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High Protein Rice Flour in the Development of Gluten-Free Muffins and Bread

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HIGH PROTEIN RICE FLOUR IN THE DEVELOPMENT OF GLUTEN-FREE MUFFINS AND BREAD

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ABSTRACT

Gluten sensitivities and intolerance disorders such as Celiac disease are being diagnosed more every day which has opened a huge market for gluten-free foods that is expected to reach nearly $7.6 billion by 2020. About 30% of the US population says they are trying to cut-down on or avoid gluten completely, with only 7% doing so for medical reasons. Although there are gluten-free alternatives available to use in cooking and baking, simple substitution of these flours for wheat does not produce acceptable texture characteristics. Gluten-free baked goods suffer from being low in nutritional value such as protein and fiber content while having a high glycemic index, which is driving the need for further research. Rice is the third most globally produced cereal and is being used regularly in gluten-free product development due to its hypoallergenicity, white color, bland taste, and easy digestibility. Rice is not a good source of protein with an average of only 6.4 percent per serving. New rice varieties are being cultivated to yield higher protein contents and overall nutritional value. The purpose of this study was to survey products in the market that contain rice flour as a predominant ingredient and replacement for wheat, in order to determine which products to focus on re-formulating with higher protein rice flour.

Grocery stores in the Baton Rouge, Louisiana area ranging from low-cost to premium were examined and product details were documented and analyzed. A total of 393 products among 5 stores listed rice flour as a primary ingredient and most were certified gluten-free. The most common product types were chips, crackers, cookies, and cakes. Thirty-five percent of the items surveyed contained one to three different gums. Sorghum flour, rice starch, potato starch, tapioca starch, and/or cornstarch were used in 60% of products in addition to rice flour. Pasta and protein bars were among the most protein dense foods with averages of 5.03 and 4.77 grams of protein per serving. There is a need for acceptable gluten-free foods that are more nutrient rich, especially with protein. Results indicated that baked goods should be the focus for development of higher protein rice flour-based products.

High protein rice flours were compared to commercial rice flours containing regular protein levels. Flour analyses included pasting properties by RVA, starch, protein, fat, and fiber content. Muffins and bread were prepared with standard bakery ingredients, a hydrocolloid, and combined in a specific manner to achieve a desirable texture. Color and texture analyses were conducted on products made with high protein rice flours and compared to commercial brown rice flour. A sensory study was done with consumers who were asked to rate their liking of the appearance, aroma, texture, and taste of the products. The only difference between the muffins was the type of rice flour used. The brown and white high protein rice flours were found to have protein contents of 8.43% and 7.12%, respectively. Results of the sensory study favored products made with high protein rice flour over commercial brown rice flour. Utilization of rice flour with greater protein content may result in comparable flavor and texture characteristics to traditional rice flour baked goods while also adding nutritional value in terms of protein for gluten-free foods.

CHAPTER 1. INTRODUCTION

Gluten sensitivity, intolerance, and autoimmune disorders such as Celiac Disease (CD) are becoming increasingly common in the United States. The only way to avoid the uncomfortable and sometimes very painful symptoms is to avoid gluten completely. Since there is still limited scientific research on this subject, there is no cure for the disease. Wheat, barley, and rye are major sources of gluten and cause damage to villi in the small intestine in people who have Celiac. Although only 1% of the population has been diagnosed with CD, it is estimated that for every person diagnosed, 5-10 people remain undiagnosed (Jones A 2017). Nonceliac gluten sensitivity presents similar signs and symptoms as Celiac Disease making it difficult to accurately diagnose and quantify its prevalence.

Understanding the pasting properties of starches helps to predict the final cooked quality of many different products. The changes in viscosity of aqueous starch mixtures as a function of time and temperature is how pasting values are quantified. Peak and final viscosities are important values to consider in product development to determine how processing may affect the final product (Shanthi K 2013). Wheat's high pasting temperature, minimal breakdown, and low final viscosity explain how the dough structure changes when being cooked/baked. When gluten-free alternatives are used in baking to replace wheat, pasting properties are drastically different. A much harder, denser, and less elastic structure is typically the result and consumer acceptability is difficult to achieve. According to Innova, "gluten-free" was among the top five claims made in 2018 on global new product launches of grain-containing food products. The availability of acceptable gluten-free alternatives for persons looking for more options has prompted an increase in research and development in this area.

Rice has some appealing characteristics that make it a suitable and widely used ingredient in gluten-free products. However, compared to gluten in wheat, rice proteins have poor functional properties in gluten-free formulations and encourages inclusion of hydrocolloids or gums to help batter rheology (Hager A, et al 2013). There are many different types of rice such as brown, white, long- or medium-grain that can be milled to flour and used in food applications. Rice generally has a high dietary glycemic index (GI) due to the nature of its composition being predominantly carbohydrates. Foods with a high glycemic index are said to be digested fast and increase blood glucose levels thus insulin secretion is also increased (Cheng Xue, et al 2017). Genetic breeding and cultivation of rice varieties to increase nutritional value are being grown and harvested for consumer use. Specifically, rice with a greater protein content would have a lower carbohydrate content thus a lower glycemic load. According to IRI, an American market research company, protein continued to be a driving force for new products in 2017.

A consumer study of product acceptability is a useful method to determine if specific characteristics of a product do or do not meet consumer standards. Attributes such as sweetness, overall liking, softness, taste, and moistness are critical in sensory discriminating (Jack A 2016). Information gathered from these studies can help guide development in the right direction toward success in the marketplace. Analysis and application of high protein rice flours could contribute to large-scale use in food products that may aim to increase nutritional value in gluten-free baked goods, naturally. The primary objectives of this research were (1) to survey the availability of rice flour based products in Baton Rouge grocery stores (2) to analyze a newly developed rice cultivar that was bred to have a higher protein content than traditional rice (3) to develop and analyze gluten-free muffins and gluten-free bread made with various rice flours (4) to determine consumer acceptability and purchase intent of GF muffins and bread. Analysis and application of high protein rice flours could contribute to large-scale use in food products that may aim to increase nutritional value in gluten-free baked goods.

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CHAPTER 2. LITERATURE REVIEW

2.1. General Introduction

2.1.1. Rice

Rice is a widely consumed food, being a major component of the most populations standard meal, some cultures use rice as a primary caloric source (Asmeda R, et al 2016). Rice (*Oryza sativa* L.) was domesticated between 8,000 to 10,000 years ago (Greenland 1997). Currently, rice is the staple food for more people than wheat, 90% of total rice production is grown and consumed in Asian countries (Fairhurst T, et al 2002). Rice is from the genus Oryza and contains about 21 species, the two most cultivated species are *Oryza sativa* and *Oryza glaberrima* (Kennedy G, 2003; USDA ERS, 2019). *Oryza sativa* originated in Southeast Asia, and *Oryza glaberrima* originated in West Africa. Currently nearly all rice varieties grown originate from *Oryza sativa* (USDA ERS, 2019). Oryza sativa can be divided into three subspecies, Indica, Japonica and Javanica. Indica and Japonica subspecies are the most common (Birla D, et al 2017). Sweet glutinous ("sticky") and non-glutinous varieties exist for all subspecies. The amylose content is a major factor in the stickiness of rice. Rice with low amylose content is considered sticky. As amylose content increases, the rice's stickiness decreases, and it becomes firmer. Japonica varieties have lower amylose content when compared to Indica rice varieties (FAO, 2000). According to the USDA ERS (2019), Five products can be produced from rough rice, these are hulls, bran, brown rice, whole kernel milled rice, and brokens. Brokens contain two categories which are second heads, that are used for flour, and brewers, are used for beer and in pet food.

2.1.2. Rice flour as a food ingredient

Rice flour is a type of flour made from milled rice, and it is used as an alternative to wheat flour. Rice flour has positive rheological benefits such as thickening and inhibiting liquid separation (Eke-Ejiofor J, et al 2016). There has been an increase in rice flour production (FAOSTAT, 2012; Asmeda R, et al 2016) for use in gluten-free foods. Rice flour is colorless in food matrices, has low sodium levels and does not contain gluten. Rice flour is currently used to make noodles and infant foods (Vongsawasdi P, et al 2009; Chou C, et al 2014).

2.1.3. Muffins

Johnson (1990), evaluated muffins containing waxy rice flour at 5, 15, and 25%, they found that the rice flour samples were "moderately close" or "very close" to wheat flour samples by sensory panel. Johnson (1990) also found that physically all rice flour products retained more moisture than wheat flour controls. Matos and others (2014) established the function of proteins on the rheological and quality properties of rice-based gluten free muffins, finding that protein type determines the rheological properties of rice muffin batter, and in the finished product. They used 6 rice-based formulations, one without added protein and five formulations with different protein sources: soy protein isolate, pea protein isolate, egg white protein, casein, and wheat gluten. Johnson stated that where muffins quality was concerned, egg white protein increased the height and specific volume. Where texture was concerned, pea protein isolate containing muffins were the softest and most springy and casein provided the hardest muffin. Muffins with best visual appearance were those containing egg white protein or casein. This research indicated that the source of protein is imperative to ensure proper texture in rice flour products.

2.1.4. Bread

The texture and other physicochemical properties of whole rice bread was evaluated by Kadan and others (2001) and they concluded that rice breads had less specific volume, harder texture, and were more prone to retrogradation during storage than whole wheat bread. From this study it can be surmised that gluten replacements such as hydrocolloids need to be added to rice breads to increase texture quality (Kadan R, et al 2001). The rheological properties of rice dough for making rice bread with hydroxypropyl methylcellulose (HPMC) added as gluten substitute was evaluated. It was found that rheological measurements from oscillation tests and creep tests of rice dough with 1.5% and 3.0% HPMC were similar to that of wheat flour dough and was acceptable for making rice bread (Sivaramakrishnan H, et al 2014).

2.2. Gluten-free movement

Between 2004 and 2011 the gluten-free market grew at an annual rate of 28% (Gaesser G, et al 2012). The FDA effectively issued a final rule on voluntary gluten-free labeling in 2014 providing standardization of the term "gluten-free" as <20 ppm of gluten (FDA, 2015). Many consumers feel that avoiding gluten can improve cholesterol levels, promote digestive health, and increase energy levels. In 2013 the NPD (National Purchase Diary market research group) cited that 30% of Americans showed interest in avoiding gluten (Jones, 2017). A 2013 study reported 65% of American adults believed gluten-free foods were healthier; 27% chose GF to assist in losing weight (Gaesser G, et al 2015; Jones A 2017). While 44% of patients with celiac disease choose rice if they include a grain or starch choice at meals (Lee A, et al 2009).

