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Sensory and Textural Evaluation of Gluten-Free Bread Substituted With Amaranth and Montina™ Flour

Karen L. Breshears[1]& Kristi M. Crowe[2]

[1] Department of Nutrition and Kinesiology, University of Central Missouri, Warrensburg, Missouri, USA

[2] Department of Human Nutrition, University of Alabama, Tuscaloosa, Alabama, USA

Correspondence: Karen L. Breshears, Department of Nutrition and Kinesiology, University of Central Missouri, Warrensburg, MO 64093, USA. Tel: 1-660-543-8737. E-mail: kbreshears@ucmo.edu

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Abstract

The objective of this study was to develop a nutrient-dense gluten-free bread (GFB) using either amaranth or Montina™ flour in a standardized gluten-free lean bread recipe for the purpose of comparing the nutritional, sensory, and objective qualities of the developed breads to a commercially-marketed GFB. Participants (n=222) included individuals who typically eat a gluten-free diet and those who eat a non-restricted diet. The non-restricted diet group was used to assess product acceptability in the general population and to determine product marketability among those without gluten restrictions. Nutritionally, both developed breads provided at least 26% more iron than the commercial GFB and $\geq$40% more fiber while the amaranth bread provided twice as much folate. Significant differences ($p < 0.05$) in sensory attributes (appearance, texture, flavor, tenderness, and overall acceptability) of both amaranth- and Montina™-based breads were not reported between the groups. Based on sensory scores using a 9-point Hedonic scale, the commercial GFB was preferred over either developed bread and the Montina™-based bread was preferred over the amaranth-based bread. Significant differences in bread hardness were not detected among the tested GFB, yet commercial GFB slices exhibited the largest and most consistent cell size throughout. Results suggest that amaranth and Montina™ flours assist in improving the nutritional quality of GFB, yet additional testing is needed to assist in formulation modifications of this standardized lean bread recipe in order to produce a product similar in sensory qualities to commercially-marketed GFB.

Keywords: sensory evaluation, gluten-free bread, amaranth, Montina™

1. Introduction

Celiac Disease (CD), an autoimmune response to foods containing gluten, presents itself in genetically-susceptible individuals resulting in lesions or total flattening of intestinal villi (Garcia-Manzanares & Lucendo, 2011; See & Murry, 2006). Currently, this disease affects one in 100 individuals worldwide or 1-2% of the general population, although not all have symptoms or are diagnosed with the disease. At present, the only treatment for CD is strict adherence to a gluten-free diet (GFD), which means abstaining from products containing offending proteins within wheat, rye, barely, spelt, and, in some cases, oats that were contaminated during processing (Alvarez-Jubete, Arendt, & Gallagher, 2009; de la Barca, Rojas-Martinex, Islas-Ruboi, & Cabrera-Chávez, 2010; Garcia-Manzanares & Lucendo, 2011; See & Murry, 2006). However, those with CD are not the only individuals who require a GFD. Gluten intolerant (GI) individuals have similar sensitivities to gluten including many of the same symptoms, and thus, must follow a GFD. According to current statistics, GI is 30 times more widespread than CD ("What is gluten intolerance", n.d.).

Among the gluten-free offerings on the market, gluten-free yeast bread (GFB) products are not known for their outstanding taste, texture or nutrient density. Likewise, giving up yeast bread is often difficult for those with CD or those who are GI because yeast bread products are a staple in the typical American diet (Anton & Artfield, 2008). As such, elimination of breads, pasta, and pastries containing the protein fractions that comprise gluten can be a challenge for those on a GFD. Another concern for individuals adhering to a GFD is the availability of GFB products. Not all grocery stores carry GF items, and thus, products must be purchased at specialty stores or on the Internet. Furthermore, the availability of GF products varies across the United States (US) with coastal cities having the greatest variety while the Midwest has more limited choices (Lee, Ng, Zivin, & Green, 2007;

Singh & Whelan, 2011).Collectively, these factors can lead to low dietary compliance rates which are currently between 50-80% (Garcia-Manzanares & Lucendo, 2011), and as a result, those with CD or who are GI may be at risk for a host of nutrient deficiencies.

