus altilis* needs to be in sand, sandy loam, or loam soil with good drainage. Additionally, soil should be neutral to alkaline with a pH value ranging from 6.1-7.4. The ideal growth temperature is 21-32°C (Sikarwar et al., 2014).

B. Harvest

The breadfruit tree produces from March to June and again from July to September (Akanbi, Nazamid, & Adebawale, 2009). The fruits vary in size, shape, and texture but are generally

round, oval, and oblong in shape ranging from 9-20cm, more the 30cm long, and weigh around 0.25-6kg. The color of breadfruit can range from light green, yellow-green, and green-brown with ripe fruits having yellow to yellow-brown skin. The flesh of the ripe fruit is creamy, white, and soft. Breadfruit has estimated yields of 6 t·ha⁻¹ on a dry-weight basis (cultivar dependent) in an orchard system, making it one of the most productive crops in the world (Bowers, 1981). Most cultivars are highly seasonal, but due to investigations normally being performed at a single location, it may be difficult to predict how they do in different regions (Fownes & Raynor, 1993; A. M. P. Jones, Murch, & Ragone, 2010; Lebegin, Lemerre Desprez, & Mademba-Sy, 2007; Morton, 1987). A single breadfruit tree can produce 250-400kg of fruit (Liu et al., 2014).

iii. Nutritional Profile

A 1,000 calorie serving of breadfruit can fulfill over 100% of carbohydrate and fiber requirements, over 50% of potassium and magnesium, over 20% protein, vitamin C, iron, calcium, and phosphorus, and over 8% of vitamin B9 (folic acid) of the daily recommended dietary allowances (RDA) (A. M. P. Jones, Baker, Ragone, & Murch, 2013; A. M. P. Jones, Ragone, Aiona, Lane, & Murch, 2011; Ragone & Raynor, 2009; Ragone et al., 2001). Some cultivars are also a good source of vitamin A carotenoids (Englberger et al., 2003; Englberger, Lorennij, & Taylor, 2013; A. M. P. Jones, Murch, Wiseman, & Ragone, 2013; Meilleur, Jones, Titchenal, & Huang, 2004). Table 2 shows a nutritional comparison between breadfruit, white potatoes, and white rice, which are comparable gluten-free crops.

Table 1-2 Nutritional Comparison of Breadfruit, White Potato and White Rice (per 100g serving)

	Breadfruit†	White Potato†	White Rice†
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Water (g)	70.65	81.58	12.89
Energy (kcal)	103	69	360
Protein (g)	1.07	1.68	6.61
Total Lipid (g)	0.23	0.1	0.58
Carbohydrate (g)	27.12	15.71	79.34
Fiber (g)	4.9	2.4	--
Sugar (g)	11	1.15	--
Calcium (mg)	17	9	9
Iron (mg)	0.54	0.52	0.8
Magnesium (mg)	25	21	35
Phosphorus (mg)	30	62	108
Potassium (mg)	490	407	86
Sodium (mg)	2	16	1
Zinc (mg)	0.12	0.29	1.16
Vitamin C (mg)	29	9.1	0
Thiamin (mg)	0.11	0.071	0.07
Riboflavin (mg)	0.03	0.34	0.048
Niacin (mg)	0.9	1.066	1.6
Vitamin B6 (mg)	0.1	0.203	0.145
Folate (µg)	14	18	9
Vitamin B12	0	0	0
Vitamin A (IU)	0	8	--
Vitamin E (mg)	0.1	0.01	--
Vitamin D (IU)	0	0	0
Vitamin K (µg)	0.5	1.6	--
Saturated Fatty Acids (g)	0.048	0.026	0.158
Monounsaturated Fatty Acids (g)	0.034	0.002	0.181
Polyunsaturated Fatty Acids (g)	0.066	0.043	0.155
Trans Fatty Acids (g)	0	0	--
Cholesterol (mg)	0	0	0

† Data for breadfruit, raw; potato, white, flesh and skin, raw; cooked, rice, white, medium-grain, raw, unenriched (U.S.

Department of Agriculture, 2016)

iv. Culinary Uses

Breadfruit is an extremely versatile fruit that can be eaten at all stages of development though mature fruit is most desirable for culinary purposes. The mature breadfruit has a potato-like texture and can be prepared similarly by steaming, frying, boiling, baking, and roasting (“Brief Breadfruit Basics,” 2013). Breadfruit can be eaten raw, commonly consumed fried like potato chips or boiled and used to make more complex recipes. A variety of dishes can make use of breadfruit such as casseroles, fritters, croquets, curries, stews, chowders, salads, breads, pancakes, as well as other baked goods. It can even be mashed and made into dips, formed vegetarian burgers, or pâté. Another way breadfruit can be used is by having the peel/core removed from the raw fruit, slice or shred the fruit, then dry and grind it into flour (Ragone, 2014).

v. Potential for Gluten Free Applications

Since breadfruit does not contain gluten, it has potential to be used as a wheat flour substitute. Breadfruit flour could feasibly replace gluten-containing flours in a variety of baked goods such as cakes, cookies, breads, muffins, pastries, and could even be used in noodle formulations (Khoiri, Muchlis, Noriandita, & Zeni, 2014). Likewise, serving as a thickening agent, powdered flavor carrier, and bulking agent could be alternative food applications where breadfruit flour could replace gluten-containing materials. Breadfruit is also a good source of potassium, dietary fiber, and other nutrients that gluten-free products typically lack due to the removal of wheat flour, making breadfruit flour an ideal choice to boost the nutritional profile of gluten-free foods. Like other gluten-free flours, breadfruit flour lacks the elasticity or leavening capacity of wheat flour. This means that other functional ingredients such as gums and hydrocolloids will be

needed to produce gluten-free products that have comparable quality to their gluten-containing counterparts (Ragone, 2014).

vi. Study Objectives and Justification

The main purpose of this study was to assess how various inclusion levels of breadfruit flour affect the quality of gluten-free breads leavened with biological or chemical leavening agents. Specific objectives were to determine an appropriate level of substitution of breadfruit flour for rice flour in a gluten-free bread formulation, evaluate the effect of these substitutions on the major quality factors of gluten-free bread, and to make recommendations as to feasible usage levels of breadfruit flour in rice flour based gluten free bread.

A team from the Pacific Business Center Program (PBCP) at the University of Hawaii Manoa won the award for Research and Analysis from the University Economic Development Association (UEDA). This top award was given to the PBCP's Pacific Region Breadfruit Initiative, whose purpose is to promote breadfruit in the Pacific and to address the lack of food security in the Pacific. PBCP's Senior Business Development Manager, C. L. Cheshire, approached food scientists at Kansas State University seeking their expertise in gluten-free food product development. The scientists from Kansas State University were added to the Pacific Region Breadfruit Initiative team, and were tasked with researching breadfruit flour's potential as an ingredient for developing gluten-free value-added food products.

Chapter 2 - The Effect of Breadfruit Flour on the Quality of Gluten-Free Bread

I. Introduction

Celiac disease is an autoimmune disorder that affects genetically susceptible individuals. It is caused by the ingestion of wheat gluten, as well as proteins in related cereals, such as barley, rye, and possibly oats. Portions of these proteins elicit an autoimmune response that causes inflammation of the upper small intestine, thus causing a variety of undesirable symptoms (Alaedini & Green, 2005). Studies in both the United States and Europe show the disease affects about 1% of the population (Wieser & Koehler, 2008). The only effective and available treatment is the lifelong avoidance of gluten-containing foods.

Breadfruit (*Artocarpus altilis*) is widely available in tropical and subtropical regions across the globe, with the genus *Artocarpus* (Moraceae) being comprised of approximately 50 species (Zerega et al., 2005). As breadfruit does not contain the gluten proteins harmful to celiac patients, it is an appropriate grain for use in gluten-free products for human consumption. Additionally many regions that grow breadfruit are dependent upon imports to support their food supply. Utilizing breadfruit flour to replace commonly imported flours such as wheat and rice could help create increased food security in these regions.

It is widely accepted that gluten proteins are responsible for the gas-holding matrix that sets the structure in wheat bread (Hosney, 1994). Without these structure-forming proteins, it is a challenge to produce high-quality gluten-free bread that is acceptable to consumers. While there

are a handful of commercially available gluten-free breads, these products have an undesirable firm texture, large crumb structure, bland taste, and poor shelf-life. Additionally, commercially available gluten-free breads may not be readily available in the tropical and subtropical regions that grow breadfruit.

Studies dating as far back as the 1920s document the effects of wheat flour composition and particle size on end-product quality (LaClerc, Wessling, Bailey, & Gordon, 1919). However, such studies have not been carried out for the purposes of improving quality of breadfruit products, and specifically, gluten-free breadfruit bread. At present, it has been observed that breadfruit flour is not commercially available, except in local farmer's markets within the regions breadfruit is grown, and there are no particular quality specifications regarding particle size, starch damage, or fiber content of the flour.

Based on the documented and well-understood effects of wheat flour properties on product quality, it was hypothesized that type and amount of leavening agent as well as inclusion level of breadfruit flour will affect breadfruit flour quality of gluten-free bread. In testing this research hypothesis, the main objective was to provide information that would assist millers in understanding the importance of milling a more value-added breadfruit flour that can be successfully used in a variety of applications, as well as to enable product developers to produce higher-quality gluten-free products from breadfruit flour. Overall, fulfillment of these objectives will most benefit producers of breadfruit flour and breadfruit flour value-added products as well as consumers who have celiac disease, gluten allergy, or gluten sensitivity.

II. Materials & Methods

i. Preliminary Experiments

A. Breadfruit Flour Properties

Since breadfruit flour is a relatively new ingredient, it was deemed necessary to first characterize the flour and perform preliminary testing to assess its functionality. The initial flour samples were milled and received from Samoa. The samples consisted of flour of the Ma'afala variety of *Artocarpus altilis*, which was selected due to its lengthy harvesting season and overall production rates compared to other breadfruit varieties. Mature breadfruit were harvested by Samoan farmers, skinned to remove the outer peel, dried, then ground into flour, and shipped to Kansas State University in airtight plastic bags. Initial experimentation with this flour yielded product that had a distinct off-flavor reminiscent of fermented products and characterized as bitter, acidic, and astringent. Microbial testing revealed too numerous to count (TNTC) yeast and mold counts. Upon further investigation by the breadfruit grant's milling expert, it was found that the drying/milling process was to blame for these undesirable characteristics. Though specific flour-processing protocol and equipment had been provided by Kansas State University milling expert, Dr. Jeff Gwartz, local Samoan mill workers preferred to use traditional methods such as peeling the fruit with the lids of tuna cans instead of using the industrial grade peeler and drying the fruit via sunlight under a tarp upon the roof of the mill. These "traditional" methods do not allow for control over processing time, temperature, relative humidity, and other variables crucial to producing consistent high-quality flour; this variability was likely the cause of the off-flavor detected in the breadfruit flour as well as bread that included the flour as an ingredient.

To reduce the chances of off-flavor as well as variability in processing the flour, it was decided that any breadfruit flour used for this study was to be milled at Kansas State University under the supervision of Dr. Jeff Gwartz. Samples of breadfruit (varying in fruit variety and maturity) were grown/harvested in Hawaii, shredded into thin (roughly 1cm long) pieces, dried in a commercially available food dehydrator, and then packaged into 5lb sealed plastic bags that were shipped to researchers at Kansas State University. In order to select the optimal breadfruit shreds for milling, preliminary tests were performed to assess off flavor, moisture content, ash content, protein content, fat content, fiber content, color, particle size, and damaged starch. Throughout the study, all proximate analyses were performed by the manager of the Analytical Lab in Weber Hall at Kansas State University. Particle size and damaged starch were performed by United States Department of Agriculture-Agricultural Research Service (USDA-ARS) scientists working at the USDA-ARS building located off of College Avenue in Manhattan, KS.

a. Proximate Analysis

ai. Moisture Content

The moisture contents of the flours were measured using the Association of Official Analytical Chemists (AOAC) approved method 930.15. The procedure determines the dry matter of the sample by oven drying at 135°C for 2 hrs. Moisture was evaporated from the sample during the drying, and then dry matter was determined gravimetrically as the residue remaining after drying. The moisture was then calculated by subtraction of dry matter from the whole sample, which was found to be anywhere between 4-6% for the breadfruit shred samples.

aii. Protein Content

The protein contents of the flours were measured using AOAC approved method 990.03: Nitrogen Determination by Combustion. Nitrogen in the sample was freed by combustion at high temperatures in pure oxygen, and then measured by thermal conductivity. This value was converted to the equivalent protein by a numerical factor of 6.25.

aiii. Fat Content

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

aiv. Fiber Content

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

b. Color

A HunterLab MiniScan (Model Mini Scan EZ 4500L, Hunter Associates Laboratory Inc., Reston, VA) was used to measure the color of flour samples. The device was calibrated with a light trap and white tile provided by Hunter Associates Laboratory Inc. The type of illuminant used was C, average daylight, with a 10° Standard Observer. “L*”, “a*”, and “b*” values were given as output. “L*” is the measurement for lightness (0 = black and 100 = white). Red and green colors are indicated by the “a*” value (+a = red and -a = green). The “b*” value indicates

yellow (+b) and blue (-b) colors. Throughout the study, color analysis was performed in the Kansas Value-Added Food Lab at Kansas State University.

c. Milling

The flour was milled in the milling lab of Shellenberger Hall at Kansas State University, using a Buhler Laboratory Mill (MLU-202, Uzwil, Switzerland). AACC Method 26-22, Buhler Method for Hard Wheat, with the appropriate roll gap settings (Table 1). The resulting flour was sieved through multiple screens, and the finest fraction was collected for use as experimental breadfruit flour. In order to characterize the experimental breadfruit flour, particle size and starch damage were measured.