2.3. Development of gluten-free foods

An overview and considerations for development of gluten-free foods was published by Jnawali and others in 2016. The key take away was that gluten-free products available in the market have low nutritional quality while being more expensive than gluten-containing food products. From this study it is clear that there is a strong need to develop gluten-free products that are nutritionally complete as well as economical. Jnawali proposed that during the developing gluten-free products it was imperative to find an alternate non-gluten source, ensure nutrition and sensory quality attributes, while maintaining compliance with regulatory guidelines (Jnawali P, et al 2016).

2.4. Benefits of high protein and low glycemic load foods

Pfeiffer and others (2006) researched the biofortification of staple food crops and found that it is technically feasible to enrich the nutrition of staple crops via plant breeding without compromising agricultural productivity. Concern about the high global prevalence of nutrient malnutrition, has led to a new focus on the nutrient density of staple crops. Nutritional genomics is used to influence the synthesis of plant compounds that have nutritional value (Tian L, et al 2001; Kennedy G, et al 2003). The benefits of a high protein rice are increasing the protein intake of people that depend on rice as a staple food (Wenefrida I, et al 2013). High protein rice has an average protein content of 10.6%, which is about a 50% increase from its original content. High protein rice also needs less heat, time, and less water to cook, these are all economically pleasing factors (Wenefrida I, et al, 2013). Proteins affect the quantity of water rice absorbs in cooking, while the availability of water during cooking determines the hydration of protein in the rice, the concentration of the dispersed and viscous phase of the starches in rice determine the texture of the cooked rice displays (Martin M, et al 2002).

Diets with low glycemic index value play a role in the prevention of coronary heart disease in diabetic and healthy subjects. In obese or overweight subjects, low-glycemic index meals increase satiety and control of food intake. There is also correlation between selecting low glycemic index foods increased postprandial glucose and lipid metabolism in healthy subjects (Rizkalla S, et al 2002). Among consumers who shopped in the natural foods channel, 47% shopped at Trader Joe's and 52% shopped at whole foods (Packaged Facts 2018a). This information can be attributed to the high availability and marketing of "healthy," "natural," or "nutritious" products at these stores. Food trends continue to focus on healthy and natural foods that can offer more nutrition such as fruit smoothies or snacks with increased protein and fiber.

2.5. Market availability

In a report providing a forecast and analysis of the global rice flour market by Transparency Market Research (2018) it was cited that North Americans and Europeans are consuming pre-gelatinized or gluten-free flour over other flours due to rising health responses. Global sales of rice flour are valued as US$ 712.9 Million in 2017. The market is expected to moderately grow at a Compound Annual Growth Rate (CAGR) of 4.4% over the forecast period, achieving a value of US $1,003.1 million by 2025 (Transparency Market Research, 2018). Rice flour is widely available in supermarkets across America. High-protein rice is currently marketed as "Cahokia" rice and is grown commercially in Illinois (Wenefrida I, et al 2013).

In a study of the perspectives of super market retailers on healthy food retail strategies by Martinez and others (2018), it was concluded that for people in higher-income neighborhoods, access to the healthier substitutes recommended for a healthy diet was relatively easy due to their access to a supermarket that made these items available. Results revealed that almost all supermarkets in higher income areas (Los Angeles, CA) stock a variety of recommended substitutes. But in small independent grocery stores, usually those found in low-income neighborhoods, there was a disparity and often these low-income stores did not have high macronutrient options or healthier food options. The items were either “never available”, or “available only some of the time”. Within these lower income neighborhoods, people who lack transportation may not have consistent access to healthier foods. From this study it can be seen that healthy is a relative term based on socioeconomic status, education and market availability.

2.6. Limitations for developing rice-based gluten-free foods

Physically and texturally rice flour products have less specific volume, harder texture, and are more prone to retrogradation during storage when compared to whole wheat bread (Kadan R, et al 2001). Another challenge to the growth of the global rice flour market is the price volatility of grains which is largely caused by climate changes. Changes in climate and seasonal variations greatly affect crops yields in many regions causing variations in input and output prices of rice flour (Transparency Market Research, 2018). The rice flour market should find efficient operational solutions to secure long-term viability of rice flour (Transparency Market Research, 2018). Although an estimated 26% of Americans believe gluten-free products are healthier, evidence supporting this claim and the effectiveness of a gluten-free diet in weight management is limited (El Khoury D, et al. 2018). Consumer acceptance and attitudes towards gluten-free items have some negative responses due to their expensive prices and inadequate availability. Consumers may be relatively satisfied with the taste and texture of available gluten-free products, but efforts to improve palatability of these products is still being urged to continue (Nascimento A, et al 2014).

2.7. Solutions for gluten-free product texture and acceptance

Rice flour products can be used with gluten replacements such as hydrocolloids and hydroxypropyl methylcellulose (HPMC) to increase texture quality (Kadan R, et al 2001; Sivaramakrishnan H, et al 2014). Rice flour in combination with other gluten-free flours like corn, and cassava flour can also increase rheological quality of rice products (Lopez A, 2004). Improvements to the flavor and aroma of gluten-free bread is being conducted by matching the volatile flavor of wheat-containing products by combining proline and glucose in gluten-free product recipes (Pacynski M, et al 2015).

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CHAPTER 3. RICE FLOUR BASED GROCERY PRODUCTS MARKET SURVEY

3.1. Introduction

Gluten sensitivity and intolerance disorders such as Celiac Disease (CD) are on the rise in the United States. Celiac Disease is an autoimmune disorder triggered by gluten proteins which account for up to 80% of the total grain protein content (Brouns F, et al, 2013). Specifically, gluten causes inflammation of the intestinal mucosa causing gastrointestinal and/or extraintestinal symptoms that are uncomfortable and sometimes very painful (Leonard MM, et al 2017). Nonceliac gluten sensitivity presents similar signs and symptoms as Celiac Disease making it difficult to diagnose and quantify its prevalence. Since there is very little scientific research on this subject, there is still no cure for the disease. The primary method suggested by doctors to prevent symptomatic gluten responses is adapting a gluten-free diet.

Wheat ranks third among cereals in global production behind maize and rice (Brouns et al, 2013). Production in the United States is nearly 3 times the consumption resulting in high incorporation of the ingredient in our food supply. The performance and usefulness of wheat in many commercialized food items, especially baked goods, makes it a common ingredient in many shelved grocery products. People following a gluten-free diet are typically limited to fresh fruits, vegetables, meat and gluten-free alternatives. The availability of acceptable gluten-free alternatives for patients looking for more options has prompted an increase in research and development in this area. Although there are other cereals that can be used as a replacement for wheat, simple substitution of naturally gluten-free flours and starches in most recipes will not yield products with similar texture characteristics.

Gluten acts as a binding agent when combined with water and is responsible for the elasticity of dough. When gluten-free alternatives are used in baking, the result is more of a batter than a dough. For this study, rice flour was the focus as a substitution for wheat flour in grocery products. Rice's white color, bland taste, and easy digestibility make it a suitable and widely used ingredient in gluten-free baking. Compared to gluten in wheat, rice proteins have poor functional properties in gluten-free formulations which encourages inclusion of hydrocolloids or gums to help batter rheology (Hager A, et al 2013). Rice generally has a high dietary glycemic index (GI) due to the nature of its composition being predominantly carbohydrates. Foods with a high glycemic index are said to be digested fast and increase blood glucose levels. Glycemic load (GL) is representative of a person’s glycemic response to the diet and is directly related to glycemic index (GL = GI x carbohydrate content) (Cheng X., et al. 2017). Genetic breeding and cultivation of rice varieties to increase nutritional value are being grown and harvested for consumer use.

Replacement of a traditional rice flour with flour milled from rice with a higher protein content is undergoing research currently. Quantitative information about commercial rice flour versus high protein rice flour were studied for nutritional and physiochemical differences in the flour alone and in application of baked gluten-free products such as muffins and bread. This survey analyzed which variation of rice flour is commonly found in the commercial marketplace and treat it as a control. The objective of this study was to survey grocery stores in Baton Rouge to determine what products utilize rice flour to drive development of products using high protein rice flour as a replacement.

3.2. Research design and methods

The availability of grocery products containing rice flour as a predominant ingredient was surveyed in Baton Rouge, Louisiana. Five chain grocery stores were chosen based on price point and convenience. Pictures were taken of product front labels, nutrition information, and ingredient lists for all items being surveyed. In addition to package details, prices were also recorded for comparison between stores. Names, prices, protein contents and important ingredients listed on the food labels were entered in an Excel spreadsheet and used to organize information and identify what product types lack nutritional value and may be able to benefit from use of a more nutritious base ingredient, specifically a high protein rice flour. Two stores with a low price point, two stores with a medium price point, and one store with a high price point were chosen to ensure diversity of products.

3.3. Results and discussion

An average of 79 products were surveyed from the 5 stores for a total of 393 items that listed rice flour as a primary ingredient. Very few items used both rice and wheat flour together. Figure 3.1 summarizes the most abundant types of food items found to contain rice flour as a predominant ingredient. Baked goods and breakfast items were among the top categories at 36% and 21% respectively. Most items were labeled "gluten-free" and about half of them were found in the gluten-free section of the store. Due to the rise in Celiac Disease, there are many all-purpose gluten-free flours and mixes that could be used as replacements to their wheat counterparts. Various flours, starches, oils, and gums were among the most common and abundant ingredients in gluten-free baked goods, mixes, and flour blends. In conjunction with rice flour, these additional ingredients help to mimic the light, soft structure consumers want in baked goods. About 35% of the food items surveyed contained one, two, and occasionally even three different gums to aid in stabilization and thickening. The two most frequently used gums were xanthan and cellulose. Roughly 60% of the items used one or more of the following ingredients in combination with rice flour: sorghum flour, rice starch, potato starch, tapioca starch, and corn starch. This information was used to help identify what additional baking ingredients were useful to try and include in the development of gluten-free products.