The GFD calls for the elimination of offending culprits, which are the protein fractions that comprise gluten: gliadin and glutenin. These proteins require hydration and manipulation in doughs and batters to form gluten which is responsible for contributing plasticity and elasticity to breads. Gluten development is critical to the finished texture of breads because of its ability to trap expanding gases during the rising and baking processes (Anton & Artfield, 2008; Arendt & Moore, 2006; Arendt, Renzetti, & Bello, 2009). Overall, gluten impacts the cell size and formation, crumb structure (Without gluten, bread is dry and crumbly.), volume, and airiness of baked goods to the extent that products made with non-gluten forming flours often have poor textural quality and mouthfeel along with a pasty flavor or aftertaste (Anton & Artfield, 2008; Arendt & Moore, 2006). As a culinary guideline, when substituting GF flours in baked goods, alterations to the recipe must be made in order to create an acceptable, aesthetically-pleasing product. In order to achieve these sensory goals, recipes need to include a polysaccharide- or protein-based hydrocolloid that forms a gel with water and mimics the properties of gluten in the final product. Examples of hydrocolloids commonly used in baking applications include guar gum, xanthan gum, or methylcellulose and either whey or egg albumin all which provide elasticity and desired textural qualities (Abdel-Aal, 2009; Anton & Artfield, 2008; Arendt et al., 2009).

Among the most common ingredients used in GFB products are rice starch, cornstarch, potato starch, and/or tapioca starch (Arendt et al., 2009; Pagano, 2006). These non-wheat starches have been used in many cultures for generations, and as a result, these starches have met with success among product formulations sold in the GF marketplace. Additional wheat-alternative grains are available on the market some of which are more nutritious than wheat. For example, amaranth, Indian rice grass (Montina™), millet, quinoa, sorghum, Timothy grass seed (Timtana®), and bean flours such as navy bean or garbanzo bean are among the more commonly used GF flours. Among these, amaranth is higher than wheat in protein, fiber, iron, calcium, magnesium, and zinc (Pagano, 2006; "USDA National Nutrient Database", 2010). Likewise, Montina™, which was a dietary staple of Native Americans prior to the introduction of maize (Barr, 2004), is higher in protein, fiber, iron, and calcium than wheat ("Mission Mountain Food Enterprise Center", n.d.; Sands, 2010). Although there is little experimental research published on the bread-making qualities of Montina™, it is known in the culinary realm to impart good textural qualities to GFB products with acceptable substitution levels of 10-30% of the total flour (Hillson, 2010; Gruss, n.d.). However, since none of the alternative grains have the exact baking qualities of wheat, a blend of flours is typically needed to attain an acceptable product.

While the nutritional quality of some GF flours may be nutritionally superior to wheat, some GFB products are nutritionally inferior to their counterparts made with wheat. This is partially due to the fact that wheat flour, by law, is enriched during the milling process in order to enhance the nutritional quality. Because the vast majority of GF flours are not enriched or fortified, products made with these ingredients are often lower in nutritional quality (Thompson, 2000, 2009). For example, GFB products have been reported to have a lower thiamin, riboflavin, niacin, iron and, sometimes, fiber content (Thompson, 2000). Thus, the nutritional quality of GFB products must be carefully considered during the development phase. Lastly, it is also important to consider that iron, folate, fiber, calcium, and vitamins D, B_{12} and B_6 are all nutrients that those with CD are commonly deficient in upon initial diagnosis (Garcia-Manzanares & Lucendo, 2011; Hallert et al., 2002; Saturni, Ferretti, & Baccheeti, 2010; See & Murry, 2006). Although some of these vitamin and mineral deficiencies correct themselves as a result of strict adherence to a GFD, many nutrient deficiencies still remain due to inadequate dietary intake or poor compliance (Garcia-Manzanares & Lucendo, 2011; Kinsey, Burden & Bannerman, 2008; Thompson, Dennis, Higgins, Lees, & Sharrett, 2005). For these reasons, the sensory qualities are not the only considerations that should be taken into consideration during the development of GFB products.