Table 2-1 Roll Gap Settings for Buhler Mill

	Break Rolls		Reduction Rolls	
	Left	Right	Left	Right
Inches	.00472	.00394	.00275	.00118
mm	.1	.08	.07	.03

d. Particle Size

A Beckman Coulter LS™ 13 320 Laser Diffraction Particle Size Analyzer (Beckman-Coulter, Inc., Miami, FL) was used to determine the particle size distribution of the flours. The dry powder system was used. The flour was placed into the load cell until it was approximately 2/3 full. The cell was then loaded into the Tornado™ Dry Powder Dispersing attachment for the instrument, and measurements were taken. The LS™ 13 320 uses light scattering properties to determine the particle size distribution.

e. Starch Damage

Starch damage was determined using the Megazyme Starch Damage Assay Procedure, K-SDAM 05/2008, AACC Method 76.31 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). In the procedure, damaged starch granules are hydrolyzed to maltosaccharides and alpha-limit dextrans through a highly controlled treatment with purified fungal alpha-amylase. This leads to nearly complete solubilization of the damaged starch granules, while minimizing breakdown of undamaged granules. The reaction was terminated with dilute sulfuric acid. Aliquots were subsequently treated with excess levels of purified amyloglucosidase to give complete degradation of starch dextrans to glucose. The resulting solution is reacted with glucose oxidase/oxidase reagent, and the glucose concentration was determined colorimetrically. Reported values are for damaged starch as a percentage of flour weight on an “as is” basis.

B. Initial Formulations

Many preliminary formulas were attempted in order to identify an optimal gluten-free bread formula to be used for experimental purposes. A panel of food science faculty and students informally evaluated all breads. Trial #1 utilized a formula (in baker’s percentages) consisting of 100.00% breadfruit flour, 1.75% salt, 1.00% sugar, 100.00% water, 2.00% yeast, and 25.00% whole eggs. Ingredients were mixed together until incorporated and a batter formed, the batter was allowed to rise for 60 minutes at 42°C, then 120g of batter was portioned into a 6 x 2 slotted mini loaf pan, loaves were baked for 40 minutes at 93.3°C, removed from oven, and allowed to cool on a wire rack for 60 minutes. Since the resulting loaves were unacceptably hard, cracked, dry, dense, and had an overwhelming fermented flavor, this formula was rejected. Trials #2-4

followed a similar protocol but incorporated nonfat dry milk at 3.00%, xanthan gum at 1.00%, additional gluten-free flours (white sorghum, white rice, and potato starch) at 70.00%, and breadfruit flour at 30.00%. These formulas produced loaves with comparable results to Trial #1 and were decidedly not optimal. Trials #5-18 utilized gluten-free flour blends consisting of combinations of gluten-free flours (white sorghum, white rice, buckwheat flour, potato starch, tapioca flour, cornstarch) with breadfruit flour in various proportions. It was found that increasing the inclusion level of breadfruit flour also increased the need for water while decreasing loaf volume. Ingredients such as hydroxypropyl methylcellulose (HPMC), eggs (whole, whites, powdered), vinegar (white, cider), sweetening agents (white sugar, brown sugar, honey), lipids sources (butter, vegetable oils, olive oil) and various gums (xanthan, guar) were also added to see if they improved overall loaf structure and quality. Additionally, it was found that a combination of rice flour, tapioca starch, cornstarch, and potato starch produced the best gluten free bread with volume, crumb structure, and flavor most similar to conventional gluten-containing bread. Trials #20-22 utilized an optimal flour blend consisting of breadfruit flour, tapioca starch, cornstarch, and potato starch, in conjunction with other ingredients found to produce acceptable gluten-free bread, to assess appropriate proportions of ingredients. Processing parameters (mixing time, fermentation time/temperature, baking time/temperature) were assessed and optimal parameters were identified. The final trials (#23-27) assessed alternative methods of leavening (yeast, baking powder), optimal cooling time, and use of a masking agent on quality of final loaves. Pictures of various trial formulations can be found in Figure 2-1.

Figure 2-1 Picture of Various Failed Gluten-Free Breadfruit Bread Formulas





a. Water Optimization

Prior to baking, the water addition necessary for each flour treatment was optimized by standardizing the batter consistency. For wheat bread, it is widely accepted that optimum water absorption may be determined with a Brabender farinograph or a mixograph. However, there are no such standard methods for water absorption optimization for gluten-free breads that do not form a dough. As a result, water optimization for this particular experiment was conducted by assessing how the bread performed during preliminary experimentation. It was discovered that a ratio of 1: 2.5 percent breadfruit flour to water was adequate for adjusting the water content in formulas containing varying amounts of breadfruit flour. This was assessed visually and organoleptically by a group of food science faculty and students.

C. Screening Criteria

The quality of bread is contingent upon numerous aspects of its formula and processing. Criteria used to evaluate overall quality of preliminary experimental bread included specific volume, color, crumb structure, and organoleptic properties such as visual appearance, flavor, texture in mouth, and aftertaste.

D. Material Selection

The materials used during experimentation were chosen based on how they performed during preliminary tests. The shreds selected for milling were of the mature Ma'afala variety, with a measured moisture content of 5%, and had mean color scores of 69.15 (L*), 3.25 (a*), 67.83 (b*), particle size of 58.21g/cm³, and were found to have 4.12% damaged starch. These shreds were most ideal because of their variety's long harvesting season and, when ground, produced the lightest colored flour.

Since preliminary experiments revealed that a blend of rice flour, tapioca starch, cornstarch, and potato starch produced a bread most similar to conventional gluten-containing bread, this blend was used as a control formula. Rice flour was replaced at various inclusion levels with breadfruit flour. The remaining ingredients were selected depending upon how well they performed in preliminary experiments. Leavening was selected as an experimental treatment because preliminary trials showed that yeast and baking powder successfully leavened bread, but could produce different sensory characteristics. This difference prompted further investigation on how each would affect the quality of gluten-free bread made from breadfruit flour.

E. Experimental Design

This experiment utilized a randomized complete block design. For this particular study, the blocks were the 6 x 2 slotted mini-loaf pans. One pan containing the 12 treatments randomly assigned to slots was baked on the top rack of the oven and a second pan containing the same 12 treatments randomly assigned (different from the pan on the top rack) was backed on the bottom

rack. This allowed for two subsamples of a treatment to be produced per replication, with three replications performed in total on separate days.

The moisture content, ash content, protein content, fat content, fiber content, starch damage, and particle size of breadfruit shreds were performed in duplicates. Measurements of batter pH, weight, and volume as well as loaf weight, volume, color (crust and crumb), texture, pH, and water activity were repeated in triplicates. Replications of each flour/leavening treatment were baked in duplicate loaves, and 2 slice views were evaluated for crumb characteristics with a C-Cell instrument. Proximate analysis was performed once on the duplicate loaves from the first replication.

All data were analyzed using SAS, Software Release 9.4 (SAS, Institute Inc., Cary, NC, 2013). When treatment effects were found significantly different, the least square means with Tukey-Kramer groupings were used to differentiate treatment means. A level of significance was observed at $\alpha \leq 0.05$. The level of significance is indicated in parentheses. Multiple linear regression was carried out to determine significance of interaction between variables. Pearson correlation coefficients were used to determine if positive or negative correlations existed between the different terms analyzed. Paired t-tests were performed on data from the sensory testing ballots to see if the two treatments being analyzed were significantly different from each other. A level of significance was observed at $\alpha \leq 0.05$.

ii. Experimentation

A. Formulation

a. Control

The control formula was selected on the basis that a majority of commercial gluten-free products contain rice flour in their formula. Rice flour is also a main import to many of the regions where breadfruit grows. Since many of these regions are food insecure, and rely upon imports to provide the majority of their food supply, an important aspect of this research was to see if breadfruit flour can substitute in part for commonly used rice flour in gluten-free bread formulations.

b. Treatments

Two variables were evaluated during this study: breadfruit flour inclusion and leavening. Breadfruit flour inclusion was the percent (baker's percent) breadfruit flour used in the gluten-free flour blend. There were four treatment levels of breadfruit flour inclusion: 0% (control), 20%, 35%, and 50%. Leavening contained three treatment levels: yeast, 15% baking powder, and 20% baking powder. A total of twelve treatments were performed in duplicate in each of the 3 replications.

Table 2-2 Formulations for Yeast Leavened Gluten-Free Bread Made with Breadfruit Flour

	Yeast/Control (YC)	Yeast/Breadfruit 20% (Y20)	Yeast/Breadfruit 35% (Y35)	Yeast/Breadfruit 50% (Y50)
Rice Flour (g)*	30.00	--	--	--
Breadfruit Flour (g)*	--	20.00	35.00	50.00
Tapioca Starch (g)*	32.68	37.68	30.18	22.68
Corn Starch (g)*	35.74	40.74	33.24	25.74
Potato Starch (g)*	1.58	1.58	1.58	1.58

Xanthan Gum (g)	2.00	2.00	2.00	2.00
Powdered Whole Egg (g)	0.79	0.79	0.79	0.79
Salt (g)	1.83	1.83	1.83	1.83
Masking Agent (g)	1.25	1.25	1.25	1.25
Nonfat Dry Milk (g)	4.87	4.87	4.87	4.87
Unsalted Butter (g)	8.79	8.79	8.79	8.79
Whole Eggs (g)	29.23	29.23	29.23	29.23
Cider Vinegar (g)	1.33	1.33	1.33	1.33
Honey (g)	17.23	17.23	17.23	17.23
Water (g)	72.00	68.00	74.00	80.00
Yeast (g)	0.70	0.70	0.70	0.70
Baking Powder (g)	--	--	--	--
Total (g)	240.02	236.02	242.02	248.02

* Components of Flour Blend

Table 2-3 Formulations for 15% Baking Powder Leavened Gluten-Free Bread Made with Breadfruit Flour

	Baking Powder 15%/Control (BP15C)	Baking Powder 15%/Breadfruit 20% (BP1520)	Baking Powder 15%/Breadfruit 35% (BP1535)	Baking Powder 15%/Breadfruit 50% (BP1550)
Rice Flour (g)*	30.00	--	--	--
Breadfruit Flour (g)*	--	20.00	35.00	50.00
Tapioca Starch (g)*	32.68	37.68	30.18	22.68
Corn Starch (g)*	35.74	40.74	33.24	25.74
Potato Starch (g)*	1.58	1.58	1.58	1.58
Xanthan Gum (g)	2.00	2.00	2.00	2.00

Powdered Whole Egg (g)	0.79	0.79	0.79	0.79
Salt (g)	1.83	1.83	1.83	1.83
Masking Agent (g)	1.25	1.25	1.25	1.25
Nonfat Dry Milk (g)	4.87	4.87	4.87	4.87
Unsalted Butter (g)	8.79	8.79	8.79	8.79
Whole Eggs (g)	29.23	29.23	29.23	29.23
Cider Vinegar (g)	1.33	1.33	1.33	1.33
Honey (g)	17.23	17.23	17.23	17.23
Water (g)	72.00	68.00	74.00	80.00
Yeast (g)	--	--	--	--
Baking Powder (g)	15.00	15.00	15.00	15.00
Total (g)	254.32	250.32	256.32	262.32

* Components of Flour Blend

Table 2-4 Formulations for 20% Baking Powder Leavened Gluten-Free Bread Made with Breadfruit Flour

	Baking Powder 20%/Control (BP20C)	Baking Powder 20%/Breadfruit 20% (BP2020)	Baking Powder 20%/Breadfruit 35% (BP2035)	Baking Powder 20%/Breadfruit 50% (BP2050)
Rice Flour (g)*	30.00	--	--	--
Breadfruit Flour (g)*	--	20.00	35.00	50.00
Tapioca Starch (g)*	32.68	37.68	30.18	22.68
Corn Starch (g)*	35.74	40.74	33.24	25.74
Potato Starch (g)*	1.58	1.58	1.58	1.58
Xanthan Gum (g)	2.00	2.00	2.00	2.00
Powdered Whole Egg (g)	0.79	0.79	0.79	0.79

Salt (g)	1.83	1.83	1.83	1.83
Masking Agent (g)	1.25	1.25	1.25	1.25
Nonfat Dry Milk (g)	4.87	4.87	4.87	4.87
Unsalted Butter (g)	8.79	8.79	8.79	8.79
Whole Eggs (g)	29.23	29.23	29.23	29.23
Cider Vinegar (g)	1.33	1.33	1.33	1.33
Honey (g)	17.23	17.23	17.23	17.23
Water (g)	72.00	68.00	74.00	80.00
Yeast (g)	--	--	--	--
Baking Powder (g)	20.00	20.00	20.00	20.00
Total (g)	259.32	255.32	261.32	267.32

* Components of Flour Blend

Figure 2-2 Picture of Randomized Experimental Bread Loaves



Figure 2-3 Picture of Crumb from BP15C



B. Methodology

a. Mixing

The control formulations are shown in Tables 2-2 (YC), 2-3 (BP15C), and 2-4 (BP20C).