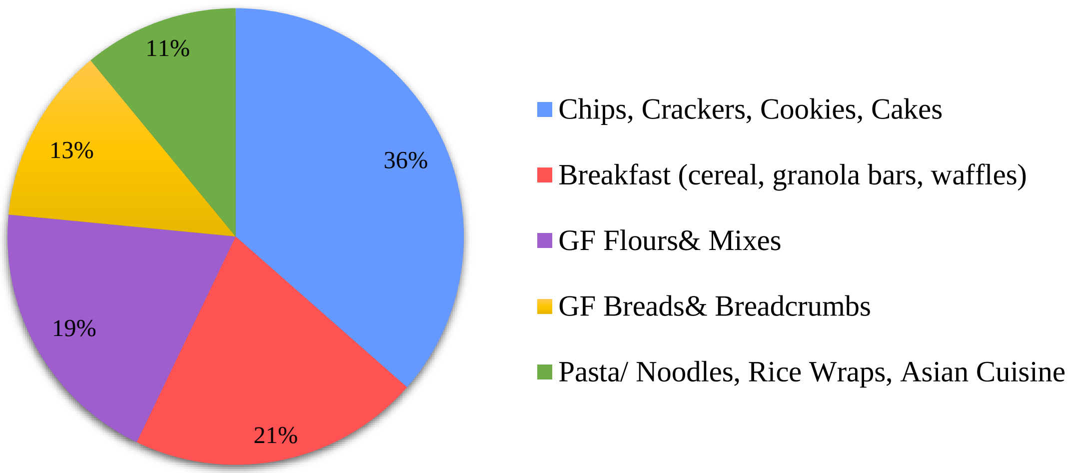

Figure 3.1. Category prevalence of product types surveyed containing rice flour

Properties of rice are dependent on variety, methods of cultivation, processing, and cooking. Nutritional value of rice is reduced with a higher degree of milling. White rice is the product of removing the husk, bran, and germ from brown rice. Proteins, fats, vitamins, and minerals are concentrated in the outer layer of the endosperm and germ (Roy, et al. 2011). Presence of the bran and germ in brown rice offers more desirable nutritional properties and contributes to reducing the cost of production compared to white rice because it does not require a polishing step (Hamada et al, 2012). Brown rice contains about five times more fiber than white rice which can be found in the bran and germ (Kondo M., et al. 2017). Products made with brown rice instead of white rice are more nutritious and have lower glycemic loads because of its high fiber and lower carbohydrate content. Figure 3.2 shows the high frequency use of brown rice flour in the commercial market which could be explained by its lower processing cost and increased nutritional value compared to white rice flour (Roy O., et al. 2011). Thirty-six percent of food items did not specify what type of rice flour was used however, an assumption could be made that most unspecified rice flours were brown since brown was the most common type recorded at 38 percent. Unlike rice flour that is ground from medium and long grain rice, sweet rice flour is made from short grain glutinous rice which yields a much higher starch content than other types of rice (Li D., et al. 2018). Foods with higher starch or carbohydrate contents have a direct correlation to high glycemic load thus resulting in faster digestion than foods with lower starch contents (Cheng G., et al 2017).

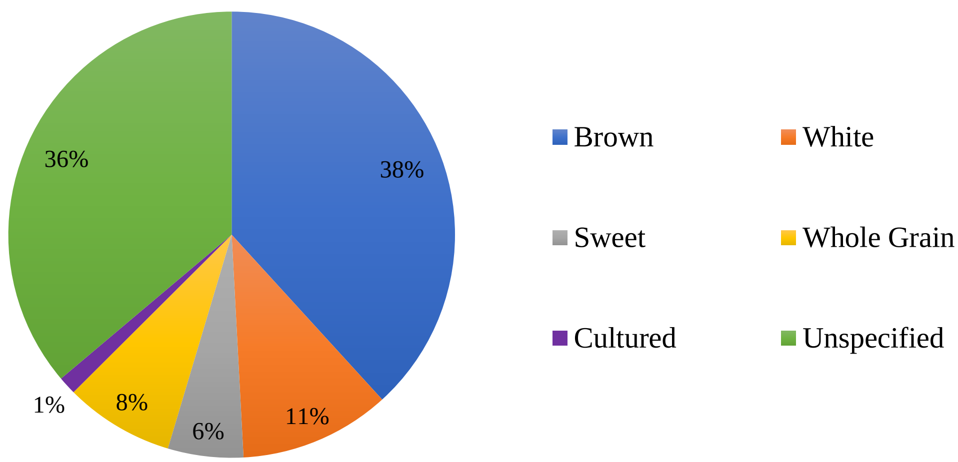

Figure 3.2. Summary of the types of rice flours listed on food labels of rice-based foods

As consumers become more aware and concerned with their health and nutrition, a trend towards protein and fiber fortification in common foods has emerged. According to IRI, an American market research company, protein continued to be a driving force for new products in 2017. About 36% of the top 76 best-selling foods and drinks in 2017 touted protein. Protein fortification can be done using soy and whey proteins, but this does influence product texture. Santarpia L, et al. (2017) stated that the optimal protein consumption for weight management should be between 1.2-1.6 grams of protein per kilogram of body weight per day. Generally, an intake of 20-30 grams of protein at breakfast, lunch, and dinner has been confirmed to promote weight loss and maintenance in young, old, and obese adults. Figure 3.3 examined the average protein content per serving of common product types found during the survey. Pasta and protein bars were among the most nutritious items with averages of 5.03 and 4.77 grams of protein per serving. Unfortunately, besides an occasional protein bar that had enough protein for a meal, all other products were lacking in nutrient density.

Another option for increasing protein content of the food items is the use of a rice flour that is cultivated to have a higher protein content than traditional rice. The rice flour product market could use research and product development to increase its nutritional value for consumers who are limited to gluten-free or other strict diets. Rice with increased protein levels will in turn have decreased carbohydrate levels thus decreasing the glycemic load on absorption and digestion in the body. Carbohydrate analysis is typically based on digestible carbohydrates. In order to calculate the amount of carbohydrates subject to digestion, Liese, et al. (2005) reported the subtraction of fiber intake from total carbohydrate intake. This value is more closely related to the glycemic index than values containing undigestible carbohydrates such as fiber.

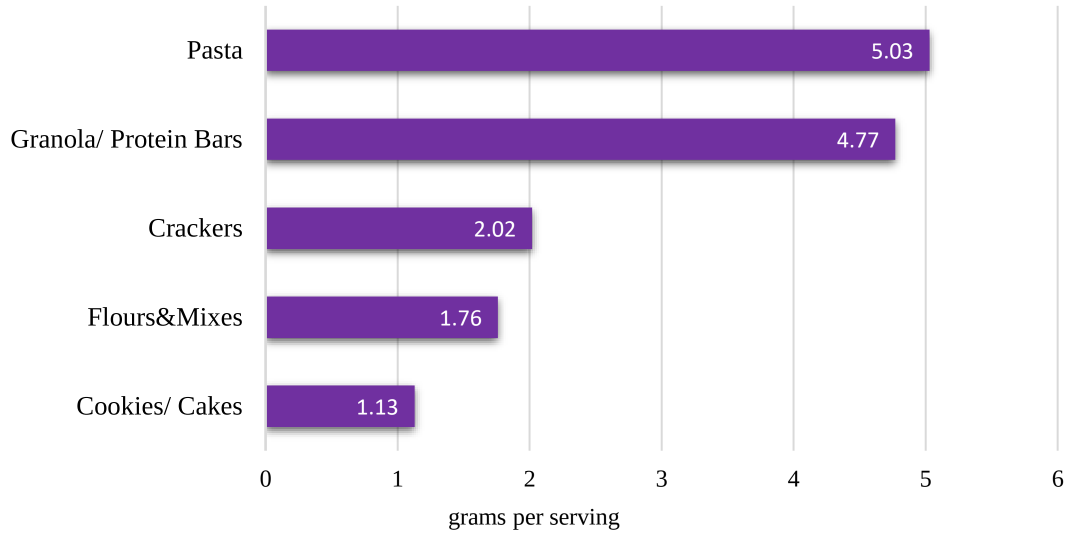

Figure 3.3. Average protein contents of 5 rice-based product types (N = 43, 79, 71, 77, 67)

3.4. Conclusion and applications

Rice flour as a gluten-free alternative to wheat flour is quickly becoming more common. Brown rice flour offers more beneficial characteristics for nutrition, baking, and process cost reduction than white rice flour thus making it the more preferred type to use in commercialization. Although traditional rice does not supply an adequate amount of protein on its own, cultivation of rice to contain more protein per serving is underway. Use of a rice flour with a higher protein content is applicable for most products on the market to easily increase nutritional value in items that typically provide little to no nutritional benefit. Developing products such as muffins and bread with a high protein rice variety will be conducted in hopes of providing producers with simple, cost-efficient, and acceptable formulations to produce more nutritious products for the food market.

3.5. References

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CHAPTER 4. HIGH PROTEIN RICE FLOUR IN THE DEVELOPMENT OF GLUTEN-FREE MUFFINS

4.1. Introduction

Wheat is the third most globally produced cereal behind maize and rice. Production in the United States is nearly 3 times the consumption resulting in high incorporation of the ingredient in our food supply. The performance and usefulness of wheat in many commercialized food items, especially baked goods, makes it a common ingredient in many shelved grocery products. Wheat's high protein content (roughly 12%) is primarily composed of gluten proteins. Gluten accounts for up to 80% of the total grain protein content and acts as a binding agent when combined with water (Brouns et al, 2013). The way gluten binds water and traps air bubbles from fermentation is unique and responsible for the elasticity and structure of dough. Application of water and heat to starch results in a gel-like paste that can be utilized for bakery products. It is important to understand starch functionality for controlling moisture, texture, mouth-feel, and shelf-life of finished products (Wang et al. 2013).

Gluten sensitivity, intolerance, and autoimmune disorders such as Celiac Disease (CD) are on the rise in the United States. In Celiac patients, gluten causes inflammation of the intestinal mucosa causing gastrointestinal and/or extraintestinal symptoms that are uncomfortable and sometimes very painful (Leonard M., et al. 2017). Nonceliac gluten sensitivity presents similar signs and symptoms as Celiac Disease making it difficult to diagnose and quantify its prevalence. Since there is still little scientific research on this subject, there is no cure for the disease. The primary solution to prevent symptomatic gluten responses is adapting a gluten-free diet.

Understanding the pasting properties of starches helps to predict the final cooked quality of many different products. The changes in viscosity of aqueous starch mixtures as a function of time and temperature is how pasting values are quantified. Peak and final viscosities are important values to consider in product development to determine how processing may affect the final product (Srivastava, Y 2013). Wheat's high pasting temperature, minimal breakdown, and low final viscosity explain how the dough structure changes when being cooked/baked. When gluten-free alternatives are used in baking to replace wheat, pasting properties are drastically different. A much harder, denser, and less elastic structure is typically the result and consumer acceptability is difficult to achieve. The availability of acceptable gluten-free alternatives for persons looking for more options has prompted an increase in research and development in this area.

Rice's white color, bland taste, and easy digestibility make it a suitable and widely used ingredient in gluten-free products. Although rice has a low protein content and poor functional properties in gluten-free formulations, it prompts inclusion of hydrocolloids or gums to help batter rheology (Hager A., et al. 2013). Cultivation of new rice varieties is underway and increased nutrition is at the forefront of research objectives. According to IRI, an American market research company, protein continued to be a driving force for new products in 2017 with six in ten consumers saying they tried to add more protein to their diet.

The availability of rice with greater protein content than traditional rice is limited. However, researchers at Louisiana State University in Baton Rouge, Louisiana have successfully cultivated rice with an average of 53% more protein than normal. This developed rice cultivar is currently being grown commercially in Illinois and sold online as Cahokia Brown or Cahokia White Rice. It is important to understand the different starch composition and behavior of this rice and as a milled flour to be used as a baking ingredient.