As the GF retail market continues to grow at a rapid rate, the use of GFB products varies among the GF population (Arendt & Moore, 2006), yet numerous studies have documented the frequency of GFB as a staple in the diet of individuals with CD (Araújo & Araújo, 2011; Black & Orfila, 2011; Gilbert et al., 2006; Lamontagne, West & Galibois, 2001). Taken collectively, these studies show that GFB is consumed frequently and is important in assisting with dietary compliance by those who follow a GFD. Therefore, substituting alternative grains with higher nutrient density than those commonly used in commercial products may be beneficial for GF consumers, especially those who wish to consume a nutritious GFD without depending on commercial products. For these reasons, the objective of this study was to develop a nutrient-dense GFB prepared from a standardized consumer recipe for the purpose of comparing the nutritional, sensory, and objective qualities of the developed bread to a commercially-marketed GFB product. After a pilot study to evaluate preferences and substitution levels, amaranth and Montina™ flour were chosen as the nutrient-dense starches for this study.

2. Methods

2.1 Development of Bread

A standardized lean bread recipe (Coppedge, 2008) was used to develop GFB with amaranth or Montina™ flour. Based on the results of the pilot study, amaranth and Montina™ were substituted at a percentage of the total starch by weight to achieve product acceptability. The final level of substitution for the amaranth and Montina™ flour was 20% (70 g) of total flour weight. Table 1 lists the ingredients and preparation procedures. A commercial GFB product served as the comparison product for this study in order to establish a comparison for consumer expectations for sensory and quality attributes of GFB.

Table 1. Recipe ingredients and preparation procedures[a] of developed gluten-free bread

White Rice Flour	95 g
Potato Starch	95 g
Tapioca Starch	50 g
Soy Flour	50 g
Amaranth or Montina™ Flour	70 g
Whey	10 g
Guar Gum	25 g
Dry Egg White Powder	30 g
Salt	9 g
Instant Yeast	12 g
Liquid Egg Whites	100 g
Carbonated Water	385 g

[a] Procedure for preparing GFB with partial flour replacement using amaranth or Montina™ flour – Prepared in a non-contaminated kitchen with utensils and ingredients which were known to be GF. Each ingredient was weighed and mixed in a Kitchen Aid Mixer (Model KS25GOX, Kitchen Aid USA, Greenville, OH) equipped with a paddle on Speed 2 for a total of 4 min. Dough was placed in 9 x 5 (23 x 13 cm) bread pans lined with parchment paper, allowed a rise time of 30 min in a warming tray, and baked for 50 min at 375 °F (190 °C) to an internal temperature of at least 210 °F (100 °C). Breads were immediately removed from pan and cooled completely on a wire rack prior to testing.

2.2 Nutritional Composition

Nutrient composition for the three breads evaluated in sensory analysis was determined using Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) for crude protein by the Kjeldahl method, crude fat, crude fiber, iron, calcium, folate, and vitamin B_{12} (Latimer, 2012). Carbohydrates were determined by difference method. Proximate analysis and mineral analyses were performed by the Agricultural Experiment Station Chemical Laboratories at the University of Missouri-Columbia and vitamin analysis was performed by Silliker, Inc.