Ingredients used included rice flour, tapioca starch, potato starch, xanthan gum (Bob's Red Mill, Milwauki, OR), cornstarch, sea salt, nonfat dry milk, butter, Grade A large eggs, cider vinegar, honey (Great Value, Wal-mart Stores, Inc., Bentonville, AR), powdered whole egg (Primavera Foods, Cameron, WI), active dry yeast (Red Star Yeast, Milwaukee, WI) or double-acting baking powder (Clabber Girl, Corporation, Terre Haute, IN), water, and a masking agent (Gold Coast Ingredients Inc., Commerce, California). The total amount of the rice/breadfruit flour, tapioca starch, cornstarch, and potato starch was interpreted as the flour weight basis. The addition of water to the formulation was modified for each flour treatment in order to standardize the consistency of each batter, as previously described.

The dried yeast was reactivated with 5 minutes of pre-hydration in the amount of water (37.8°C) appropriate for each flour treatment. The flour blend (rice or breadfruit flour, tapioca starch, cornstarch, and potato starch), xanthan gum, dried egg powder, salt, nonfat dry milk, and masking agent were mixed separately, breaking up any clumps, and then whole eggs, butter, cider vinegar, and honey were added. If the formula contained baking powder, it was added at this step along with the other dry ingredients. The batter was mixed with a 300 W Kitchen Aid mixer (Ultra Power, St Joseph, MI) with a flat beater attachment for 30 seconds at the lowest speed, and then scraped. The yeast and water mixture (or just water if the formula was leavened with baking powder) was then added to the batter and mixed for 2 minutes on speed 5. After mixing, 120.00g of each batter was weighed into greased mini loaf baking pan (randomized as

previously described) and proofed at 42°C and 85% relative humidity in a proofing cabinet (National Manufacturing Co., Lincoln, NE). Each batter was proofed to height, corresponding to 1cm above the edge of the pan. Approximate proof time was about 60 minutes. After proofing, the batters were baked for 35 minutes in an electrically-powered reel-type test baking oven (National Manufacturing Co., Lincoln, NE) preheated to 218.3°C (425°F) with convection. After baking, the loaves were removed from the pan and cooled for 1.15 hours on a wire rack at ambient temperature. All analyses were performed on the loaves immediately following the 1.15-hour cooling time.

b. Analysis

bi. Specific Volume

After cooling, loaves were weighed and loaf volume was measured by rapeseed displacement (AACC Method 10-05). Loaf specific volume (loaf volume [mL]/loaf weight [g]) was calculated.

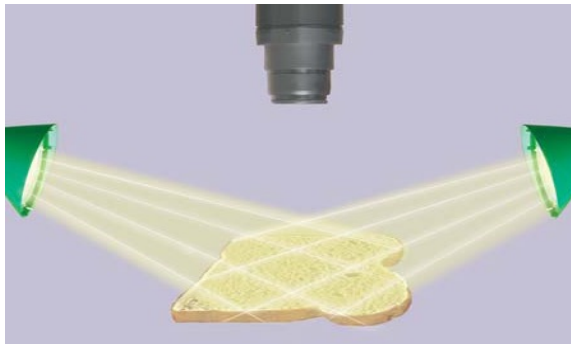
bii. Color

A HunterLab MiniScan (Model Mini Scan EZ 4500L, Hunter Associates Laboratory Inc., Reston, VA) was used to measure the color of the crust as well as crumb of each treatment sample post cooling. The device was calibrated with a light trap and white tile provided by Hunter Associates Laboratory Inc. Reading were taken from three spots on the loaf (end, middle, and opposite end). The type of illuminant used was C, average daylight, with a 10° Standard Observer. “L*”, “a*”, and “b*” values were given as output. “L*” is the measurement for lightness (0 = black and 100 = white). Red and green colors are indicated by the “a*” value (+a = red and -a = green). The “b*” value indicates yellow (+b) and blue (-b) colors.

biii. Crumb Structure

Once the specific volume of each treatment loaf was determined, the loaf was sliced transversely using an in-house manufactured slice regulator and bread knife to obtain four slices of 25 mm thickness. The 3rd bread slices from each experimental loaf were assessed for crumb grain characteristics using a C-Cell Instrument (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). C-Cell uses high definition imaging and controlled illumination to obtain images, as illustrated by Figure 2-1. A C-Cell Instrument has the capability to determine important bread crumb attributes, including average cell diameter and volume, average cell wall thickness, average crumb fineness (number of cells/cm²), and slice brightness (Chen, Feng, Seabourn, & Caley, 2007).

Figure 2-4 Illustration of C-Cell Imaging Process



<http://www.c-cell.info>

biv. Texture

Texture Profile Analysis (TPA) (Bourne 1978) of the crumb was performed on the second slice from each experimental loaf using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) equipped with a 38 mm Perspex cylinder probe along with a 30 kg load cell. TPA was carried out with a constant speed of 2.0 mm/s (applying to the pre-test speed, test speed, and post-test speed) for a distance of 10.0 mm, corresponding to 40% compression of

the 25 mm slices. There was a 5 second wait time between the first and second compression cycles; the trigger force was 20.0 g. Texture analysis was performed in the Kansas Value-Added Foods Lab at Kansas State University.

bv. pH

After cooling, bread pH was analyzed using 15 g of crust-free crumb of applicable baked product separated into small pieces) in dry Erlenmeyer flask and add 100 ml cooled, distilled water. The flask was agitated until bread was suspended and free of lumps. The suspension was maintained for 30 min using a magnetic stirrer. The suspension was left to stand and settle for 10 min then the supernatant liquid was decanted into electrode vessel and pH was immediately determined, using potentiometer and electrodes that have been calibrated against known buffer solutions (AACC Method 02-52.01). Analysis of pH was performed in the Kansas Value-Added Foods Lab at Kansas State University.

bvi. Water Activity

Water activity (a_w) was determined for each loaf by putting it into a plastic sample dish then inserting it into a calibrated Aqua Lab Series 3 water activity meter (Decagon Devices Inc., Pullman, WA). A small fan circulates the air above the sample, speeding vapor equilibrium. An infrared sample measures the samples surface temperature, eliminating the need for temperature equilibration. A small internal mirror is cooled until water condenses at the dew point temperature. The mirror and sample temperatures are used to compute water activity of the sample. A microprocessor controls the heating and cooling of the mirror and allows precise a_w readings to be made. Both a_w and sample temperature are displayed on the instrument's screen

and were recorded (Czuchajowska, Pomeranz, & Jeffers, 1989). Analysis of water activity was performed in the Kansas Value-Added Foods Lab at Kansas State University.

bvii. Proximate Analysis

Moisture Content

The moisture contents of the finished loaves were measured using the Association of Official Analytical Chemists (AOAC) approved method 930.15. The procedure determines the dry matter of the sample by oven drying at 135°C for 2 hrs. Moisture was evaporated from the sample during the drying, and then dry matter was determined gravimetrically as the residue remaining after drying. The moisture was then calculated by subtraction of dry matter from the whole sample.

Protein Content

The protein contents of the flours finished loaves were measured using AOAC approved method 990.03: Nitrogen Determination by Combustion. Nitrogen in the sample was freed by combustion at high temperatures in pure oxygen, and then measured by thermal conductivity. This value was converted to the equivalent protein by a numerical factor of 6.25.

Fat Content

The fat contents of the finished loaves were measured using AOAC approved method 920.39. This method determines crude fat in the samples by ether extraction with subsequent solvent evaporation. The fat content is reported as a percentage of the original sample weight.

Fiber Content

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

bviii. Initial Informal Sensory

To assess the acceptance and quality of these products an informal consumer study was carried out in Call Hall of Kansas State University. A total of 5 randomly selected panelists in the building were asked to evaluate each of the 12 treatments for liking of organoleptic sensory properties including appearance, color, flavor, texture in mouth, aftertaste, overall acceptance. The loaves were prepared the same day as the initial informal sensory tests as described in the section titled “Mixing”. Each participant was served one interior slice from each treatment loaf, on a 3-digit coded plate, one treatment at a time, in random order. At the time the treatment samples were distributed, numbered ballots bearing identical 3-digit codes, matching those on the sample plates, were given to the panelists. Panelists were instructed to evaluate each sample in the order they were provided to them (to eliminate possible bias) and complete the ballots according to the instructions listed on them. Each ballot contained a 9-point hedonic scale for the previously listed organoleptic sensory properties (appearance, color, flavor, texture in mouth, aftertaste, and overall acceptance). These 9-point hedonic scales displayed degree of liking corresponding to the specific attributes (9 being “like extremely, 5 being “neither like nor dislike”, and 1 being “dislike extremely). The four treatments with the highest overall acceptance score (YC, Y20, Y35, BP2020) were selected for additional initial sensory testing.

To further identify the most acceptable treatment, a total of 10 randomly selected panelist in the building were asked to evaluate each of the top four treatments (YC, Y20, Y35, BP2020) from the first initial sensory evaluation, using the same procedures described above. The two treatments with the highest overall acceptance score (YC, Y20) were selected for a final 100 consumer sensory study.

bix. Consumer Sensory Study

To assess the acceptance and quality of the two most preferred products, a consumer study was carried out in Call Hall of Kansas State University. A total of 108 untrained panelists volunteered to participate in this study, including 5 suffering from celiac disease and/or a gluten allergy or sensitivity. Prior to participating in the study, each panelist signed an Informed Consent Statement that informed them of the purpose and guidelines of the study (Appendix A). Panelists were also required to complete a numbered pre-screening form containing information about their age, gender, highest education completed, if they suffer from any food allergies, the frequency they purchase bread products, frequency they purchase gluten-free products, and frequency they purchase gluten-free bread products (Appendix B). Any participant who indicated they had a food allergy, intolerance, or sensitivity to anything other than gluten was not allowed to participate in the study. Degree of liking of organoleptic sensory properties including appearance, color, flavor, texture in mouth, aftertaste, and overall acceptance were again assessed. The loaves were prepared the same day as the initial informal sensory tests as described in the section titled “Mixing”. Each participant was served one interior slice from each of the four treatment loaves, on a 3-digit coded plate, one treatment at a time, in random order. At the time the treatment samples were distributed, numbered ballots bearing identical 3-digit codes, matching those on the sample plates, were given to the panelists. Panelists were instructed to

evaluate each sample in the order they were provided to them (to eliminate possible bias) and complete the ballots according to the instructions listed on them. Each ballot contained a 9-point hedonic scale for the previously listed organoleptic sensory properties (appearance, color, flavor, texture in mouth, aftertaste, and overall acceptance). These 9-point hedonic scales displayed degree of liking corresponding to the specific attributes (9 being “like extremely, 5 being “neither like nor dislike”, and 1 being “dislike extremely). When panelists were finished tasting and rating the samples, they had the opportunity to write additional comments to suggest improvements, and make any other comments concerning the samples (Appendix C).

c. Evaluation of Top 2 Treatments

The top two treatments (YC, Y20) were evaluated for sensory characteristics (as previously described in the Consumer Sensory Study) as well as shelf life. Initial shelf life assessment indicated visible mold growth as the mode of failure of these products. Therefore, to perform shelf life, a single slice of bread from each treatment was placed into a sealed, airtight, quart sized plastic bag. The treatment samples were observed for visible molds, which occurred 5-7 days after the shelf life study had been initiated.

II. Results & Discussion

i. Loaf Analysis

A. Specific Volume

A significant effect was noted ($p < 0.05$) for the specific volume of breads produced with all levels of leavening studied (Table 2-5). Values ranged from 1.91 mL/g (YC) to 4.29 mL/g (BP2050).

Within all leavening treatments, breads leavened with yeast had significantly higher specific

volumes when compared to all other leavening treatments. Additionally, there was no significant difference ($p = 0.470$) in specific volume between both baking powder treatments. A significant effect was also noted ($p < 0.05$) for the specific volume of breads produced with all levels of breadfruit flour studied (Table 2-5). Within all breadfruit flour treatments, breads containing 0% breadfruit flour (30% rice flour) had significantly higher specific volumes when compared to all other leavening treatments. Treatments of 20%, 35%, and 50% breadfruit flour were all significantly different ($p < 0.05$) from one another, with specific volume decreasing as percent breadfruit flour increases. This finding may affect consumer acceptability of gluten-free bread made with breadfruit flour, since higher specific volume has been associated with higher acceptability of gluten-free bread (De Morais, Cruz, & Bolini, 2013).