The objective of this study was to analyze and utilize a newly developed rice variety with a greater protein content to develop gluten-free muffins which are more nutritious and consumer acceptable than a commercial rice flour muffin. Analysis and application of high protein rice flours could contribute to large-scale use in food products that may aim to increase nutritional value in gluten-free baked goods, naturally.

4.2. Materials and methods

4.2.1. Rice flour analysis

4.2.1.1. Moisture content and pasting properties

Moisture contents of various flours were quantified using an OHAUS MB45 rapid moisture analyzer (Switzerland). One-gram samples were heated to 190°C until all moisture was evaporated. The difference in initial and final weight corresponded to moisture content. All samples were analyzed in triplicate and used in characterizing pasting properties. A Newport Scientific RVA-4 Rapid Visco Analyzer (Australia) attached to a Thermo Electron Corporation Neslab RTE 7 cooling system was used to monitor pasting characteristics over a range of time and varying temperatures in triplicate replications for each flour type. A flour weight of 3 grams and 25 ml of deionized water were combined in an aluminum canister with a polycarbonate stirring paddle and attached to the RVA which follows the AACC Approved Method 61-02 (AACC 2000a).

4.2.1.2. Protein, fat, and fiber content

Nitrogen contents of long grain brown high protein rice flour, long grain white high protein rice flour, and a control white rice flour were quantified in duplicates and used to determine crude protein contents. Using the Dumas method with 100-gram samples at 900°C, the nitrogen was converted into protein content using a conversion factor of 5.95 according to the 2016 USDA

National Nutrient Database for Standard Reference Release 28. A second protein quantification method known as the Kjeldahl method was used to compare to Dumas nitrogen results. This AOAC Official Method 976.06 for Protein (Crude) in Animal Feeds and Pet foods (AOAC, 16th Edition, Vol 1) semi-automated method has three steps: digestion, distillation, and titration. Previous studies favor a combustion method because of the greater number of samples that can be run in a day unattended unlike the Kjeldahl method which requires many manual steps. The use of hazardous reagents such as sulfuric acid and sodium hydroxide in the Kjeldahl method, Dumas method is safer and more cost efficient (Marco 2002).

Quantification of crude fat was done using the AOAC Methods 2003.05 and 2003.06 Randall/Soxtec modification of the conventional Soxhlet solvent extraction procedure.

Crude fiber content was identified using the AOCS approved Ba6a-05 Filter Bag method. The sample was digested with sulfuric acid and sodium hydroxide to remove proteins, sugars, starch, fats, and portions of carbohydrates. The organic residue left after digestion was used to quantify crude fiber.

4.2.2. Muffin formulation and analysis

Ingredients listed in Table 4.1 were combined in a specific manner to create a desirable batter rheology for all three muffins this recipe makes . First, the eggs were whipped for 5 minutes to entrap air bubbles and aid in a leavened final product. The egg, sugar, soybean oil, and xanthan gum were beaten together using the creaming method. This method incorporates more air in the batter and the xanthan gum aids in holding its structure together. The next five dry ingredients were combined and alternately added with water to the creamed mixture. It was important to not overmix the dry or wet ingredients by hand, so the final texture was crumbly like a muffin while still retaining air bubbles for lightness. A standard muffin pan was lined with paper liners and lightly greased with vegetable oil spray. A standard batter of 45 grams per muffin was weighed into each greased liner and the tops were smoothed down for an even bake. The muffins were cooked in a Baxter mini rotating rack gas oven (model OV310G) preheated to 350°F for 12 minutes. Liners were removed as soon as the muffins were cool enough to handle.

Table 4.1. Percentages of gluten-free rice flour muffin ingredients

Ingredient	
Sugar	14.03%
Soybean oil	14.03%
Egg (whole, beaten)	14.03%
Xanthan gum	0.21%
Rice flour*	35.06%
Baking powder	1.19%
Baking soda	0.56%
Salt	0.70%
Powdered milk	2.66%
Water	17.53%

*muffin treatments varied only by rice flour type used; makes 3 standard 45-gram samples

4.2.2.1. Color and texture analysis

A Konica Minolta BC-10 Baking Contrast Meter (Japan) was calibrated using a white tile before testing. Crust color was measured on the top of each type of muffin in triplicate replications. The crumb color was analyzed by first slicing off the muffin top then placing the colorimeter on the flat surface to record the data. Color, lightness, and darkness were quantified as L, a*, and b* values.

Using a TA.XT*Plus* texture analyzer (made in Scarsdale, NY), triplicates of each type of muffin were tested for hardness, cohesiveness, springiness, gumminess, and chewiness. It was important to cut the rounded muffin tops to yield a muffin height of 2.5 centimeters to standardize and conduct texture analysis. After calibration and sanitation of the equipment, individual samples were centered on a metal stand underneath a 2” cylinder probe. A program specific for muffins was chosen to run a 2-bite test with a 0.049 N trigger force and a wait time of 5 seconds between bites.

4.2.3. Muffin consumer sensory testing

Once the muffin formulation was refined and standardized, a sensory study was conducted to measure consumer acceptability. First, the formulation was multiplied to accommodate 100 panelists. Each panelist evaluated three rice flour muffins including a control and recorded their answers on the Louisiana State University Qualtrics online survey program. Questions were centered around specific attributes such as appearance, texture, taste, and overall acceptance. The likelihood that the consumer would purchase each muffin type was assessed before and after a health message was displayed. The message informed panelists about the nutritional benefits of consuming muffins made with high protein rice flour and that the products were gluten-free. Sample assignments followed a randomized complete block design (RCBD) where each group/block received one set of three muffin treatments. All attributes were ranked on a 9-point hedonic scale and some were also ranked on a just about right (JAR) scale.

4.2.4. Statistical analysis

Statistics were done on color and texture data using SAS to conduct an analysis of variance (ANOVA) test coupled with a Tukey test on all treatments. This would determine their means and distinguish whether the muffin colors and textures were significantly different or if samples were comparable. An analysis of variance (ANOVA) test was also done between sensory treatments to calculate mean acceptances and to see if there were any significant differences between samples types. A Tukey test was used to compare treatments parametrically for statistical differences for color, texture, and sensory data.

4.3. Results and discussion

4.3.1. Moisture and pasting properties

Quantifying moisture content of a food ingredient helps predict how it may act in the presence of other ingredients and during processing. The moisture content of these four flours varied by less than 0.50% (Table 4.2). Greater pasting temperatures seen with the commercial flours show that their starch granules swell and gelatinize at greater temperatures than the high protein rice flours. According to Chen Y, et al. (2003) this could mean the high protein rice flours have a greater amylopectin content than the two commercial flours. Total setback (TSB) is the difference between final viscosity and minimum or trough viscosity. This value reflects the retrogradation tendency of a starch (Jacobs et al. 1995). Retrogradation is defined as the reassociation of starch components after processing or cooking. The higher TSB in the brown high protein rice flour compared to other flours means that there is greater retrogradation potential.

On their own, brown and white high protein rice flours did not show similar behavior in terms of peak, breakdown, and total setback viscosities to wheat flour. The composition of wheat is significantly different from rice as far as protein and starch content. Wheat flour forms an elastic structure when cooked which makes it the preferred base of fresh baked goods. Trough viscosity of brown rice flours was closest to wheat flour. Therefore commercial brown rice flour was used as the standard control for comparison in further analysis.

Table 4.2. Moisture and starch gelatinization behavior of various flours

Flour Type	*Moisture Content* *(%)*	*Pasting Temp* *(°C)*	*Peak Viscosity* *(cP)*	*Trough Viscosity* *(cP)*	*Breakdown* *(cP)*	*Final Viscosity* *(cP)*	*Total Setback* *(cP)*
CW	13.05 $\pm 0.55_A$	89.48 $\pm 0.51_A$	1790 $\pm 40.95_D$	1186.33 $\pm 17.95_D$	570.33 $\pm 39.80_C$	2430 $\pm 94.06_D$	1243.67 $\pm 76.20_E$
CBR	12.69 $\pm 0.46_A$	89.93 $\pm 0.40_A$	2347.33 $\pm 39.80_B$	1433 $\pm 25.87_C$	947.67 $\pm 21.22_B$	3174.67 $\pm 36.94_C$	1741 $\pm 20.60_C$
CWR	12.90 $\pm 0.39_A$	84.83 $\pm 0.06_C$	3477.67 $\pm 63.22_A$	1897.33 $\pm 79.65_A$	1547 $\pm 29_A$	3264.33 $\pm 52.44_C$	1367 $\pm 34.70_D$
BHPR	12.77 $\pm 0.52_A$	82	2278	1221	1057	3748	2527
WHPR	13.15 $\pm 0.17_A$	76	3331	1773	1558	3281	1508

(A) CW-Commercial Wheat, CBR-Commercial Brown Rice, CWR-Commercial White Rice, BHPR-Brown High Protein Rice, WHPR-White High Protein Rice (B) *Same subscript letter within a column signifies no significant difference at 95% confidence

4.3.2. Protein, fat, and fiber content

Nitrogen content was converted to crude protein by multiplying the percent nitrogen by a conversion factor which is 5.95 for rice flour. It is considered “crude” protein due to the possibility of nitrogen derivatization from sources other than protein. Results of the Dumas method of combustion confirmed that both high protein rice flours do have more protein than commercial rice flour (Table 4.3). As hypothesized, white rice flours had lower protein contents than their brown counterparts for commercial and both batches of high protein rice flours. Brown high protein rice flours had over a 2 percent difference from the commercial brown rice flour. In comparison to protein quantification by Kjeldahl method, values for commercial rice flours were about 2% higher while high protein rice flour protein contents were lower than Dumas values.

Table 4.3. Proximate analysis of rice flours

Type of Rice Flour	*Nitrogen (ppm)*	*Dumas Protein (%)*	*Kjeldahl Protein (%)*	*Crude Fat (%)*	*Crude Fiber(%)*
Commercial Brown	1.06 ± 0.02C	6.29	9.15 ± 0.07	2.45 ± 0.07	0.86 ± 0.36
Commercial White	0.98 ± 0.01D	5.81	7.25 ± 0.21	0.35 ± 0.07	0.12 ± 0.06
Brown High Protein	1.44 ± 0.05A	8.55	8.3	2.8	0.24
White High Protein	1.23 ± 0.04B	7.34	6.9	0.7	0.44

(A) mean ± SD, N=3 for commercial rice flour, N=1 for high protein rice flour due to limited batch size

The Kjeldahl method of protein quantification was done for comparison to results of the combustion method. Variability was less than 0.5% between the two test methods.