2.3 Sensory Evaluation of Developed Breads

Two hundred and twenty-two untrained volunteers (58 males and 164 females ages 19 to 85) recruited from faculty, staff, and students at a university and three Celiac support groups in the Midwestern US participated in sensory analysis. Among these, 44.6% were between 19 and 24; 9% between 25-34; 6.3% between 35-44; 15.8% between 45-54; 10.8% between 55-64; and 13.5% over age 65. Participants recruited for the study included individuals who typically eat a GF diet and those who eat a non-restricted diet. The non-restricted diet group was used to assess product acceptability in the general population and to determine product marketability among those without gluten restrictions. Ninety-two participants reported eating GF products at least 2-3 times per week and were grouped as GF consumers. The remaining 129 were grouped in the non-restricted diet group because they did not eat gluten-free products. Power calculations revealed 92 participants per group were necessary for statistical significance in comparing groups. The study was approved by the Institutional Review Board of the partnering institution and panelists signed consent forms per human subjects protocol.

Sensory analysis was conducted according to guidelines by Larmond (1991), Lawless and Heymann (2010, Chapter 14) and Stone and Sidel (2004) using a 9-point Hedonic scale ranging from *like extremely* to *dislike extremely* to assess appearance, texture, flavor, tenderness, and overall acceptability. A five-point Hedonic scale was used to assess usage and marketability. To control variability, panelists were seated at individual booths at which randomly coded and presented samples were provided on disposable white plates. After the products were tested, the unconsumed product and plate were removed prior to the next product presentation. In accordance with standard sensory testing procedures, panelists cleansed their palettes between samples with filtered tap water (25 °C).

2.4 Objective Measures

Developed breads were compared to the commercial GFB product to assess differences in texture, cell size, and cell uniformity. Texture analysis was completed on a Brookfield LFRA-100 Texture Analyzer (Brookfield Engineering Laboratories, Middleboro, MA). A TA 4/1000 cylinder probe (38.1 mm diameter, 20 mm length, AOAC Standard) was used to measure hardness of the bread by measuring force in compression on a 1.75cm slice of bread (Mezaize, Chevallier, Le Bail, & De Lamballerie, 2009; Krupa, Rosell, Sadowska, &Soral-Smietana, 2010). Hardness was defined as the maximum force in grams at peak load measured at velocity of 0.5 mm/s (Hoye & Ross, 2011). Texture analysis was performed in triplicate for each bread type. Uniformity and cell size of the bread were measured using a flatbed scanner at 350 dpi according to standard procedures (de la Barca et al., 2010; McWilliams, 2008; Schober, Bean, & Boyle, 2007; Yano, 2010).

2.5 Statistical Methods

Group difference toward bread preferences and bread textures were analyzed for statistical significance ($p < 0.05$) with repeated measures ANOVA and Tukey's HSD post hoc testing using SPSS Statistics 19 (2010, IBM Company).

3. Results and Discussion

3.1 Nutritional Comparison

Compared to the commercial product, the developed GFB products had nutritional advantages for total carbohydrate provision along with iron, fiber, and folate content (Table 2). The energy content of the commercial GFB product was higher than either developed GFB likely due to added sugars and/or fat. For example, the commercial GFB had twice as many carbohydrates per 100 g than either developed GFB. This could be significant for glycemic control if managing a diabetic diet along with a GFD. Both CD and Type 1 Diabetes are autoimmune diseases which occur together in 3.9 -12.3% of all CD cases ("Do You Have Celiac Disease", n.d.). Both developed breads contained 52-60% less calcium than the commercial product, and were similarly lower in calcium than most commercial wheat breads ("Arnold Bread", 2012; "Nature's Own", 2012). The iron content of amaranth and Montina™ breads was 45% and 26% higher, respectively, than the iron content in the commercial product. Additionally, the fiber content of the bread developed with Montina™ flour was 59% higher than the commercial GFB product whereas the amaranth-based bread provided approximately 40% more fiber than the commercial GFB product. The vitamin B_{12} content was two to three times higher in the commercial bread than in the amaranth and Montina™- based breads. Although none of the products were significant sources of vitamin B_{12}, each would contribute less than 5% to the overall dietary intake ("Dietary Supplement Fact Sheet: Vitamin B_{12}", 2011). The folate content was 50% higher in the amaranth bread compared to the commercial product, whereas Montina™ was 8% lower in folate than the commercial product. Since a food is considered a good source of folate if it contains 10% of the recommended daily amount ("Folate Fact Sheet", 2004), one serving of the amaranth-based bread would be considered a good source of folate (16% of Daily Value). Nevertheless, since iron, fiber, vitamin $B_{12,}$ and folate are four of the nutrients commonly deficient among newly diagnosed individuals, the breads developed with these nutritious alternative GF flours may assist in providing these critical nutrients.