Table 2-5 Comparison of Specific Volumes in Bread Produced from Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

Specific Volume (mL/g)			
Flour Inclusion Level	Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%
0%	3.95 ± 0.24^{Aa}	2.97 ± 0.28^{Ab}	3.04 ± 0.40^{Ab}
20%	3.14 ± 0.06^{Ba}	2.59 ± 0.49^{Bb}	2.74 ± 0.43^{Bb}
35%	2.60 ± 0.48^{Ca}	2.36 ± 0.27^{Cb}	2.37 ± 0.29^{Cb}
50%	2.31 ± 0.25^{Da}	2.03 ± 0.04^{Db}	2.08 ± 0.22^{Db}
<p>For each column, mean values with the same <u>uppercase</u> superscript are not significantly different ($p > 0.05$). For each row, mean values with the same <u>lowercase</u> superscript are not significantly different ($p > 0.05$).</p>			

a. Effect of Fiber on Specific Volume

Specific volume is affected by many factors, including dough composition (including amounts of water, fiber, starch, protein, processing aids, etc.), processing conditions, and dough rheology—

all properties that impact gas retention capabilities. While there is a nutritional benefit to the incorporation of dietary fiber into gluten-free products (as well as other baked goods), this is met with the limitation of decreased volume (Chen, Rubenthaler, Leung, & Baranowski, 1988; Krishnan, Pang, & Brown, 1987; Pomeranz, Shogren, Finney, & Bechtel, 1977; Sievert, Pomeranz, & Abdelrahman, 1990). In the present study, fiber contributed significantly to specific volume ($p < 0.0001$). Additionally, it was observed that due to concentrated fiber content, bran particles from brown rice flour and buckwheat flour swelled extensively during dough mixing, causing a weakened structure and a decreased volume of bread (Pomeranz et al., 1977). Gan and others suggested that bran particles would disturb the homogeneity of the starch gel and prevent uniform gas cell formation. In wheat bread, the dough and bread structures are stabilized and strengthened by a gluten network, yet decreased volume is still seen when fiber is incorporated (Gan, Ellis, & Schofield, 1995). It was therefore hypothesized by Moore and others that this deleterious effect on volume could be expected to be even worse in gluten-free baked products (Moore, Schrober, Dockery, & Arendt, 2004). These results add validity to the observations in the present study that show a decrease in specific volume with an increase in fiber content.

b. Effect of Particle Size on Specific Volume

Flour particle size has also been shown to affect overall baked product quality, but specifically loaf volume. For this study, particle size was kept constant between all treatments by using breadfruit flour identified as having a particle size of 58.21 μm . Yamazaki and Donelson reported a correlation coefficient of -0.94 between median diameter of patent flour and cake volume (Yamazaki & Donelson, 1972). A similar relationship was noted by Chaudhary and others with a correlation coefficient of -0.85 for the same relationship (Chaudhary, Yamazaki, &

Gould, 1981). Additionally, Kim and others discovered that rice flour with a particle size $<95\mu\text{m}$ yielded cupcakes with the highest specific volume. Air cell size as well as homogeneity were found to decrease as particle size decreased (J.-M. Kim & Shin, 2014). Such results may explain why loaves containing rice flour had significantly higher ($p<0.001$) specific volume than those containing breadfruit flour. Likewise, loaves containing lower levels of breadfruit flour (20%) had significantly higher specific volume than those containing higher levels of breadfruit flour (35%: $p<0.001$, 50%: $p<0.001$). As previously stated, this finding may affect consumer acceptability of gluten-free bread made with breadfruit flour, since specific volume has been associated with higher acceptability of gluten-free bread (de Morais et al., 2013).

c. Effect of Starch Damage on Specific Volume

Because increased starch damage is a result of decreasing flour particle size, its synergistic effects with particle size must not be ignored; Farrand observed that loaf volume and crumb structure were significantly correlated with variations in starch damage (Farrand, 1972). In the study at hand, starch damage of the breadfruit flour used was found to be 4.12%; for comparison, wheat flour was found to be 8.35%. Miller and others reported that an increase in starch damage which negatively affected cake quality (Miller, Trimbo, & Powell, 1967). Excessive starch damage leaves swollen starch granules susceptible to attack by alpha-amylase (Tipples, 1969). An increase in the hydrolysis of starch by alpha-amylase will decrease the viscosity of the dough/batter matrix and affect end-product quality. The result is a sticky, heavy crumb texture with low volume (Evers & Stevens, 1985). De Morais and others noted that higher specific volume has been associated with higher acceptability of gluten-free bread (de Morais et al., 2013). Since it seems that starch damage may have a diminishing return effect on specific

volume, further investigation is needed to determine the appropriate level for production of gluten-free breadfruit bread.

B. Color

a. L* Values

Results for crust L* values for all leavening and breadfruit flour treatments studied are shown in Table 2-6 and crumb L* results are shown in Table 2-7. Crust L* values ranged from 31.12 (BP15C) to 62.23 (BP2035) and crumb L* values from 56.80 (BP2020) to 78.53 (YC). The L* value indicates the measure of lightness of a sample and is considered to be an expression of the sample's whiteness. The value ranges from 0 (black) to 100 (perfect white), with higher values indicating brighter samples (Hutchings, 1994; Kurimoto & Shelton, 1988). Leavening did not have a significant effect on crust L*. Breadfruit flour inclusion was found to have a significant effect ($p \leq 0.05$) on crust L. Significant differences between inclusion levels were found between 0 and 35%, 0 and 50%, as well as 20% and 50%. Significant effects ($p < 0.05$) in crumb L* values were found for both leavening and breadfruit flour treatments. Significant differences ($p < 0.0001$) were found between all leavening treatment levels. Crumb L* values were significantly higher in breads leavened with yeast compared to baking powder (15%, 20%), meaning that yeast leavened breads had a whiter crumb color. Significant differences ($p < 0.05$) were also found between breadfruit flour treatments, specifically between 0% and 20% ($p < 0.0001$) as well as 35% ($p < 0.0112$) breadfruit flour. Additionally, 20% and 50% breadfruit flour showed significant differences ($p < 0.0001$). L* values decreased significantly ($p < 0.05$) with increasing levels of breadfruit flour. It should also be noted that no significant difference ($p < 0.05$) was

found between 0% breadfruit flour and 50%, suggesting that the crumb of bread made from rice flour is similar in lightness to that of bread made from 50% breadfruit flour.

Previous studies have shown that fiber content has an effect on the brightness of a sample. Oh and others noted a decline in flour brightness when wheat flour fiber content was increased by 8% (Oh, Seib, Ward, & Deyoe, 1985). In this study, fiber significantly contributed to crust L* ($p < 0.05$) as well as crumb L* ($p < 0.0001$) value. Particle size has been shown to have an impact on flour color, and particularly L* values. Kurimoto and Shelton examined the effect of wheat flour particle size on flour attributes. Results for L* values showed a significant increase with decreasing particle size, with a correlation coefficient of -0.98 ($p < 0.01$), suggesting that finer flour appears to be brighter or whiter (Kurimoto & Shelton, 1988). Further research would need to be done to assess if the particle size of breadfruit flour affects crust and crumb L* values.

b. a* Values

Results for crust a* values for all leavening and breadfruit flour treatments studied are shown in Table 2-6 and crumb a* results are shown in Table 2-7. Crust a* values ranged from 11.41 (BP1535) to 17.34 (BP20C) and crumb a* values from 0.74 (YC) to 11.80 (BP20C). The a* value is a measure of the degree of redness or greenness of a sample, ranging from -100 to +100 (Hutchings, 1994). A positive value indicates redness, and a negative value expresses greenness. A value of 0 is indicative of a grey sample (Kurimoto & Shelton, 1988). No significant effect ($p < 0.05$) on crust a* values was found for leavening or breadfruit inclusion. A significant effect ($p < 0.0001$) on crumb a* values was found for leavening as well as breadfruit flour inclusion. Crumb a* values were significantly different between all leavening treatments, with yeast leavened

bread having significantly lower a^* values (redness) than those leavened with baking powder. Crumb a^* values were also significantly different between breadfruit flour treatments, specifically between breadfruit flour levels 50% and 0% ($p < 0.0001$), 50% and 20% ($p < 0.0001$), and 50% and 35% ($p < 0.0005$). Breads containing 50% breadfruit flour had significantly lower a^* values (redness) than those containing lower amounts of or even lacking breadfruit flour. While results for the collection of samples exhibited positive values, overall, the values were close to zero, thus indicating a grey appearance.

Fiber content has been implicated in impacting a^* values of flour samples. Ash content—an indication of bran contamination in flour—has been correlated with flour color (Kim & Flores, 1999). A correlation coefficient of -0.20 was observed in this study between fiber and crust a^* values and 0.20 between fiber and crumb a^* values. Ramirez-Wong and others found significant differences in a^* values with variation in fiber content. Specifically, as the rate of extraction increased, the a^* values decreased (became more negative) (Ramirez-Wong et al., 2007). However, in the aforementioned study by Kurimoto and Shelton, samples of varying fiber content and particle sizes showed no significant change with respect to a^* values (Kurimoto & Shelton, 1988). Again, further research would need to be done to assess if the particle size of breadfruit flour affects crust and crumb a^* values.

c. b^* Values

Results for crust b^* for all leavening and breadfruit flour treatments studied are shown in Table 2-6 and crumb b^* results are shown in Table 2-7. Crust b^* values ranged from 17.76 (YC) to 39.53 (BP2020) and crumb b^* values from 21.25 (YC) to 35.64 (BP2020). The b^* value is a

measure of the degree of yellowness (positive values) or blueness (negative values) of a sample, ranging from -100 to +100 (Hutchings, 1994). A value of 0 is indicative of a grey sample (Kurimoto & Shelton, 1988). A significant effect ($p < 0.05$) on crust b^* values was found for both leavening and breadfruit flour treatments. Leavening treatment had significant differences ($p < 0.05$) between crust b^* values, specifically with yeast leavened breads having significantly decreased b^* (yellowness) compared to those leavened with baking powder. No significant difference ($p > 0.05$) was found between 15% baking powder and 20% baking powder. Significant differences were also found for breadfruit treatments, with 0% having significantly lower b^* values than both 35% and 50%. Inclusion of 20% breadfruit was additionally found to have significantly lower b^* values than 50%. A significant effect on crumb b^* values was found for both leavening and breadfruit flour treatment levels. Crumb b^* values (yellowness) were significantly lower for yeast leavened breads ($p < 0.0001$) compared to those leavened with baking powder. No difference was found between the two baking powder treatments. Crumb b^* values were also significantly different between breadfruit flour treatments, specifically between breadfruit flour levels 0% breadfruit flour and 20% ($p < 0.0001$), 35% ($p < 0.0001$), and 50% ($p < 0.0001$). Breads containing 0% breadfruit flour had significantly lower b^* values (yellowness) than those containing higher amounts of breadfruit flour. While results for the collection of samples exhibited positive values, overall, the values were below 50, thus indicating a paler yellow appearance.

In this study, correlation coefficients of 0.10 for crust b^* and 0.21 for crumb b^* were found; this indicates no correlation between fiber content and b^* values. Little research was found on the effect of fiber or flour particle size on color of a sample, specifically on yellowness and b^*

values. However, Kurimoto and Shelton noted a significant decrease as the sample particle size decreased, with a correlation coefficient of 0.99 ($p < 0.01$) (Kurimoto & Shelton, 1988). As previously stated, further research would need to be done to assess if the particle size of breadfruit flour affects crust and crumb a^* values.

The L^* , a^* , and b^* values together describe flour color, the dominant factor in determining crumb color. In fact, Pomeranz observed that flour color was correlated with crumb color with a coefficient of 0.987 (Pomeranz, 1960). As discussed within each attribute of color, flour is influenced by composition, and most notably freedom from bran particles (Pyler, 1988). Color, either in crumb or crust, is a central characteristic for acceptance of baked products (Sabanis, Lebesi, & Tzia, 2009).

Table 2-6 Crust L^* , a^* , and b^* Values of Gluten-Free Breads Made From Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

	L^*			a^*			b^*		
Flour Inclusion Level	Leavening Treatment			Leavening Treatment			Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%
0%	39.11 ± 6.63 ^{Aa}	42.74 ± 7.42 ^{Aa}	44.15 ± 10.59 ^{Aa}	15.08 ± 1.56 ^{Aa}	15.24 ± 1.31 ^{Aa}	14.84 ± 1.53 ^{Aa}	24.58 ± 6.03 ^{Aa}	30.11 ± 6.24 ^{Ab}	30.51 ± 6.23 ^{Ab}
20%	43.96 ± 5.14 ^{ABa}	43.92 ± 5.93 ^{ABa}	45.69 ± 6.94 ^{ABa}	15.67 ± 0.44 ^{Aa}	15.53 ± 1.28 ^{Aa}	15.04 ± 0.61 ^{Aa}	30.14 ± 2.63 ^{ABa}	32.39 ± 2.74 ^{ABb}	33.95 ± 3.45 ^{ABb}
35%	46.56 ± 5.90 ^{Ba}	50.89 ± 7.34 ^{Ba}	51.75 ± 7.23 ^{Ba}	15.62 ± 0.42 ^{Aa}	14.31 ± 2.29 ^{Aa}	14.09 ± 1.45 ^{Aa}	31.75 ± 3.34 ^{BCa}	34.78 ± 1.09 ^{BCb}	35.89 ± 2.00 ^{BCb}
50%	49.14 ± 5.02 ^{Ca}	53.36 ± 5.66 ^{Ca}	52.81 ± 1.78 ^{Ca}	15.70 ± ±	13.96 ± ±	14.76 ± 1.78 ^{Aa}	33.56 ± ±	36.03 ± 2.20 ^{Cb}	37.04 ± 1.58 ^{Cb}

				0.45 ^{Aa}	1.53 ^{Aa}		2.35 ^{Ca}		
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For each column, mean values with the same uppercase superscript are not significantly different within each variable (p>0.05).