4.3.3. Color and texture

Color and texture characteristics of food are some of the most important qualities to perfect in production. Consumers eat with their eyes first and if a product color is off enough for the consumer to notice, it is very likely they will not find it acceptable enough to purchase. Similarly, and more detrimental to the likelihood of gaining repeat buyers, texture and mouthfeel of food products are important to measure and compare to desired sample values.

A greater L value represents a "lighter" color such as the white high protein rice muffin crust and crumb. As Table 4.4 shows, the brown rice muffins had similar crumb lightness, but were darker than the white high protein rice muffins. This could have been caused by a greater rate of Maillard reaction in the brown rice muffin crust due to the higher protein/ amino acid content (Bolarinwa, Lim et al. 2018). The two brown rice muffins were similar in color for both crust and color. The negative a* value for the white high protein rice muffin crumb means it has a little greener hue than the others that were more red. All b* values favored a more yellow hue than blue (Table 4.4). These results suggest that the brown high protein rice flour could be substituted for a commercial brown rice flour with minimal visual differences while adding nutritional value.

Table 4.4. Gluten-free muffin crust and crumb colors

Crust color	*L*	*a**	*b**
Commercial BR	$69.51 \pm 1.72_{B}$	$5.18 \pm 2.08_{A}$	$27.72 \pm 1.91_{A}$
Brown HPR	$72.14 \pm 2.7_{B}$	$4.67 \pm 0.95_{A}$	$26.23 \pm 1.82_{A}$
White HPR	$77.56 \pm 4.5_{A}$	$1.90 \pm 2.02_{B}$	$27.90 \pm 2.30_{A}$
Crumb color			
Commercial BR	$71.27 \pm 2.05_{B}$	$1.46 \pm 0.34_{B}$	$24.14 \pm 1.13_{A}$
Brown HPR	$72.18 \pm 1.46_{B}$	$2.11 \pm 0.40_{A}$	$24.37 \pm 0.86_{A}$
White HPR	$77.37 \pm 0.61_{A}$	$-1.19 \pm 0.24_{C}$	$22.23 \pm 0.49_{B}$

(A) Commercial BR: brown rice flour, Brown HPR: brown high protein rice flour, White HPR: white high protein rice flour (B) mean ± SD, N=6 (C) Same subscript letter within a column signifies no significant difference at 95% confidence

Texture characteristics of the muffins were comparable between the two brown rice treatments (Table 4.5). Commercial white rice muffins were the least hard and chewy while being the most cohesive compared to all other muffin treatments. Springiness is associated with freshness, or lack of staling, of a product and is not significantly different between commercial brown and white rice muffins. Greater springiness values are representative of a greater muffin quality (Tess M et al, 2015). The high protein rice flour muffins had greater hardness and lower springiness than commercial rice flour muffins which could be explained by the higher fat, and fiber compositions of the high protein rice flours (Cakir E et al, 2011).

Table 4.5. Texture profile analysis of gluten-free rice flour muffins

	Hardness (N)	*Cohesion*	*Springiness (%)*	*Chewiness(N)*
Commercial BR	$22.63 \pm 3.15_{AB}$	$0.75 \pm 0.04_{B}$	$95.64 \pm 1.58_{A}$	$16.10 \pm 1.39_{AB}$
Commercial WR	$14.63 \pm 1.08_{B}$	$0.84 \pm 0.01_{A}$	$94.47 \pm 1.97_{AB}$	$11.57 \pm 1.02_{B}$
Brown HPR	$26.08 \pm 3.63_{A}$	$0.68 \pm 0.04_{C}$	$90.25 \pm 1.46_{C}$	$14.25 \pm 2.88_{AB}$
White HPR	$26.08 \pm 9.17_{A}$	$0.77 \pm 0.03_{B}$	$91.49 \pm 3.6_{BC}$	$18.66 \pm 6.26_{A}$

(A) Commercial BR: brown rice, Commercial WR: white rice, Brown HPR: brown high protein rice, White HPR: white high protein rice (B) Same subscript letter within a column signifies no significant difference at 95% confidence

4.3.4. Consumer sensory study

A total of 97 panelists participated in the muffin sensory study. Figures 4.1 and 4.2 show the demographics of participants with most being between the age of 18 and 35 years old due to the location of the study being at Louisiana State University. People of all ages may indulge in a muffin for breakfast or a sweet snack. Children may be more likely to consume muffins because they tend to consume sweet foods without concern of negative health effects. However, specific attributes and the effect of a health message on purchase intent is applicable for adults who pay close attention to product labels and composition.

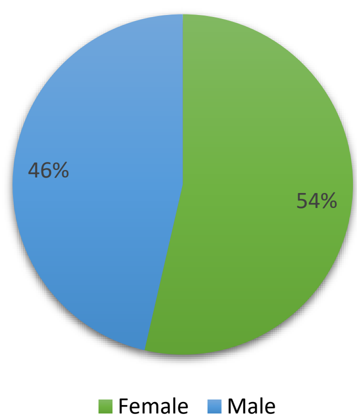

Figure 4.1. Gender of participants (N = 97)

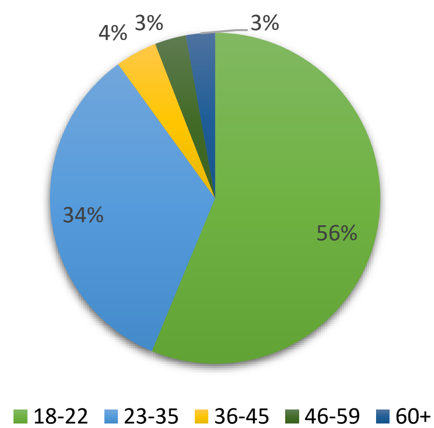

Figure 4.2. Age of participants (N=97)

The specific product characteristics listed in Table 4.6 were analyzed to see if the samples differed significantly or not. Acceptability of muffin color was the only product descriptor that favored one treatment over the others and that was the white high protein rice muffin. All other product features and overall acceptance were not statistically different between the three treatments. Although not statistically different, acceptability ratings of the high protein rice muffins tended greater than the commercial brown muffins. A study conducted by Bhaduri (2013), showed acceptance of muffins with lighter colored flour were greater than those with a darker color. This is also true for the white high protein rice flour muffins having the lightest L value and highest color acceptance.

Table 4.6. Acceptance of muffin characteristics

	Color	***Crumbliness***	***Moistness***	***Softness***	***Aroma***	***Flavor***	***Overall Acceptance***
CBR	5.90 $\pm 1.82_{B}$	6.13 $\pm 1.79_{A}$	6.00 $\pm 1.74_{A}$	6.19 $\pm 1.64_{A}$	5.52 $\pm 1.52_{A}$	5.97 $\pm 1.82_{A}$	5.94 $\pm 1.77_{A}$
BHPR	6.06 $\pm 1.71_{B}$	6.30 $\pm 1.54_{A}$	6.27 $\pm 1.78_{A}$	6.53 $\pm 1.64_{A}$	5.74 $\pm 1.45_{A}$	6.44 $\pm 1.64_{A}$	6.36 $\pm 1.67_{A}$
WHPR	6.97 $\pm 1.45_{A}$	6.41 $\pm 1.48_{A}$	6.09 $\pm 1.7_{A}$	6.28 $\pm 1.65_{A}$	5.88 $\pm 1.45_{A}$	6.07 $\pm 1.69_{A}$	6.28 $\pm 1.61_{A}$

(A) CBR: 100% brown rice flour; BHPR: 100% brown high protein rice flour; WHPR: 100% white high protein rice flour (B) N= 97; mean ± SD based on 9-point hedonic scale (C) Same subscript letter within a column signifies no significant difference at 95% confidence

The previous evaluation was conducted on a 9-point hedonic scale and three of the 6 product characteristics were also tested for their level of appropriateness. Table 4.7 summarizes how panelists perceived the suitability of muffin crumbliness, moistness, and softness for each type. Even though the only difference between treatments was the type of rice flour used, there is noticeable variability in product ratings. Overall, these three product parameters were said to be present in about the right amount for most panelists. The high protein rice flour muffins had higher frequencies of appropriateness than the commercial control (Table 4.7). All three product characteristics were found to be "too much" more often in the commercial brown rice muffins which contributed to it having the lowest levels of suitability in terms of acceptability across the board. Future improvements to the white high protein rice flour could lead to enough increase in moistness and softness to increase the intensity of "just about right" perceptibility for those characteristics.

Table 4.7. Attribute intensities of gluten-free muffins

	Product Characteristic	***Not Enough***	***Just About Right***	***Too Much***
Commercial BR	Crumbliness	16.49	67.01	16.49
Brown HPR		14.43	72.16	13.40
White HPR		17.53	76.29	6.19
Commercial BR	Moistness	37.11	57.73	5.15
Brown HPR		25.77	73.20	1.03
White HPR		37.11	60.82	2.06
Commercial BR	Softness	28.87	65.80	5.15
Brown HPR		19.59	79.38	1.03
White HPR		28.87	69.07	2.06

(A) Commercial BR: 100% brown rice flour; Brown HPR: 100% brown high protein rice flour; White HPR: 100% white high protein rice flour (B) N= 97; based on a JAR scale; 1=not enough, 2=just about right, 3=too much

The last two survey questions were centered around the panelists' intent to purchase the individual muffins (Table 4.8). Both high protein rice muffins had lower frequencies of "no" responses while the commercial brown rice muffins had the highest. A message was displayed after the first purchase intent question which stated that the products were gluten free and the high protein rice flour muffins also stated that a rice flour with a naturally higher protein content was used. The effect of a product health claim has been proven to impact consumer purchase intent. In a study by Cori Navarro (2016), products were perceived as healthier when labeled gluten-free resulting in a higher rating. Health benefit statements has been proven to have a positive effect on overall purchase intent of muffins formulated to be healthier (Jack A 2015; Wardy W et al 2017), As seen in Table 4.8, there was a 9% and 12% increase in "yes" purchase intent response for the high protein rice muffins and only a 5% increase for the commercial brown rice muffins. The addition of a higher protein rice flour resulted in an even larger increase in purchase intent beyond that of the gluten-free message. Essentially, any food or ingredient company could use this information to market their products if claims made are related to increased protein and non-gluten containing ingredients.