Table 2. Nutrient composition[a] of developed gluten-free breads as compared to a commercially-marketed gluten-free bread

Per 100 g	Amaranth	Montina™	Commercial
Energy (kcal)	214.28	192.2	351.16
Crude Fat (g)	5.04	0.64	5.52
Carbohydrate (g)	31.86	36.52	65.1
Crude Protein (g)	10.37	10.09	10.27
Crude Fiber (g)	0.74	1.07	0.44
Calcium (mg)	46.7	38.7	96.8
Iron (mg)	1.71	1.29	0.95
B_{12} (mcg)[b]	0.14	0.12	0.36
Folate (mcg)[b]	65.7	34.1	37.8

[a] Proximate, mineral, and vitamin analyses performed according to approved AOAC methods.

3.2 Sensory Evaluation

The research population is similar to those with CD, as up to 70% of those diagnosed with CD are women (Green & Cellier, 2007; Shah & Leffler, 2010), and although CD can occur in any ethnicity, it is most common in those from European decent (See & Murry, 2006). Published data on GI demographics is not yet available, so comparisons with this population are yet to be determined (Di Sabatino & Corazza, 2012); however, the demographics are considered to be similar to the CD population as a whole. As such, the finding of this study may be generalized to the CD population.

There were no statistically significant differences ($p > 0.05$) between participant groups in overall acceptance of the two developed GFB products suggesting that the developed breads were equally acceptable to both participant groups (results not shown). Using a 9-point Hedonic scale, the mean acceptance scores for the GF/ non-restricted groups were 5.8/5.9 for amaranth and 6.5/6.2 for Montina™. In agreeance with these study results, Zandonadi, Botelho and Araújo (2009) used similar comparison groups in a study evaluating psyllium and GF flours in place of wheat flour to create a GFB product. No statistical differences in preference between groups were detected indicating that the developed products may be acceptable to those individuals with or without GI.

When comparing overall acceptance of the commercial product to the two developed GFB containing Montina™ or amaranth, significant differences were observed ($p < 0.001$). Across both participant groups (N=222), the commercial GFB was significantly preferred over either developed bread and the Montina™-based bread was significantly preferred over the amaranth-based bread (Table 3). Likewise, the commercial bread product received statistically higher scores than either developed bread in all sensory attributes evaluated. The Montina™- and amaranth-based breads were acceptable products with means > 5.5 out of 9 for all categories of evaluation (Table 3). The mean scores associated with likelihood of buying and comparison to similar gluten-free products on the market between the two developed breads were “neutral/no preference” (mean of 3.0 and 3.1 out of 5, respectively) for Montina™ and “somewhat unlikely/ prefer less” (mean of 2.8 out of 5, for both categories) for amaranth. Since sensory ratings and acceptance were lower than expected for both developed GFB, the lean bread recipe may need further modifications if these flours are to be used for in-home preparation of GFB.