For each row, mean values with the same lowercase superscript are not significantly different within each variable (p>0.05).

Table 2-7 Crumb L*, a*, and b* Values of Gluten-Free Breads Made From Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

	L*			a*			b*		
Flour Inclusion Level	Leavening Treatment			Leavening Treatment			Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%
0%	77.40 ± 0.97 ^{Aa}	61.85 ± 1.07 ^{Ab}	58.63 ± 2.17 ^{Ab}	0.96 ± 0.23 ^{Aa}	10.41 ± 0.54 ^{Ab}	11.23 ± 0.68 ^{Ac}	23.26 ± 1.32 ^{Aa}	33.75 ± 1.03 ^{Ab}	34.68 ± 0.36 ^{Ab}
20%	73.01 ± 0.76 ^{Ba}	60.70 ± 0.86 ^{Bb}	58.63 ± 2.06 ^{Bb}	2.62 ± 1.11 ^{Aa}	10.10 ± 0.31 ^{Ab}	10.40 ± 0.83 ^{Ac}	26.86 ± 1.11 ^{Ba}	34.66 ± 0.66 ^{Bb}	34.58 ± 0.78 ^{Bb}
35%	70.47 ± 0.53 ^{BCa}	62.28 ± 0.84 ^{BCb}	61.97 ± 0.85 ^{BCb}	3.94 ± 0.29 ^{Aa}	8.88 ± 0.33 ^{Ab}	9.02 ± 0.60 ^{Ac}	29.46 ± 1.21 ^{Ba}	34.50 ± 0.78 ^{Bb}	34.10 ± 0.61 ^{Bb}
50%	69.45 ± 0.77 ^{ACa}	63.93 ± 1.39 ^{ACb}	63.87 ± 0.93 ^{ACb}	4.29 ± 0.24 ^{Ba}	7.44 ± 0.61 ^{Bb}	7.95 ± 0.68 ^{Bc}	29.11 ± 0.67 ^{Ba}	33.71 ± 0.70 ^{Bb}	33.65 ± 0.66 ^{Bb}

For each column, mean values with the same uppercase superscript are not significantly different within each variable (p>0.05).

For each row, mean values with the same lowercase superscript are not significantly different within each variable ($p>0.05$).

C. Crumb Structure

a. Cell Diameter and Volume

Results for cell diameter and cell volume in breads produced with all breadfruit flours studied are shown in Table 2-8. Values for cell diameter ranged from 3.16 mm (YC) to 1.25 mm (BP1550). Values for cell volume ranged from 11.37 mm³ (YC) to 3.28 mm³ (BP2050). Leavening treatment had a significant effect ($p < 0.05$) on bread cell diameter with significant differences between yeast and 15% baking powder ($p < 0.033$), but not between yeast and 20% baking powder or 15% baking powder and 20% baking powder ($p < 0.858$). Leavening treatment had no significant effect on cell volume ($p < 0.858$). Control breads had significantly higher cell diameter and volume than all breads made from breadfruit flour. However, 20% breadfruit flour tended to have significantly larger cell diameters and volumes than those made with a higher percentage of breadfruit flour. Breads produced from 50% breadfruit flour had significantly lower cell diameter and volume than all other samples. Similarly, breads produced from yeast had a significantly larger cell diameter compared to those leavened with baking powder (15%, 20%).

In wheat bread, the extent to which cells are formed is a function of the protein-starch interactions (specifically from gluten) that provide viscoelastic properties to the dough. As gluten-free bread lacks the means necessary to produce such a network, another mechanism is utilized to form gas cells. Air cells, or alveoli, are created during mixing. Carbon dioxide, which

is produced as a byproduct of yeast fermentation, diffuses into these air cells, causing them to expand (Gan et al., 1995). Overall, a smaller cell diameter is indicative of a smaller cell volume. In fact, in the present study, the correlation coefficient between cell diameter and cell volume was 0.97 (Table 2-9). Quality white pan breads are characterized by small, elongated gas cells with thin cell walls (Hayman, Hosney, & Faubion, 1998). Smaller cells, whether defined by volume or diameter, are desirable in gluten-free bread products, as greater numbers of small gas cells have been found to produce loaves of higher specific volumes (Gallagher, Gormley, & Arendt, 2003). Larger cell diameters are typically indicative of gas cell coalescence. Ahlborn and others found that gas cell coalescence diminishes the presence of a web-like structure which, if achievable in gluten-free bread, improves both visual and eating properties of the product (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005). Cell diameter (0.79) and cell volume (0.72) were both found to positively correlate with specific volume indicating that, as both attributes increase, so does specific volume (Table 2-9).

As corroborated by results for specific volume and crumb firmness, the small cell diameter and volume noted for breads produced from breadfruit flour, especially when used at a higher percentage, are indications of the extreme density of the products. To further this hypothesis, Figures 2-5, 2-6, and 2-7 illustrate the poor crumb structure of breads produced from breadfruit flours at a higher level (50%) compared to a lower level (20%). These increased levels of breadfruit flour clearly resulted in a weak crumb structure that hindered gas cell formation resulting in dense loaves.

Table 2-8 Comparison of Cell Diameter and Volume in Bread Produced from Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

	Cell Diameter (mm)			Cell Volume (mm ³)		
Flour Inclusion Level	Leavening Treatment			Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%
0%	2.76 ± 0.26 ^{Aa}	2.23 ± 0.12 ^{Ab}	2.46 ± 0.13 ^{Aab}	8.87 ± 1.38 ^{Aa}	7.90 ± 0.58 ^{Aa}	9.12 ± 0.51 ^{Aa}
20%	1.95 ± 0.17 ^{Ba}	1.82 ± 0.14 ^{Bb}	1.80 ± 0.12 ^{Bab}	5.91 ± 0.79 ^{Ba}	6.08 ± 0.68 ^{Ba}	6.28 ± 0.41 ^{Ba}
35%	1.93 ± 0.10 ^{Ca}	1.52 ± 0.04 ^{Cb}	1.52 ± 0.08 ^{Cab}	5.94 ± 0.50 ^{Ca}	4.44 ± 0.25 ^{Ca}	4.62 ± 0.34 ^{Ca}
50%	1.54 ± 0.04 ^{Da}	1.36 ± 0.07 ^{Db}	1.40 ± 0.10 ^{Dab}	4.35 ± 0.30 ^{Da}	3.71 ± 0.28 ^{Da}	3.90 ± 0.50 ^{Da}

For each column, mean values with the same uppercase superscript are not significantly different (p>0.05).

For each row, mean values with the same lowercase superscript are not significantly different (p>0.05).

Table 2-9 Correlation Coefficients Between Key Crumb Structure Attributes

Variable	Cell Diameter	Cell Volume	Cells per Slice Area	Cell Wall Thickness	Loaf Volume
Cell Diameter	1	0.97	-0.09	0.94	0.79
Cell Volume	0.97	1	-0.10	0.93	0.72
Cells per Slice Area	-0.09	-0.10	1	-0.11	-0.08
Cell Wall Thickness	0.94	0.93	-0.11	1	0.75
Loaf Volume	0.79	0.72	-0.08	0.75	1

Figure 2-5 C-Cell (Top) and Volume (Bottom) Images. From Left: Y20, Y50

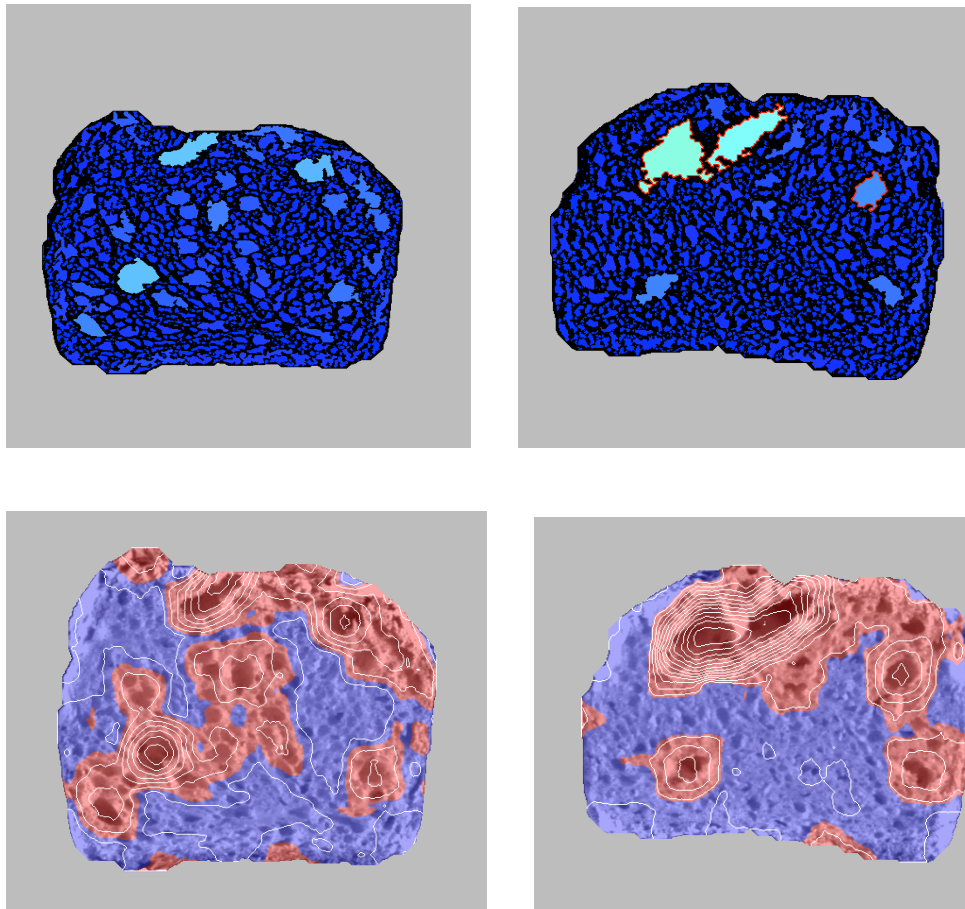
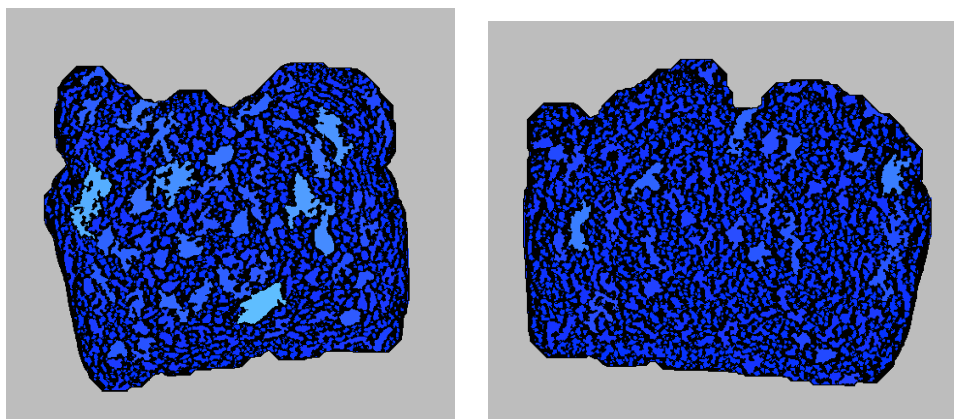


Figure 2-6 C-Cell (Top) and Volume (Bottom) Images. From Left: BP1520, BP1550



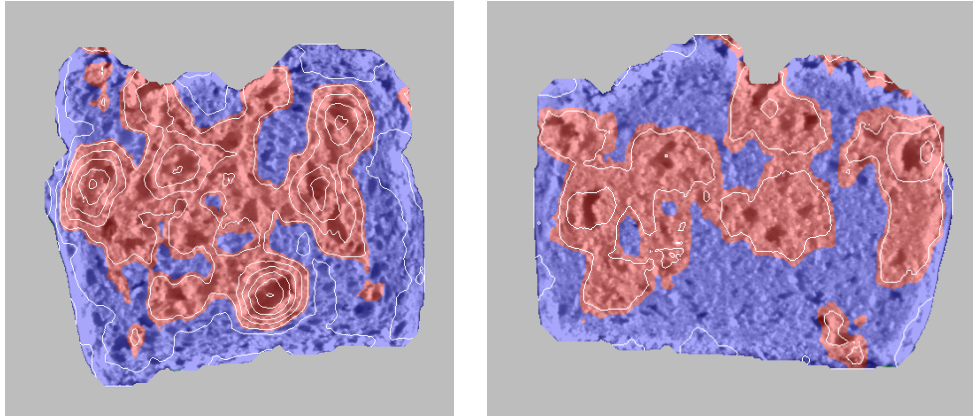
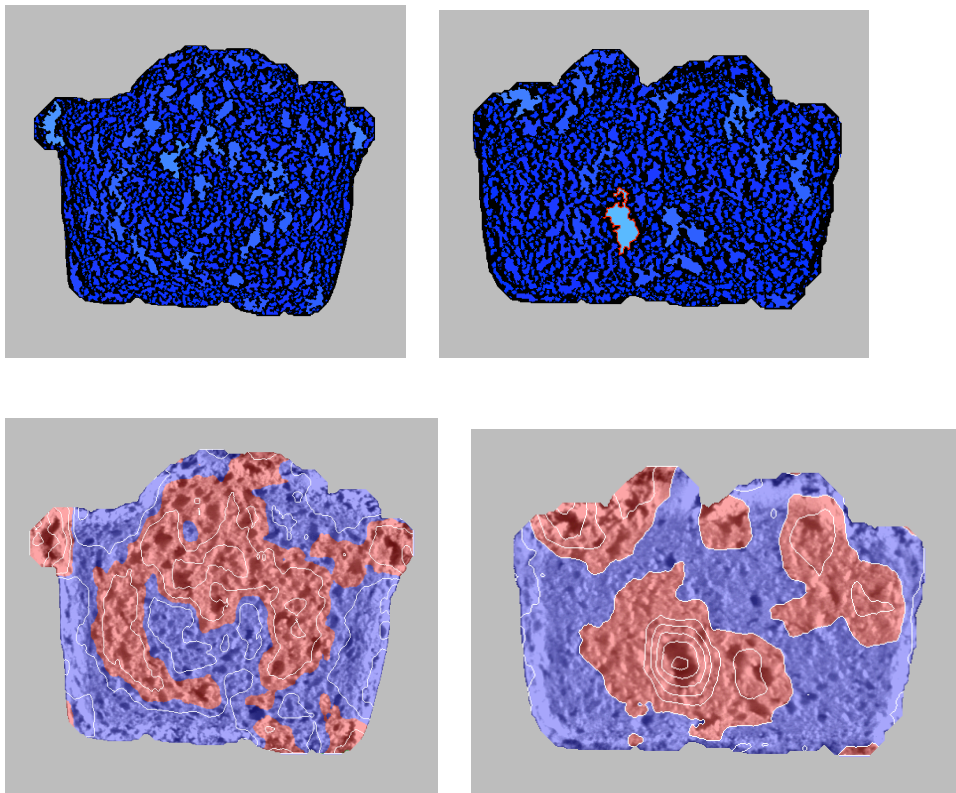