Table 4.8. Effect of health message on purchase intent of gluten-free rice flour muffins

Treatment	***Panelist Response***	***Purchase Intent BEFORE message***	***Purchase Intent AFTER message***	***% difference***
Commercial Brown Rice Flour	Yes	20.62	25.77	+5.15
	Maybe	42.27	41.24	-1.03
	No	37.11	32.99	-4.12
*Brown High Protein Rice Flour**	Yes	32.99	42.27	+9.28
	Maybe	44.33	45.36	+1.03
	No	22.68	12.37	-10.31
*White High Protein Rice Flour**	Yes	31.96	44.33	+12.37
	Maybe	41.24	31.96	-9.28
	No	26.80	23.71	-3.09

(A) health message stated "This is a **GLUTEN-FREE** muffin made with **RICE FLOUR**" or "***HIGH PROTEIN RICE FLOUR"**

4.4. Conclusion

Based on pasting properties and wheat as the control, the commercial brown rice flour would have the closest behavior to wheat. Although the brown high protein rice flour had the closest peak and trough viscosity to wheat, it also had the highest total setback and final viscosity of all samples indicating a potential for increased retrogradation. Protein contents were greatest for both high protein rice flours. Fat contents were found to be greater for brown high protein rice flour with the control brown rice and white high protein rice flour having the second and third highest values. The lightness of the white high protein rice flour muffins had a direct effect in the greater acceptance of their color compared to brown rice flour. Muffin hardness and chewiness were statistically the same for all three muffin types. The consumer sensory study favored the two high protein rice flour muffins over control brown rice flour muffins in all survey questions but not enough to be statistically different. Results show that the replacement of commercial rice flour with a rice flour that has greater protein content has a positive effect on consumer acceptance of gluten-free muffins. The statistical indifferences of muffin acceptability for all product attributes (other than color) confirms that high protein rice flours can be used as a substitution for commercial rice flour to increase nutritional value.

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CHAPTER 5. HIGH PROTEIN RICE FLOUR IN THE DEVELOPMENT OF GLUTEN-FREE BREAD

5.1. Introduction

The ever-growing demand for gluten-free options has driven research in product development to create and improve new food items. Unfortunately, people who follow a strict gluten-free diet often feel that their availability of a variety of food options is limited. Gluten's functional role in baked goods and pasta poses technological challenges for gluten-free alternatives and is ultimately the reason GF products have poor sensory properties (Sandri L 2017). Replacement of wheat with gluten-free alternatives often results in a loss of nutritional value such as protein and important vitamins and minerals. Further research is needed to develop healthier and better-quality gluten-free options; especially for those suffering from a gluten allergy, intolerance, or Celiac Disease.

The 2017 report by Sandri and others named bread as the most globally studied gluten-free item. Bread is a staple food in many cultures so with an increase in the prevalence of gluten-sensitivities and disorders comes a need for acceptable gluten-free bread. Rice is widely used as a gluten-free flour, but its poor functional properties requires the addition of other food ingredients to aid in batter consistency. Different varieties of rice may be more or less suitable for use as rice bread flour thus requires research on available varieties of rice flour (Han H, et al 2012). Gluten-free bread formulations that use rice as a base are often combined with maize, potato, or cassava starch. The addition of starch in GF baking can help form a matrix to entrap gas bubbles and increase the capacity of a batter to hold gas like gluten does in wheat batters (Loan L, et al 2017). Starch can also enhance crumb softness, maintain batter consistency during mixing, and influence starch gelatinization during baking (Abdel-Aal E 2009). Use of a hydrocolloid such as xanthan gum is also incorporated regularly in gluten-free processing due to their ability to impart specific functional properties to various food products. Texture, starch retrogradation, moisture retention, and overall quality of a product can be improved with the addition of a hydrocolloid (Gomez M, et al 2007).

The objectives of this study were (1) to analyze high protein rice flours for physical/chemical properties, (2) to develop and analyze a gluten-free bread formulation, and (3) to survey consumer acceptance of GF bread samples made with high protein rice flour or commercial rice flour.

5.2. Materials and methods

5.2.1. Rice flour analysis

5.2.1.1. Moisture content and pasting properties

Moisture contents were found using an OHAUS MB45 rapid moisture analyzer (Switzerland) in triplicate replications. One-gram samples were heated to 190°C until all moisture was evaporated. The difference between initial and final weight corresponded to moisture content. All samples were analyzed in triplicate and used in characterizing pasting properties. A Newport Scientific RVA-4 Rapid Visco Analyzer (Australia) attached to a Thermo Electron Corporation Neslab RTE 7 cooling system was used to monitor pasting characteristics over a range of time and varying temperatures. A flour weight of 3 grams and 25 ml of deionized water were combined in an aluminum canister with a polycarbonate stirring paddle and attached to the RVA which follows the AACC Approved Method 61-02 (AACC 2000a). Samples were heated then cooled to mimic cooking and measure changes in viscosity over time in triplicate replications.

5.2.1.2. Protein, fat, and fiber content

Nitrogen contents of long grain brown high protein rice flour, long grain white high protein rice flour, and a control white rice flour were quantified in duplicate and used to determine crude protein contents. The Dumas method was used to combust 100-gram samples at 900°C to quantify nitrogen which was then converted into protein using a conversion factor of 5.95 according to the 2016 USDA National Nutrient Database for Standard Reference Release 28. The AOAC Official Kjeldahl Method 976.06 for Protein (Crude) in Animal Feeds and Pet foods (AOAC, 16th Edition, Vol 1) semi-automated method was used to compare protein values. Previous studies favor a combustion method because of the greater number of samples that can be run in a day unattended unlike the Kjeldahl method which requires many manual steps. The use of hazardous reagents such as sulfuric acid and sodium hydroxide in the Kjeldahl method also makes the Dumas method safer and more cost efficient (Marco 2002).

Crude fat was quantified in duplicate using AOAC Method 2003.06 Randall/Soxtec modification of the conventional Soxhlet solvent extraction procedure. Crude fiber content was determined using the AOCS approved Ba6a-05 Filter Bag method. Samples were digested with sulfuric acid and sodium hydroxide to remove proteins, sugars, starch, fats, and portions of carbohydrates. The organic residue left after digestion was used to measure crude fiber.

5.2.2. Bread formulation and analysis

Replacement of gluten in GF bread formulations typically requires use of a mix of GF flours, starches, dairy products, proteins, hydrocolloids, and other functionl ingredients (Zannini E, et al 2012). Fleischmann’s active dry yeast (ACH Food Companies, Inc. Memphis, TN), sugar, and warm distilled water measured according to values in Table 5.1 were combined and set aside to allow the yeast to activate. The remaining dry ingredients were combined in a Globe stand mixer (model SP5 Global Food Equipment Dayton, OH) with whisk attachment and stirred on low for 30 seconds to evenly distribute all dry components. Use of a mixture of cornstarch, rice flour, and cassava/tapioca starch has been proven to result in high-quality, gluten-free bread with good taste and appearance (Sanchez H, et al 2002). In a separate small bowl, room temperature eggs, vegetable oil spread, and apple cider vinegar were weighed according to percentages listed in Table 5.1 then added to the mixer and whisked on low for 1 minute. The activated yeast produced CO_2 bubbles which was used to create a light airy texture in the bread. The yeast mixture was added in increments while mixing on low until incorporated. Whisk speed was increased slowly to high setting and beat for 7 minutes. Rather than being a dough that can be kneaded, gluten-free bread typically has a more batter-like consistency which was the case for this formulation. One Professional by Chef Made mini loaf pan (15.4 x 8.6 x 4.7 cm) was lightly sprayed with vegetable oil. A standard loaf batter of 150 grams was weighed in the greased pan and the top was smoothed down evenly with a small spatula. The pan was placed in a full-size Metro proofing cabinet (C599-SDS-U Intermetro Industries Corporation Wilkes-Barre, PA) set at 100 F and 90% relative humidity for 30 minutes. Height more than doubled and met the top of the pan after proofing. Very carefully, the pan was transferred to the center rack of a Baxter mini rotating rack gas oven (model OV310G) that was preheated to 345 F and allowed to bake for 20 minutes. It was important to refrain from opening the oven at any point to avoid temperature variability which could have negative impact on the bread structure. Once the loaf was cooked and removed from the oven, it was left to cool in the pan for 5 minutes then carefully flipped to release the loaf and placed right side up to cool further. An hour cooling time was enough to solidify the bread and begin slicing.

A sanitized electric meat slicing machine (model S-4 Sanitary Scale Company Belvidere, IL) was adjusted to 25 mm to cut slices for color and texture analysis.

Table 5.1. Percentages of gluten-free bread ingredients

Ingredient	
Active dry yeast	0.90%
Water	32.92%
Sugar	2.74%
Rice flour*	17.46%
Tapioca flour	14.96%
Cornstarch	16.46%
Baking powder	0.50%
Xanthan gum	0.80%
Salt	1.50%
Egg	9.98%
Vegetable oil spread	1.50%
Apple cider vinegar	0.30%

*bread treatments varied only by rice flour type used; makes 1 standard 150-gram mini loaf

5.2.2.1. Color and Texture Analysis

A Konica Minolta BC-10 Baking Contrast Meter was calibrated using a white calibration tile before testing. Crust color of three bread slices per loaf were measured on the top in three areas – middle and middle edge of each short side. This was repeated for 2 loaves from 4 different batches. The bread was sliced evenly to 2.5 cm thick pieces and crumb color was analyzed on the flat surface. Color, lightness, and darkness were quantified as L, a*, and b* values.

Using a TA.XT*Plus* texture analyzer, triplicate samples of bread from three different batches of the same flour type were tested. Crust of the bread pieces were cut and discarded to measure the crumb texture only. Standardization of 2.5 cm thick bread slices complies with height parameters for analysis. A P/2 TA-25 2” diameter cylindrical probe was used together with an Exponent test method for bread. Height was calibrated for a load cell of 30 kg. Test parameters were set to compress the piece of bread 40% with the probe at a rate of 1.7 mm/sec which follows the AACC International Approved Method 74-09.01 for bread firmness.

5.2.3. Bread consumer sensory testing

Four bread treatments were prepared for a minimum of 100 consumers. Eleven loaf replications of each treatment were prepared by multiplying Table 5.1 ingredients by 11 and combined in the same manner as previously described. Miniature loaves were sliced into 10-1 cm pieces not including the ends. Panelists were untrained and recruited on Louisiana State University's campus over the course of one day. Sample assignments followed a randomized complete block design (RCBD) where each group/block received one set of four bread treatments. Unsalted crackers and water were provided to cleanse their palette between samples. An online Qualtrics survey was created to prompt panelists to visually and physically assess samples for specific product characteristics, overall liking, and purchase intent. All attributes were ranked on a 9-point hedonic scale and some were also ranked on a just about right (JAR) scale to determine what adjustments can be made in the future.

5.2.4. Statistical analysis

Color and texture data were analyzed using analysis of variance (ANOVA) to determine if treatments were comparable or significantly different. An ANOVA test was also done between sensory treatments to calculate mean acceptances and to see if there were any significant differences between samples types. A Tukey test was used to compare treatments parametrically for statistical differences for color, texture, and sensory data.