Figure 2-7 C-Cell (Top) and Volume (Bottom) Images. From Left: BP2020, BP2050



b. Cells Per Slice Area & Cell Wall Thickness

Results for measurements of cells per slice area and cell wall thickness in breads produced with all breadfruit flours and leavening treatments examined are shown in Table 2-10. Values for cells

per slice area ranged from 45.01 cells/cm² (YC) to 94.49 cells/cm² (BP1550). No significant effect was found for leavening or breadfruit flour level on cells per slice area. Values for cell wall thickness ranged from 0.393 mm (BP2050) to 0.538 mm (YC). A significant effect was found for leavening ($p < 0.0045$) as well as breadfruit flour level ($p < 0.001$) on cell wall thickness. Bread leavened with yeast had significantly thicker cell walls than both baking powder treatments (15%: $p < 0.0096$, 20%: $p < 0.0139$). All breads were found to have significantly ($p < 0.05$) thicker cell walls at all levels of breadfruit flour inclusion. Among all breadfruit flour levels, those produced with rice flour (control) had the thickest cell walls. Of the samples produced with breadfruit flour, breads with 20% breadfruit flour had significantly thicker cell walls than those with 35% ($p < 0.0083$) and 50% ($p < 0.0001$) flour.

The ratio of cells per slice area is calculated by dividing the number of cells in each slice by the total slice area. The measurement attempts to provide standardization for variations in specific volume per loaf. However, this standardization effect has a tendency to diminish visible quality differences and should not be taken out of context. For example, the ratio of cells per slice area for bread produced from breads (produced with rice flour) leavened with yeast, 15% baking powder, and 20% baking powder are not significantly different, indicating that the three breads do not differ in porosity. However, by examining Figure 2-8 it can again be seen that there are marked differences in crumb structure of the breads for each leavening treatment. As such, it is this researcher's opinion that cells per slice area is not an accurate determinate of crumb quality for this particular study. It seems to be more appropriate for evaluating breads that are expected to have similar overall crumb characteristics, but slight differences in number of cells or slice area.

Additionally, it should be noted that the measurement of cells per slice area may be able to provide some degree of insight into crumb structure. Variation in this ratio may be accompanied by a variation in cell wall thickness and cell diameter (and therefore, cell volume). In this study, the correlation coefficient between cells per slice area and cell wall thickness was -0.10 (Table 2-9); this is interpreted to mean that breads with thicker cell walls were not more likely to have a lesser amount of cells per standardized slice area, or vice versa. As such, it was concluded that cell diameter and cell volume are related to cells per slice area within the scope of this study. In the present study, correlation coefficients between cells per slice area and cell diameter and cell volume were both -0.09, thus cell diameter and cell volume are not correlated with cells per slice area. Also, cell wall thickness was found to correlate with cell volume and cell diameter; the coefficients being 0.93 and 0.94, respectively. Cell wall thickness has been shown to correlate with crumb grain character. Thin cell walls predominate in fine-grained, fine-textured crumbs, and thicker cell walls are typically found in coarse-grained crumbs (Hayman et al., 1998).

Table 2-10 Comparison of Cells Per Slice Area and Cell Wall Thickness in Bread Produced from Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

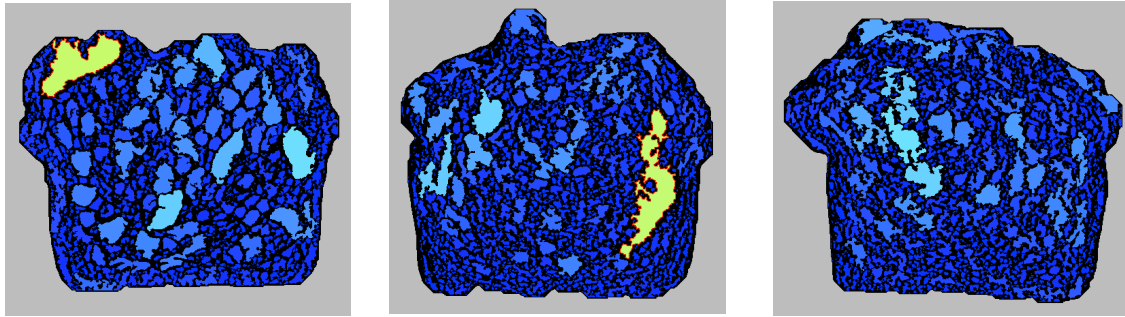
	Cells per Slice Area (cells/cm ²)			Cell Wall Thickness (mm)		
Flour Inclusion Level	Leavening Treatment			Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%
0%	69.81 ± 13.82 ^{Aa}	68.29 ± 15.22 ^{Aa}	64.18 ± 9.06 ^{Aa}	0.509 ± 0.015 ^{Aa}	0.489 ± 0.011 ^{Ab}	0.501 ± 0.007 ^{Ab}
20%	65.06 ± 12.70 ^{Aa}	64.21 ± 10.31 ^{Aa}	72.33 ± 15.15 ^{Aa}	0.469 ± 0.013 ^{Ba}	0.458 ± 0.011 ^{Bb}	0.453 ± 0.008 ^{Bb}

35%	70.71 ±13.05 ^{Aa}	75.19 ± 12.69 ^{Aa}	79.38 ± 14.71 ^{Aa}	0.471 ± 0.005 ^{Ca}	0.427 ± 0.007 ^{Cb}	0.423 ± 0.007 ^{Cb}
50%	76.91 ± 9.05 ^{Aa}	71.91 ± 17.03 ^{Aa}	65.77 ± 10.48 ^{Aa}	0.443 ± 0.005 ^{Da}	0.403 ± 0.006 ^{Db}	0.404 ± 0.008 ^{Db}
<p>For each <u>column</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).</p> <p>For each <u>row</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).</p>						

Table 2-11 Comparison of Slice Area and Number of Cells in Bread Produced From Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

	Slice Area (mm ²)			Number of Cells (mm)		
Flour Inclusion Level	Leavening Treatment			Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%	Yeast	Baking Powder 15%	Baking Powder 20%
0%	3524 ± 506 ^{Aa}	3146 ± 147 ^{Aa}	3191 ± 156 ^{Aa}	1790 ± 299 ^{Aa}	1861 ± 104 ^{Aa}	1696 ± 84.5 ^{Aa}
20%	2916 ± 276 ^{Ba}	2694 ± 175 ^{Ba}	2765 ± 112 ^{Ba}	1806 ± 98.5 ^{Aa}	1832 ± 106 ^{Aa}	1878 ± 116 ^{Aa}
35%	2577 ± 138 ^{Ca}	2272 ± 139 ^{Ca}	2160 ± 92.1 ^{Ca}	1681 ± 104 ^{Aa}	1816 ± 145 ^{Aa}	1745 ± 75.9 ^{Aa}
50%	2230 ± 165 ^{Da}	2063 ± 118 ^{Da}	1946 ± 78.2 ^{Da}	1755 ± 128 ^{Aa}	1840 ± 99.6 ^{Aa}	1718 ± 84.2 ^{Aa}
<p>For each <u>column</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05). For each <u>row</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).</p>						

Figure 2-8 C-Cell images. From left: YC, BP15, and BP20



D. Texture

a. Crumb Hardness

Significant differences were found ($p < 0.05$) in leavening and breadfruit flour level for hardness of bread slices (Table 2-13). Values ranged from 2.73 N (Y20) to 63.51 N (BP2050). Among leavening treatments, there was a significant effect on bread texture ($p < 0.0001$). Yeast leavened breads were significantly softer in crumb texture compared to baking powder treatments. Among breadfruit flour treatment levels, there was an overall significant effect ($p < 0.0001$) on crumb texture. Breads increased in crumb hardness as the level of breadfruit flour inclusion increased, Bread made from rice flour proved to have the softest crumb texture while bread containing 50% breadfruit flour had the hardest crumb texture.

Hardness is a textural attribute associated with bread crumb, and is defined as the resistance of the bread crumb to deformation (He & Hosney, 1990). Crumb firmness is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread freshness (Ahlborn et al., 2005). In white pan bread, most consumers prefer a soft, resilient, and short crumb (Pylar, 1988). As breads produced from yeast as well as lower inclusion levels of breadfruit flour had the softest crumb, these treatments are recommended for production of gluten-free breadfruit bread. While it seems that a use of rice flour would be most beneficial in producing high quality

gluten-free bread, the use of breadfruit flour at lower inclusion levels of 20% does yield high quality bread. Additionally, substitution of 20% breadfruit flour can serve as an extender in regions where shipments of wheat or gluten-free flours are infrequent.

Table 2-12 Comparison of Hardness of Crumb in Bread Produced From Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

Hardness (N)			
Flour Inclusion Level	Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%
0%	1.43 ± 2.14 ^{Aa}	2.24 ± 2.37 ^{Ab}	2.32 ± 3.91 ^{Ab}
20%	2.27 ± 6.98 ^{Ba}	3.03 ± 6.30 ^{Bb}	2.87 ± 7.13 ^{Bb}
35%	2.60 ± 2.79 ^{Ca}	3.40 ± 11.33 ^{Cb}	3.51 ± 10.01 ^{Cb}
50%	3.01 ± 6.86 ^{Da}	3.74 ± 9.38 ^{Db}	3.85 ± 11.60 ^{Db}
<p>For each column, mean values with the same uppercase superscript are not significantly different (p>0.05).</p> <p>For each row, mean values with the same lowercase superscript are not significantly different (p>0.05).</p>			

b. Effect of Protein Content on Crumb Firmness

Previous researchers have determined that firming of wheat bread crumb is influenced by numerous variables, including protein and fiber content, moisture, baking temperature, and loaf volume (Maleki, Hosoney, & Mattern, 1980; Moore et al., 2004; Ponte, Titcomb, & Cotton, 1962). Gluten-free bread has been shown to have much higher crumb firmness than other bread products. In a study by Ahlborn and others, crumb firmness values for gluten-free rice bread were four times higher than for standard wheat or low-protein starch breads (Ahlborn et al., 2005). In the present study, it appears that protein content had a somewhat significant effect on

crumb firmness. In a study on the incorporation of protein powders into gluten-free bread, Gallagher and others found that breads produced with more concentrated protein powders tended to have the firmest crumb compared to the control—produced with no additional protein. Additionally, Oh and others determined that internal firmness of cooked, hard wheat noodles was highly significant when correlated with protein content (Oh et al., 1985).

c. Effect of Fiber Content on Crumb Firmness

Increased fiber content is an outcome in flours with higher extraction rates. As such, the earlier reported observation from Ramirez-Wong and others showing an increase in firmness with an increase in extraction rate can partially be attributed to fiber content as well (Ramirez-Wong et al., 2007). Sabanis and others observed that fiber addition level significantly impacted crumb firmness of gluten-free bread at the $p < 0.0001$ level (Sabanis et al., 2009). Gomez and others also reported an increase in crumb firmness upon the addition of wheat fiber into wheat bread. The researchers cited an explanation for increased firmness based upon the possible thickening of the cell wall due to fiber content. Another possible explanation for increased firmness in bread crumb due to fiber is due to increased gelatinization temperatures (Gomez, Ronda, Blanco, Caballero, & Apesteguia, 2003). The addition of pea hull, lentil, and chickpea fibers were found to cause an increase in the gelatinization of wheat breads (Dalgetty & Baik, 2006; Santos, Rosell, & Collar, 2008). Higher gelatinization temperatures have been shown to have association with a higher degree of starch crystallization, which would increase bread firmness (Singh and others 2003).

d. Effect of Flour Particle Size on Crumb Firmness

Limited information exists on the effects of flour particle size and starch damage on the texture of bread, and especially gluten-free bread. In the present study, due to using a flour of consistent particle size and starch damage, the researcher could not effectively assess the how particle size or starch damage affects bread firmness. Hatcher and others noted that both particle size and starch damage influenced white salted noodle quality. More specifically it was found that flours with fine particle size produced noodles with more acceptable textural attributes than noodles produced from coarser flour. Finer particle size flours with higher degrees of starch damage may have experienced increased swelling, and therefore softening of the cooked noodles (Hatcher, Bellido, & Anderson, 2009). Flour particle size was also noted to be a major contributing factor to tortilla texture (L. Wang & Flores, 2000).

e. Effect of Loaf Volume on Crumb Firmness

Loaf volume also impacts crumb firmness. Sabanis and others noted a negative correlation between crumb firmness and loaf volume of -0.89 ($p < 0.05$). In the present study, the correlation between specific volume and firmness was -0.74 (Sabanis et al., 2009). Similar results have also been reported (Gallagher et al., 2003; He & Hosoney, 1990). In each of these studies, including the present one, loaves with lower specific volumes had denser and more tightly packed crumb structures, resulting in higher values for crumb firmness. Indeed, crumb texture is affected by the cell structure; finer, thin-walled, uniformly sized cells produce breads with a softer and more elastic texture (Hayman et al., 1998; Pylar, 1988).