5.3. Results and discussion

5.3.1. Moisture content and pasting properties of flours

Compared to wheat, both high protein rice flours and the commercial white rice flour had significantly lower pasting temperatures which denotes minimum temperature required to cook the sample (Srivastia Y., et al. 2013). Peak and breakdown viscosities were not statistically different for the commercial brown rice flour and white high protein rice flour. This could be used to infer that CBR and WHPR may have similar cooking stability. Retrogradation tendencies, or total setback, of all rice flours were significantly greater than that of wheat and high protein rice flours had the greatest setback viscosities. Peak and final viscosities of rice flour were much greater in a study that characterized the quality of different flour mixtures using wheat, rice, and maize flour in varying ratios (Rai S et al, 2012).

Table 5.2. Moisture and starch gelatinization behavior of various flours

Flour Type	***Moisture Content (%)***	***Pasting Temp (°C)***	***Peak Viscosity (cP)***	***Trough Viscosity (cP)***	***Breakdown (cP)***	***Final Viscosity (cP)***	***Total Setback (cP)***
CW	13.05 ±0.55$_{A}$	89.48 ±0.51$_{A}$	1790 ±40.95$_{D}$	1186.33 ±17.95$_{D}$	570.33 ± 39.80$_{C}$	2430 ±94.06$_{D}$	1243.67 ±76.20$_{E}$
CBR	12.69 ±0.46$_{A}$	89.93 ±0.40$_{A}$	2347.33 ±39.80$_{B}$	14330 ±25.87$_{C}$	947.67 ±21.22$_{B}$	3174.67 ±36.94$_{C}$	1741 ±20.60$_{C}$
CWR	12.90 ±0.39$_{A}$	84.83 ±0.06$_{C}$	3477.67 ±63.22$_{A}$	1897.33 ±79.65$_{A}$	1547 ±29$_{A}$	3264.33 ±52.44$_{C}$	1367 ±34.70$_{D}$
BHPR	12.70 ±0.36$_{A}$	87.55 ±0.43$_{B}$	2030 ±14.80$_{C}$	1442.67 ±18.90$_{C}$	587.33 ±33.29$_{C}$	4531 ±9.64$_{A}$	3088.33 ±16.92$_{A}$
WHPR	12.89 ±0.40$_{A}$	82.73 ±0.54$_{D}$	2450.33 ±29.50$_{B}$	1572 ±22.11$_{B}$	911.67 ±34.27$_{B}$	3743 ±34.04$_{B}$	2171 ±14$_{B}$

(A) CW: commercial wheat, CBR/CWR: commercial brown/white rice, BHPR/WHPR: brown/white high protein rice (B) Same subscript letter within a column signifies no significant difference at 95% confidence

5.3.2. Protein, fat, and fiber content of flours

Both methods of protein quantification favored high protein rice flours for protein content. Table 5.3 shows a 1-2% difference between the high protein rice flours than their commercial counterparts. Fat and fiber values were the same statistically in high protein rice flours as commercial when comparing brown rice flours separately from white rice flours. The brown high protein rice flour had the highest average values for all analyses and the only double-digit protein content. Compared to a study by Hager A, et al in 2012 that reported an average protein content of 7.33 for rice flour, both methods of protein quantification yielded much higher contents for both commercial and high protein rice flours. High protein rice flours having greater protein, fat, and fiber contents will also have a decrease in carbohydrate content compared to commercial controls thus reducing glycemic response (Moghaddam E, et al 2006).

Table 5.3. Proximate analysis of rice flours

Type of Rice Flour	***Nitrogen*** *(ppm)*	***Dumas Protein*** *(%)*	***Kjeldahl Protein*** *(%)*	***Crude Fat*** *(%)*	***Crude Fiber*** *(%)*
Commercial Brown	$1.06 \pm 0.02_C$	6.29	$9.15 \pm 0.07_B$	$2.45 \pm 0.07_A$	$0.86 \pm 0.36_A$
Commercial White	$0.98 \pm 0.01_D$	5.81	$7.25 \pm 0.21_C$	$0.35 \pm 0.07_B$	$0.12 \pm 0.06_B$
Brown High Protein	$1.46 \pm 0.01_A$	8.66	$10.1 \pm 0.42_A$	$2.5 \pm 0.14_A$	$0.9 \pm 0.13_A$
White High Protein	$1.34 \pm 0.02_B$	8.00	$9.25 \pm 0.07_B$	$0.45 \pm 0.07_B$	$0.27 \pm 0.18_B$

(A) mean ± SD, N = 4 (B)Same subscript letter within a column signifies no significant difference at 95% confidence

5.3.3. Bread Color and Texture Analysis

Gluten-free bread crust lightness, red, and yellow color values were not significantly different between treatments (Table 5.4). Crumb lightness was also similar among all samples. The biggest change in color was seen with the a* value. The crust being exposed more to direct heat and browning caused the bread to retain a greener hue than the bread crumb. This data suggests that minimal color differences between GF breads may not be visually noticeable by consumers.

Table 5.4. Gluten-free bread crust and crumb colors

Crust color	*L**	*a**	*b**
Commercial BR	77.07 ± 1.20$_A$	4.53 ± 1.04$_A$	18.47 ± 1.07$_A$
Commercial WR	72.87 ± 3.95$_A$	7.20 ± 0.26$_A$	20.47 ± 1.37$_A$
Brown HPR	77.13 ± 0.74$_A$	4.93 ± 1.84$_A$	19.17 ± 1.70$_A$
White HPR	76.20 ± 0.75$_A$	4.57 ± 0.67$_A$	17.93 ± 0.92$_A$
Crumb color			
Commercial BR	72.73 ± 1.01$_A$	-0.27 ± 0.12$_A$	12.83 ± 0.61$_A$
Commercial WR	75.07 ± 1.59 $_A$	-1.10 ± 0.09$_B$	9.53 ± 0.23$_B$
Brown HPR	73.80 ± 0.26$_A$	-0.35 ±0.07$_A$	12.90 ± 0.66$_A$
White HPR	74.90 ± 0.62$_A$	-1.13 ± 0.12$_B$	11.63 ± 0.40$_A$

(A) BR: brown rice; WR: white rice; HPR: high protein rice (B) mean ± SD, N = 6 (C) Same subscript letter within a column signifies no significant difference at 95% confidence

Rice flour gluten-free breads could be characterized by an open aerated structure like maize and rice breads in a study by Hager A and others in 2012. All loaf types had similar heights. Long mixing times for GF batters was shown to produce more gas in early stages of proofing which could explain the growth of these breads after proofing and before baking (Gomez M, et al 2013). Bread was less firm for samples made with white rice flours compared to brown rice flours. Brown high protein rice bread tended to be the firmest and could be due to the greater levels of protein. Firmness increases over time due to staling as mentioned by Gallagher and others in 2014 and was expected to do the same for these rice-flour breads.

Table 5.5. Texture analysis and loaf height

	Height (cm)	***Firmness (N)***
Commercial Brown Rice Bread	4.57±0.83$_A$	37.24±2.09$_{AB}$
Commercial White Rice Bread	4.87±0.82$_A$	25.32±2.55$_C$
Brown High Protein Rice Bread	4.60±0.63$_A$	41.92±7.51$_A$
White High Protein Rice Bread	4.83±0.43$_A$	31.84±3.02$_{BC}$

(A) mean ± SD, N = 9 (B) Same subscript letter within a column signifies no significant difference at 95% confidence

5.3.4. Consumer sensory study

A total of 111 untrained panelists participated in the consumer study. Most of the participants were 18-22 years old due to the location being on a college campus. Female panelists outnumbered male panelists having 68 and 42, respectively. Crumb color and structure were the only parameters assessed without physical touch or tasting. Bread made with white high protein rice flour had the greatest acceptance of crumb structure. The following 4 bread characteristics were evaluated once panelists were instructed to taste the samples. Results show that all four bread treatments were not significantly different in acceptance of softness, moistness, flavor, and overall liking. Although rice flours may yield breads with lower volume than breads made with maize starch, sensory acceptance is typically improved for rice flour breads (Mancebo M, et al 2015).

Table 5.6. Acceptance of bread characteristics

	Crumb Color	***Crumb Structure***	***Softness***	***Moistness***	***Flavor***	***Overall Acceptance***
CBR	5.30 $\pm 1.42_{A}$	5.17 $\pm 1.61_{B}$	5.59 $\pm 1.65_{A}$	5.26 $\pm 1.85_{A}$	5.29 $\pm 1.82_{A}$	5.27 $\pm 1.70_{A}$
CWR	5.66 $\pm 1.52_{A}$	5.66 $\pm 1.42_{AB}$	5.39 $\pm 1.71_{A}$	5.03 $\pm 1.88_{A}$	5.39 $\pm 1.84_{A}$	5.26 $\pm 1.75_{A}$
BHPR	5.48 $\pm 1.48_{A}$	5.36 $\pm 1.48_{AB}$	5.15 $\pm 1.72_{A}$	4.63 $\pm 1.92_{A}$	4.93 $\pm 1.90_{A}$	4.84 $\pm 1.90_{A}$
WHPR	5.76 $\pm 1.42_{A}$	5.74 $\pm 1.46_{A}$	5.32 $\pm 1.61_{A}$	4.77 $\pm 1.79_{A}$	5.06 $\pm 1.90_{A}$	4.98 $\pm 1.89_{A}$

(A) CBR: 100% brown rice flour; BHPR: 100% brown high protein rice flour; WHPR: 100% white high protein rice flour (B) N= 97; mean ± SD based on 9-point hedonic scale (C) Same subscript letter within a column signifies no significant difference at 95% confidence

Determining what specific bread characteristics could be improved is an important part of product development. Using the 3-point just about right scale, panelists reported that white high protein rice flour bread samples may have been softer than commercial white flour bread samples with similar moistness. A study in 2015 reported similar acceptability scores for rice flour and wheat starch-based breads compared to lower acceptability of maize starch breads which may deter use of maize starch in gluten-free bread formulations. Inclusion of a gluten-free wheat starch poses many risks but if it is tested and certified gluten-free, this addition can have positive effects on consumer acceptance and physical properties (Mancebo M et al 2015).