E. pH

Significant differences were found ($p < 0.05$) for the effect of leavening treatment on pH of bread (Table 2-14). Values ranged from 5.24 (Y50) to 7.64 (BP20C). Yeast leavened breads were significantly lower ($p < 0.0001$) in pH compared to baking powder treatments. Baking powder treatments were not considered significantly different ($p > 0.05$) from each other. Among breadfruit flour treatment levels, there was no significant effect on bread pH.

The attribute pH is associated with leavening, and thus is a major factor selecting leavening agents to achieve optimal volume in bread. Holmes and others noted that yeast is relatively tolerant to pH changes, and obtained a substantial rate of gas production (80% of optimum or better) between pH ranging from 3.7-8.0. When yeast is subjected to high pH ($pH > 9.7$), its gas producing ability is impaired (Holmes & Hosoney, 1987). Bread volume is also affected by pH, with higher volume being achieved with increasing pH; this is due to increased gas production by yeast and thermal decomposition of NaHCO_3 in formulas where no leavening acid was added. Chemical leavening produced higher loaf volume when the final bread pH was 5.62 and less loaf volume at a pH of 7.04 (Holmes & Hosoney, 1987). The pH is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread flavor. In a study by Semić and others on sourdough bread, breads with a higher percentage of sourdough (lower pH) are aromatic, of a very strong scent and flavor, but consumers found them less acceptable and likeable (Semić et al., 2009).

Table 2-13 Comparison of Bread pH in Bread Produced from Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

pH	
Flour Inclusion	Leavening Treatment

Level	Yeast	Baking Powder 15%	Baking Powder 20%
0%	5.60 ± 0.24 ^{Ab}	7.16 ± 0.18 ^{Aa}	7.25 ± 0.29 ^{Aa}
20%	5.59 ± 0.15 ^{Ab}	7.11 ± 0.18 ^{Aa}	7.32 ± 0.23 ^{Aa}
35%	5.69 ± 0.14 ^{Ab}	7.26 ± 0.24 ^{Aa}	7.36 ± 0.19 ^{Aa}
50%	5.61 ± 0.23 ^{Ab}	7.16 ± 0.20 ^{Aa}	7.28 ± 0.14 ^{Aa}

For each column, mean values with the same uppercase superscript are not significantly different (p>0.05).

For each row, mean values with the same lowercase superscript are not significantly different (p>0.05).

F. Water activity

Significant differences were found ($p < 0.05$) for the effect of leavening treatment on water activity of bread (Table 2-15). Values ranged from 0.83 (BP20C) to 0.97 (YC). Breads leavened with 20% baking powder were significantly lower in water activity compared to breads leavened with 25% baking powder or yeast. Yeast leavening was not significantly different from 15% baking powder. Among breadfruit flour treatment levels, there was no significant effect on water activity.

Water activity is an attribute associated with bread quality and shelf life (Saldívar & Othón, 2012). Water activity and glass transition are used to define the role of water in quality baked goods, as it is strongly associated with consumers' perception of bread freshness. Water is crucial to the plasticizing and solvent transformations that occur in baked bread. The organoleptic properties of baked goods change during storage, mostly due to moisture migration, and result in the quality loss known as "staling". Staling, or retrogradation of starch, causes the crumb and crust of bread to increase in hardness (Cauvain, 2003). As previously discussed,

crumb hardness is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread freshness (Ahlborn et al., 2005). In white pan bread, most consumers prefer a soft, resilient, and short crumb (Pylar, 1988). Additionally, water activity is an important factor in food spoilage. Food products with water activities >0.90 – 0.93 are generally more subject to rapid bacterial spoilage than to fungal spoilage. Below 0.90 to 0.85 aw, only some bacteria (cocci, lactic bacteria) can still grow, and spoilage by yeasts and molds becomes predominant (Richard-Molard, Lesage, & Cahagnier, 1985). In this study, all samples were stored in airtight plastic bags at ambient temperature and observed over the course of fourteen days. At day 7, visible molds were observed on all treatment samples and served as the mode of failure for gluten-free breadfruit bread shelf life.

Table 2-14 Comparison of Water Activity in Bread Produced from Varying Inclusion Levels of Breadfruit Flour and Leavening Treatments

Water Activity			
Flour Inclusion Level	Leavening Treatment		
	Yeast	Baking Powder 15%	Baking Powder 20%
0%	0.933 ± 0.02 ^{Aa}	0.908 ± 0.02 ^{Aa}	0.876 ± 0.03 ^{Ab}
20%	0.910 ± 0.01 ^{Aa}	0.899 ± 0.01 ^{Aa}	0.885 ± 0.02 ^{Ab}
35%	0.904 ± 0.02 ^{Aa}	0.906 ± 0.02 ^{Aa}	0.895 ± 0.01 ^{Ab}
50%	0.920 ± 0.01 ^{Aa}	0.904 ± 0.01 ^{Aa}	0.891 ± 0.01 ^{Ab}
<p>For each column, mean values with the same uppercase superscript are not significantly different (p>0.05). For each row, mean values with the same lowercase superscript are not significantly different (p>0.05).</p>			

ii. Proximate Analysis

Figure 2-9 Nutrition Facts Panel for 100g of Breadfruit Flour from Genesis R&D

Nutrition Facts

Serving Size (100g)

Amount Per Serving

Calories 330 **Calories from Fat 5**

% Daily Value*

Total Fat 0.5g	1%
Saturated Fat 0g	0%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 5mg	0%
Total Carbohydrate 86g	29%
Dietary Fiber 15g	60%
Sugars 35g	

Protein 3g

Vitamin A 0% • Vitamin C 40%

Calcium 6% • Iron 10%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:

Fat 9 • Carbohydrate 4 • Protein 4

Figure 2-10 Nutrition Facts Panel for One Loaf (95 g) of YC Breadfruit Bread from Genesis R&D

Nutrition Facts

Serving Size (95g)

Amount Per Serving

Calories 250 **Calories from Fat 40**

% Daily Value*

Total Fat 4.5g	7%
Saturated Fat 2.5g	13%
Trans Fat 0g	
Cholesterol 40mg	13%
Sodium 380mg	16%
Total Carbohydrate 50g	17%
Dietary Fiber 2g	8%
Sugars 5g	

Protein 3g

Vitamin A 4% • Vitamin C 0%

Calcium 4% • Iron 2%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:

Fat 9 • Carbohydrate 4 • Protein 4

Figure 2-11 Nutrition Facts Panel for One Loaf (95g) of Y20 Breadfruit Bread from Genesis R&D

Nutrition Facts	
Serving Size (95g)	
Amount Per Serving	
Calories 250	Calories from Fat 45
% Daily Value*	
Total Fat 5g	8%
Saturated Fat 3g	15%
Trans Fat 0g	
Cholesterol 45mg	15%
Sodium 430mg	18%
Total Carbohydrate 51g	17%
Dietary Fiber 2g	8%
Sugars 7g	
Protein 3g	
Vitamin A 4%	• Vitamin C 0%
Calcium 4%	• Iron 4%
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.	
	Calories: 2,000 2,500
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	

Table 2-15 Proximate Analysis of Breadfruit Flour and Experimental Breadfruit Bread Treatments

	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)
Breadfruit Flour	7.35	3.24	0.45	31.93
YC	36.11	4.70	4.30	5.62
Y20	33.95	3.57	4.99	3.52
Y35	33.99	3.81	4.98	2.96
Y50	34.72	3.93	4.58	6.52
BP15C	33.04	3.94	5.00	2.13

BP1520	32.48	3.27	5.10	4.04
BP1535	33.09	2.59	4.63	12.30
BP1550	33.09	3.53	4.38	16.01
BP20C	30.72	4.01	4.62	12.14
BP2020	30.54	3.20	4.35	2.49
BP2035	32.8	2.52	4.7	14.84
BP2050	34.2	3.5	3.99	2.9

A. Moisture Content

Moisture content (Table 2-16) of the loaves was found to be highest in the control sample (YC) and lowest in formula BP2020. Water is crucial to the plasticizing and solvent transformations that occur in baked bread. The organoleptic properties of baked goods change during storage, mostly due to moisture migration, and result in the quality loss known as “staling” Staling, or retrogradation of starch, causes the crumb and crust of bread to increase in hardness (Cauvain, 2003). As previously discussed, crumb hardness is a key attribute in baked goods, as it is strongly associated with consumers’ perception of bread freshness (Ahlborn et al., 2005). In white pan bread, most consumers prefer a soft, resilient, and short crumb (Pylar, 1988).

B. Protein Content

Protein content (Table 2-16) of the loaves was found to be highest in the control sample (YC) and lowest in formula BP2035. Higher protein content is associated with increased elasticity improved gelatinization, increased loaf volume, improved crumb regularity, and increased

sensory characteristics, which is why protein is often added to gluten-free formulations (E.K. Arendt & Dal Bello, 2008; Gallagher et al., 2003; Moore et al., 2004). It should be noted that protein content reported from proximate analysis is almost equal to that generated for breadfruit flour (Figure 2-9) as well as formulas YC (Figure 2-10) and Y20 (Figure 2-11). This indicates that Genesis R&D can successfully predict the protein content of formulas containing breadfruit flour.

C. Fat Content

Fat content (Table 2-16) of the loaves was found to be highest in BP15C and lowest in formula BP2050. Lipids in baked goods serve multiple purposes including shortening, lubrication, aeration, help with heat transfer, extension of shelf life, as well as provide structure and desirable textural properties such as tenderness, richness, and improved mouth feel (Huschka, Challacombe, Marangoni, & Seetharaman, 2011). It should be noted that fat content reported from proximate analysis is almost equal to that generated for breadfruit flour (Figure 2-9) as well as formulas YC (Figure 2-10) and Y20 (Figure 2-11). This indicates that Genesis R&D can successfully predict the fat content of formulas containing breadfruit flour.

D. Fiber Content

Fiber content (Table 2-16) of the loaves was found to be highest in BP15C and lowest in formula BP2050. As previously discussed, fiber has been shown to affect volume as well as crumb firmness of gluten-free bread. Sabanis and others observed that fiber addition level significantly impacted crumb firmness of gluten-free bread at the $p < 0.0001$ level (Sabanis et al., 2009). Additionally, while there is a nutritional benefit to the incorporation of dietary fiber into gluten-

free products, this is limited by fibers effect of decreased loaf volume (H. Chen, Rubenthaler, Leung, & Baranowski, 1988; Krishnan, Pang, & Brown, 1987; Pomeranz, Shogren, Finney, & Bechtel, 1977; Sievert, Pomeranz, & Abdelrahman, 1990). It should be noted that fiber content reported from proximate analysis is not equal to that generated for breadfruit flour (Figure 2-9) as well as formulas YC (Figure 2-10) and Y20 (Figure 2-11). This indicates that Genesis R&D cannot successfully predict the fiber content of formulas containing breadfruit flour and proximate analysis should be used to assess fiber content of products made from breadfruit flour.

iii. Consumer Sensory Study

Out of 108 volunteers in the consumer sensory testing, 74 were females, 32 were males, and 2 did not identify a gender. The age of the panelists ranged from 18 to 80 years with 71.3% of the panelists belonging to the 18-25 age group. The general population can be divided into two distinct subgroups: 5 persons suffering from celiac disease, gluten allergy, or gluten sensitivity, and 103 persons not suffering from celiac disease, gluten allergy, or gluten sensitivity.