Table 5.7. Attribute intensities of gluten-free bread

	Product Characteristic	***Not Enough***	***Just About Right***	***Too Much***
Commercial BR	Softness	26.13%	72.07%	0.90%
Commercial WR		38.74%	59.46%	0.90%
Brown HPR		30.06%	63.06%	5.41%
White HPR		26.13%	68.47%	4.50%
Commercial BR	Moistness	29.73%	67.57%	1.80%
Commercial WR		39.64%	55.86%	3.60%
Brown HPR		43.24%	52.25%	3.60%
White HPR		40.54%	56.76%	1.80%

(A) Commercial BR: 100% brown rice flour; Brown HPR: 100% brown high protein rice flour; White HPR: 100% white high protein rice flour (B) N= 97; based on a JAR scale; 1=not enough, 2=just about right, 3=too much

Information about health benefits of a product is a huge marketing strategy in the food industry. Based on visual and physical perception alone, panelists' intention to purchase any one of the samples was evenly distributed between yes, maybe, and no. A health message was prompted on the survey specifying that samples were gluten-free. In addition to the GF claim, the inclusion of a naturally high protein rice flour was given for both bread samples made with high protein rice flour. Purchase intent was not drastically changed for commercial rice flour breads, however, Table 5.8 shows the almost 11 percent increase in "yes" responses for brown high protein bread and the frequency of "no" responses for white high protein bread was reduced 8 percent. The inclusion of a health benefit message makes consumers more inclined to purchase a product despite having lower acceptance of appearance, texture, and taste.

Table 5.8. Effect of health message on purchase intent of gluten-free rice flour bread

Treatment	***Panelist Response***	***Purchase Intent BEFORE message***	***Purchase Intent AFTER message***	***% difference***
Commercial Brown Rice Flour	Yes	33.33%	35.14%	+1.81
	Maybe	33.33%	36.04%	+2.71
	No	32.43%	27.93%	-4.50
Commercial White Rice Flour	Yes	33.33%	30.63%	-2.70
	Maybe	35.14%	41.44%	+6.30
	No	30.63%	27.03%	-3.60
Brown High Protein Rice Flour	Yes	24.32%	35.14%	+10.82
	Maybe	41.44%	33.33%	-8.11
	No	33.33%	30.64%	-2.69
White High Protein Rice Flour	Yes	31.53%	36.94%	+5.41
	Maybe	32.43%	35.14%	+2.71
	No	35.14%	27.03%	-8.11

(A) health message stated "This is a **GLUTEN-FREE** muffin made with **RICE FLOUR**" or "***HIGH PROTEIN RICE FLOUR"**

5.4. Conclusion

The greater protein, fat, and fiber contents of high protein rice flours may have resulted in a firmer bread texture, but sensory scores were not significantly affected. Despite differences in physiochemical properties, gluten-free breads made with high protein rice flours were comparable to bread that was made with commercial rice flours. Formulation improvements may focus on increasing moistness and thus softness of the loaves by either using additional starches such as potato starch or inclusion of other hydrocolloids. Inclusion of a health benefit message on food packaging to market products made with more nutritious ingredients can have a positive effect on consumer purchase intent. Use of a high protein rice flour in gluten-free baking should be considered by companies who want to provide more nutritious options.

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CHAPTER 6. CONCLUSIONS

Rice flour as a predominant ingredient in gluten-free products has shown success in the early stages and will continue to be improved with research. The availability and variety of gluten-free food items made with rice flour is growing. Carbohydrate-rich foods such as bread, pasta, and on-the-go snacks are the most predominant food items to consider producing or improving when developing gluten-free alternatives.

Cultivation of more nutrient dense rice varieties are showing promise in chemical analyses and applications. Little to no physical or textural differences can be detected in gluten-free muffins and bread made with high protein rice flour when compared to GF muffins and bread made with commercial rice flours. The lightness of white high protein rice flour may have more appeal to consumers even though its protein content is less than that of brown high protein rice flour.

Formulation improvements of bread and muffins made with rice flour should focus on increasing moistness and softness to increase consumer acceptance. Inclusion of health benefit information has a significant impact on the intent of purchase by consumers and should be considered when constructing packaging. Use of high protein rice flours shows promise in developing more nutritious gluten-free products for people on a strict gluten-free diet.

If given the opportunity to continue this project, future studies should focus on continued measurements of the macronutrient composition of each new harvest of the higher protein rice to compare and calculate a true composition average. Formulation improvements would also be worthwhile to increase moistness and softness of the rice flour muffins and bread to consumer acceptance. Extending the shelf life of these products without compromising quality may also be an important step in development to commercial availability.

APPENDIX A. CONSENT FORMS

Muffin research consent form

I, _______________________, agree to participate in the research entitled "Consumer study of Muffins" which is being conducted by Witoon Prinyawiwatkul of the School of Nutrition and Food Sciences at Louisiana State University Agricultural Center, (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. One hundred consumers will participate in this research. For this particular research, about 10 minutes participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.
2. The reason for the research is to evaluate consumer liking of different muffins. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems related to such examination.
3. The procedures are as follows: one set of three coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risk: **The only risk may be an allergic reaction to the following ingredients: rice product, egg, milk product, common baking ingredients, xanthan gum, or unsalted crackers**. However, because it is known to me beforehand that all those foods and ingredients are to be tested, the situation can normally be avoided.
5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

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

Bread research consent form

I, _______________________, agree to participate in the research entitled “Consumer study of Bread” which is being conducted by Witoon Prinyawiwatkul of the School of Nutrition and Food Sciences at Louisiana State University Agricultural Center, (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. One hundred consumers will participate in this research. For this particular research, about 10 minutes participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.
2. The reason for the research is to evaluate consumer liking of different breads. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems related to such examination.
3. The procedures are as follows: one set of four coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on the online score program. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of Institute of Food Technologists.
4. Participation entails minimal risk: **The only risk may be an allergic reaction to the following ingredients: yeast, apple cider vinegar, butter, rice product, cassava/ tapioca, cornstarch, egg, common baking ingredients, xanthan gum, or unsalted crackers**. However, because it is known to me beforehand that all those foods and ingredients are to be tested, the situation can normally be avoided.
5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

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

APPENDIX B. IRB APPROVAL FORM

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

Application for Exemption from Institutional Oversight

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

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

- A Complete Application Includes All of the Following:
  (A) The original and a copy of this completed form and a copy of parts B through E.
  (B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1 & 2)
  (C) Copies of all instruments and all recruitment material to be used.
    - If this proposal is part of a grant proposal, include a copy of the proposal.

  (D) The consent form you will use in the study (see part 3 for more information)
  (E) Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing and handling data, unless already on file with the LSU AgCenter IRB.
  Training link: (http://grants.nih.gov/grants/policy/hs/training.htm)

1) Principal Investigator: Dr. Witoon Prinyawiwatkul **Rank:** Professor **Student? Y/N** NO
Dept: School of Nutrition & Food Sciences **Ph:** (225)578-5188
E-mail: wprinya@lsu.edu
2) Co-Investigator(s): please include department, rank, phone and e-mail for each
- If student as principal or co-investigator(s), please identify and name supervising professor in this space
  - Ashley Gutierrez, Research Associate, School of Nutrition & Food Sciences
  - (225)578-5423, agutierrez@AgCenter.lsu.edu

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

6) PI signature ____ ****Date** 10/23/18 (no per signatures)
****I certify that my responses are accurate and complete.** If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I leave the LSU AgCenter before that time the consent forms should be preserved in the Departmental Office.

Committee Action: Exempted ✓ Not Exempted ____ **IRB#** HE18-22
Reviewer Michael Keenan **Signature** Michael Keenan **Date** 11-5-2018

APPENDIX C. CODES

Muffin texture SAS code

```
Title1 'Muffin Texture';
Data Muffin Texture;
Input Hardness @@ Adhesiveness @@ Cohesion @@ Springiness @@ Gumminess @@ Chewiness
@@ trt;
datalines;
28.24 0.542 0.687 93.89 19.45 18.26 1
22.19 1.795 0.775 97.525 17.20 16.77 1
20.68 -0.038 0.754 95.792 15.60 14.95 1
19.44 -0.749 0.794 97.525 15.41 15.03 1
21.21 -0.125 0.739 94.554 15.69 14.83 1
24.03 0.801 0.737 94.554 17.72 16.76 1
14.46 0.612 0.839 95.792 12.14 11.62 2
13.54 0.38 0.838 92.327 11.35 10.48 2
16.13 1.31 0.842 97.525 13.57 13.24 2
15.05 -0.242 0.823 92.574 12.38 11.46 2
13.31 -0.002 0.844 94.059 11.23 10.56 2
15.29 0.657 0.835 94.554 12.76 12.07 2
30.04 0.136 0.645 91.99 19.40 17.84 3
22.99 -1.036 0.656 91.098 15.08 13.74 3
28.56 -0.401 0.691 90.347 19.75 17.84 3
21.14 -0.715 0.646 90.099 13.65 12.30 3
24.75 0.277 0.736 87.624 13.17 11.54 3
28.97 -0.971 0.705 90.347 13.52 12.22 3
37.72 0.165 0.748 93.23 28.20 26.30 4
23.67 0.009 0.773 91.832 18.30 16.81 4
31.71 0.337 0.74 92.822 23.45 21.77 4
13.17 0.165 0.807 84.406 10.63 8.97 4
20.62 0.493 0.799 92.079 16.48 15.17 4
33.38 -0.025 0.727 94.554 24.26 22.94  4
;
run;
proc sort; by trt;
proc means mean std; by trt;
ods graphics off;
proc anova;
class trt;
model Hardness Adhesiveness Cohesion Springiness Gumminess Chewiness = trt;
means trt / tukey ;
run;
ods graphics on;
ods rtf close;
run;
```

Bread texture SAS code

```
Title1 'Bread Texture';
Data Bread Texture;
Input Height @@ Firmness @@ trt;
datalines;

5.3 36.80 1
4.5 35.18 1
3.4 36.62 1
5.5 40.94 1
3.8 35.67 1
4.9 38.25 1
4.3 30.16 2
5.7 26.08 2
3.6 24.37 2
5.6 23.72 2
4.7 24.23 2
5.3 23.36 2
3.5 50.10 3
5.3 42.16 3
5.0 49.94 3
4.4 30.90 3
4.9 36.54 3
4.5 41.86 3
4.0 29.43 4
4.8 33.99 4
5.0 36.42 4
5.1 28.22 4
4.9 32.16 4
5.2 30.84 4

;
run;
proc sort; by trt;
proc means mean std; by trt;
ods graphics off;
proc anova;
class trt;
model Height Firmness = trt;
means trt / tukey ;
run;
ods graphics on;
ods rtf close;
run;
```

VITA

Gabriella Marie Paz was born in Slidell, Louisiana on December 8th, 1994. She left her hometown to attend the Louisiana School for Math, Science, and the Arts in Natchitoches, Louisiana as a high school junior. She graduated from LSMSA in May of 2013 where she also had the honor of singing the alma mater in front of her friends, family, and fellow graduates. The following August of 2013, Gabriella began her undergraduate career at Louisiana State University in Baton Rouge, Louisiana. Gabriella obtained a Bachelor of Science in Nutrition and Food Science with a concentration in Food Science and Technology in May of 2017. She enrolled in the Graduate School at Louisiana State University in August of 2017 where she is pursuing a Masters in Food Science and Technology. She is a candidate for convocation in summer of 2019.