Out of the 103 panelists without celiac disease and/or gluten sensitivity, 70 were females while 31 were males. The age of these non-celiac panelists ranged from 18 to 80 years with 73% of panelists in the 18-25 age group. These consumers had widespread GF product consumption habits. About 40% of them claimed to never consume gluten-free products. Of the 60% remaining individuals, 17% indicated that they consumed GF products once a year, 24% once a month, 16% once a week and, finally, 4% of them claimed to eat GF items at least once daily. More than half of the non-celiac disease, gluten allergy, or gluten sensitivity population indicated that they consume GF products. This corroborates with the report from the NPD Group, which

indicated that some consumers eat GF products for the supposed wholesomeness of that type of food (NPD Group, 2013). Within the 5 panelists having celiac disease, 4 were females and 1 was male. The age of these celiac panelists ranged from 18 to 55. For the population suffering from celiac disease, gluten allergy, or gluten sensitivity, 40% indicated they consumed GF products once a week while the remaining 60% consume them daily.

Table 2-17 presents the average scores from the consumer study for the general population, comprised of both celiac and non-celiac panelists, of 108 panelists. As previously described, the treatment most preferred during preliminary experimentation (Y20) was tested against the control to see if panelists preferred one bread more than the other in the various sensory categories described. Significant differences ($p < 0.05$) were found for most sensory parameters tested. YC was found to be significantly different ($p < 0.0001$) in overall acceptability compared to Y20, with mean YC scores being 1 point higher than Y20. YC was also found to be significantly different in appearance ($p < 0.05$) compared to Y20, with mean YC scores being 0.45 point higher than Y20. Likewise, YC was found to be significantly different ($p < 0.05$) in color compared to Y20, with mean YC scores being 0.45 point higher than Y20. YC was found to be significantly different ($p < 0.0001$) in flavor compared to Y20, with mean YC scores being 1.81 point higher than Y20. Similarly, YC was found to be significantly different ($p < 0.05$) in aftertaste compared to Y20, with mean YC scores being 0.85 point higher than Y20. There was a significant difference ($p < 0.0001$) in likelihood to purchase; YC had a higher mean score by 1.96 point. Despite YC scoring significantly better than Y20 in most categories, there was no significant difference in texture likability between the two breads. This observation contests the observations we made during the crumb texture analysis: bread made with rice flour was found

to be significantly softer than bread made with any treatment level of breadfruit flour. It is important to note that the significant difference in crumb hardness does not affect likability of these two breads by consumers. Both YC and Y20 had good overall acceptability (YC: 6.46 out of 9, Y20: 5.42 out of 9) considering that these products were GF. Though YC scored well in likelihood to purchase (6.52 out of 9), the same cannot be said for Y20 (4.56 out of 9). In order for a product to be launched on the market, it generally has to score an average of 7 or more for overall acceptability (Lawless & Heymann, 1999). However, this may not necessarily apply to launching of gluten free foods.

High variation was observed for most of the parameters. According to Lawless and Heymann, difference in perception of sensory parameters is often an issue with untrained panelists testing (Lawless & Heymann, 1999).

When comparing scores from panelists suffering from celiac disease, gluten allergy, or gluten sensitivity to the scores of non-suffering panelists (Table 2-18), it is noticeable that the two categories perceive sensory parameters in a different manner. Statistically significant comparisons between the two groups were attempted despite the small size of the group who identified as suffering from celiac disease, gluten allergy, or gluten intolerance ($n = 5$), keeping in mind that the findings may not be reproducible. Overall acceptability and appearance of the control (YC) and experimental (Y20) was significantly different for non-suffering consumers ($p < 0.05$), but no significant difference between the two breads was found for consumers suffering from celiac disease, gluten allergy, or gluten sensitivity. Mean scores for overall acceptability as well as appearance were higher for YC in non-suffers, while scores were higher for Y20 in

suffering consumers. Flavor was significantly different ($p < 0.0001$) between YC and Y20 for non-sufferers but not significantly different in suffering consumers; mean flavor scores for YC were higher among non-sufferers while among sufferers Y20 had a higher mean score. Texture was not found to be significantly different between YC and Y20 for neither sufferers nor non-sufferers. Mean texture scores were higher for YC among sufferers, while Y20 had higher a mean score among non-sufferers. Color was found to be significantly different ($p < 0.05$) between YC and Y20 for non-sufferers; sufferers found no significant difference between the two breads color. Non-sufferers had a higher mean color score for YC and sufferers had a higher mean score for Y20. Aftertaste was significantly different ($p < 0.05$) for non-sufferers but not significantly different in suffering consumers; mean aftertaste scores for YC were higher among non-sufferers while among sufferers Y20 had a higher mean score. Likelihood to purchase was found to be significantly different between YC and Y20 among non-sufferers, but not significantly different among sufferers. For non-suffering panelists, the mean likelihood to purchase score was of higher for the control roll, while for sufferers, the mean score was higher for Y20. A conclusion of this comparison is that the 5 suffering panelists were not a large enough sample size for significant differences to be determined and thus, do not give a good representation of which bread would be preferred by people suffering from celiac disease, gluten allergy, or gluten sensitivity.

Based on the entire population (108 panelists) surveyed, sensory testing revealed that the control (YC) was preferred over the experimental treatment (Y20) in overall acceptability, appearance, color, flavor, aftertaste, and likelihood to purchase. Neither YC nor Y20 bread was preferred over the other in the category of texture. In their comments consumers often stated that they

preferred that control (YC) because it most resembled conventional gluten-containing bread.

Hence, it can be inferred that breadfruit containing bread would not sell better than current GF breads available on the market, especially considering rice flour is the main component of these commercially available GF breads.

Table 2-16 Response of General Panelist Population to Control (YC) and Experimental Breadfruit Treatment (Y20)

	Overall Acceptability	Appearance	Color	Flavor	Texture	Aftertaste	Likelihood to Purchase
Control (YC)	6.46 ± 1.67A	6.72 ± 1.70A	6.75 ± 1.60A	6.51 ± 1.81A	6.12 ± 2.06A	6.01 ± 2.01A	6.52 ± 3.22A
Breadfruit Treatment (Y20)	5.42 ± 2.16B	6.27 ± 1.68B	6.30 ± 1.58B	5.34 ± 2.31B	5.92 ± 2.11A	5.16 ± 2.25B	4.56 ± 3.60B
A-D Values with a common uppercase letter are not significantly different (p>0.05)							

Table 2-17 Comparison of Response of Celiac Disease, Gluten Allergy, or Gluten Sensitivity Suffering and Non-Suffering Panelist Populations to Control (YC) and Experimental Breadfruit Treatment (Y20)

	Consumers <u>Not</u> Suffering from Celiac Disease, Gluten Allergy, or Gluten Sensitivity		Consumers <u>Suffering</u> from Celiac Disease, Gluten Allergy, or Gluten Sensitivity	
	Control (YC)	Breadfruit Treatment (Y20)	Control (YC)	Breadfruit Treatment (Y20)
Overall Acceptability	6.50 ± 1.64A	5.34 ± 2.15B	5.60 ± 1.95C	7.00 ± 1.73C

Appearance	6.75 ± 1.72A	6.25 ± 1.67B	6.00 ± 1.22C	6.60 ± 2.07C
Color	6.77 ± 1.60A	6.26 ± 1.59B	6.40 ± 1.82C	7.00 ± 1.41C
Flavor	6.56 ± 1.79A	5.27 ± 2.29B	5.60 ± 2.19C	6.80 ± 2.39C
Texture	6.17 ± 2.07A	5.86 ± 2.09B	5.20 ± 1.92C	7.00 ± 2.55C
Aftertaste	6.06 ± 2.02A	5.11 ± 2.21B	5.00 ± 1.41C	6.20 ± 3.03C
Likelihood to Purchase	6.63 ± 3.19A	4.46 ± 3.59B	4.20 ± 3.35C	6.60 ± 3.58C
A-D Values with a common uppercase letter are not significantly different (p>0.05)				

III. Conclusion

Overall, this research demonstrates that breadfruit flour inclusion level affects the quality of gluten-free bread. To an extent, breads with lower amount of breadfruit flour contain lower amounts of fiber, which will produce breads with more acceptable characteristics, including volume, crumb structure, color, and texture. However, it is important to note that these flour characteristics do not exert their influences independently of one another. Leavening treatment was also found to greatly affect how these characteristics develop. In fact, this research highlights to the importance in understanding the impact of starch damage, particle size, and fiber content on bread performance. Additionally, sensory attributes inherent to breadfruit bread need improvement to be competitive with commercially available GF breads. Even though the number of sufferers who evaluated the bread was only 5 people, it was very promising to note their preference was higher for the breadfruit at 20% inclusion than the control. This result needs to be confirmed with a higher number of panelists who suffer from celiac disease, gluten allergy,

or gluten sensitivity. Ultimately, breadfruit flour holds promise for use as a flour extender in the food insecure regions where it is grown. Breadfruit flour can be successfully used or substituted at 20% or less inclusion for wheat or gluten-free flours. Because breadfruit flour has an inherently high fiber content, which is detrimental to loaf quality, it may be ideal for use as a fiber supplement rather than as flour. The information from this study may assist the breadfruit industry in producing value-added gluten free breadfruit products, but will ultimately benefit millers who have yet to solidify methods for milling breadfruit flour.

Chapter 3 - Recommended Future Work

While foundations for studying gluten-free breadfruit bread have been laid, questions still remain unanswered in the search for an increasingly acceptable product. First, growers of breadfruit need to employ a method to quantitatively determine the ripeness of breadfruit. Ripeness of fruit is generally determined by measuring the amount of soluble solids (Brix value) throughout the growing process. It is recommend that breadfruit growers identify and utilize such a method to track fruit ripeness in order to harvest the fruit at a consistent stage of growth. Consistent harvesting will yield a more consistent flour post milling.

Second, the milling of breadfruit flour should be extensively studied and further honed to produce and exceptional quality flour. As seen in this study, qualities of the flour such as particle size, starch damage, and fiber content were possible causes for lower quality of gluten-free breadfruit bread. In order for quality valued-added breadfruit products to be produced and sold commercially, consistent high-quality breadfruit flour will first need to be commercially milled.

One of the main outstanding issues is staling; breadfruit breads stale more than twice as quickly as wheat bread (Hugo, Waniska, & Rooney, 1997). Investigating a delay in staling is essential in order for production of gluten-free breadfruit breads to become commercialized as opposed to daily home baking. To this note, work is moving forward to investigate how milling of breadfruit flour can improve baking quality, including a softer crumb structure and resistance to staling (Eleyinmi & Fashakin, 2011).

Additionally, the results of this study suggest that breadfruit flour with low fiber content may be favored for the production of gluten-free bread with acceptable volume. However, there are concerns about the sufficient incorporation of fiber into the gluten-free diet, as it is often filled with starch-based products lacking in complex carbohydrates and dietary fiber (Thompson, Dennis, Higgins, Lee, & Sharrett, 2005). Indeed, tracking of adults with celiac disease that follow a gluten-free diet has shown a lower daily intake of fiber than is recommended (Grehn and others 2001). As such, the incorporation of fiber into gluten-free bread would be invaluable to the celiac consumer. Since breadfruit flour was found to contain 30% crude fiber, it may serve well as a fiber supplement versus as flour. Much headway has been made in the baking industry with wheat bread formulations that include soluble fibers, such as fructooligosaccharides or resistant starches, and these technologies, along with the fiber found in breadfruit flour, should be investigated for the development of gluten-free breadfruit bread.

Finally, further research should be done to assess if consumers who identified as sufferers of celiac disease, gluten sensitivity, or a gluten allergy truly prefer gluten-free bread, produced with breadfruit flour, over conventional gluten-free bread. The present study indicated a preference may exist, but due to the small number of suffering panelists (n=5), this preference cannot be statistically validated.

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Appendix A

INFORMED CONSENT STATEMENT FOR CONSUMER SENSORY ANALYSIS OF GLUTEN FREE BREAD

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

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

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

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

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

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

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

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

SIGNATURE: _____

DATE: _____

Appendix B

CONSUMER PRE-SCREENING FORM FOR GLUTEN FREE BREAD

Please complete the information below:

Age:

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

Gender:

- Male Female

Education Completed:

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

Do you suffer from any food allergies?

- Yes No

How often do you consume bread products?

- Daily About once a week About once a month About once a year Never

How often do you consume gluten-free products?

- Daily About once a week About once a month About once a year Never

How often do you consume gluten-free bread products?

- Daily About once a week About once a month About once a year Never

If you have any food allergies besides a gluten allergy or intolerance, you cannot participate in this study. Thank you for your willingness to help.

Appendix C

CONSUMER BALLOT FOR GLUTEN FREE BREAD

Panelist # _____

Instructions:

You will be testing two samples of gluten-free bread. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Additional comments are highly encouraged and may be written on the back of this sheet. Take a drink of water and/or bite of cracker before you start and as needed throughout testing.

SAMPLE: 626

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

1. Please rate your overall acceptability of this sample

Dislike	Neither						Like	
Extremely	Like nor Dislike						Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

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

Dislike	Neither						Like	
Extremely	Like nor Dislike						Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

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

Dislike	Neither						Like
Extremely	Like nor Dislike						Extremely

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

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

Dislike	Neither						Like	
Extremely	Like nor Dislike						Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

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

Dislike	Neither						Like	
Extremely	Like nor Dislike						Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

6. How much do you like or dislike the aftertaste of this sample?

Dislike	Neither						Like	
Extremely	Like nor Dislike						Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

7. Is this a product that you would consume/purchase?

Yes No Unsure

Additional Comments: _____