Table 3. Sensory analysis of developed gluten-free breads as compared to a commercially-marketed gluten-free bread as evaluated by gluten-free and non-restricted diet consumers

	Amaranth		Montina		Commercial	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Appearance	6.9	±1.7	6.9	±1.6	7.8	±1.0
Tenderness	6.8	±1.9	7.2	±1.5	7.3	±1.4
Texture	6.5	±1.9	6.8	±1.6	7.4	±1.2
Taste	5.6	±2.1	6.2	±1.8	7.4	±1.4
Overall Acceptance	5.9	±2.0	6.3	±1.6	7.4	±1.3
Purchasing Potential	2.8	±1.4	3	±1.3	3.8	±1.3
Compared to other gluten-free breads on the market[a]	2.8	±1.1	3.1	±1.0	3.7	±1.1

[a] Survey question: "Compared to similar gluten-free products on the market, how would you rate this product?"

Since the commercial GFB contained a higher sugar and fat content, the preference for this product may be the result of these two ingredients. For example, according to Schoenlechner, Mandala, Kiskini, Kostaropoulos, and Berghofer (2010), increasing the fat content in a GF amaranth bread to 2% decreased the bitter and moldy taste of amaranth and improved sensory evaluation and acceptance ratings. Although experimental research on the sensory qualities of Montina™-based bread products is lacking, incorporation of both sugar and fat into either of the developed GFB products would be expected to improve sensory evaluation scores.

3.3 Objective Evaluation

Objective testing results indicate no significant difference ($p > 0.05$) in hardness/tenderness among breads (Figure 1). Thus, although there were differences in sensory preference and acceptability among the commercial and developed breads, these differences were not likely attributed to the textural quality of the bread. This could be attributed to the fact that most GFB includes a hydrocolloid in the product formulation or recipe for the purpose of imparting textural characteristics similar to gluten and improving the loaf quality by reducing crumb hardness (Crockett, Ie, & Vodovotz, 2011). In this study, both developed breads contained guar gum as the hydrocolloid. Since no significant differences were detected between hardness/tenderness attributes, results indicate an adequate amount of hydrocolloid in the recipe formula.

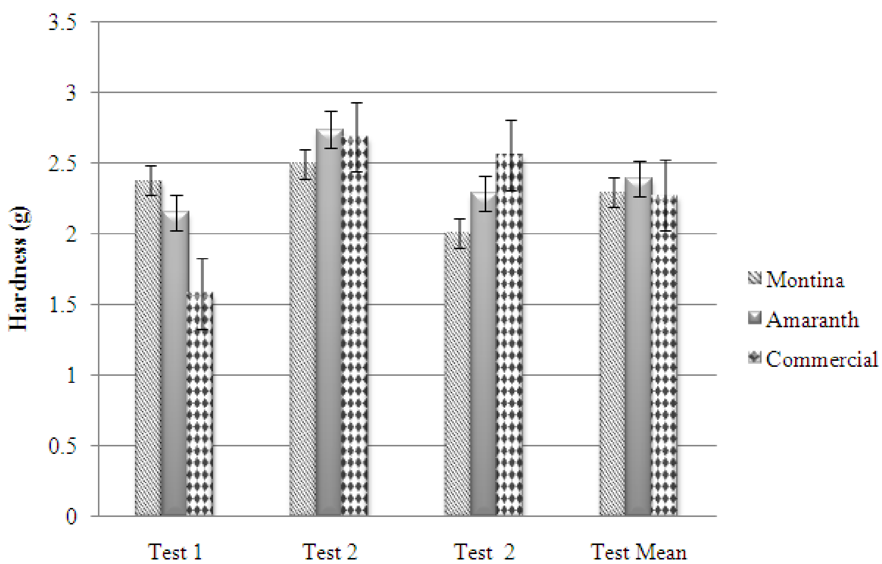

Figure 1.Tenderness of developed gluten-free breads compared to a commercially-marketed gluten-free bread. Bars depict results of triplicate testing and the mean value of tests combined

Since bread tenderness would be influenced by the rise of the bread, the density of each bread was assessed by visually comparing cell size of bread slices (Figure 2). The GFB containing amaranth exhibited a compact cell structure and a dip in the top surface where the bread collapsed while baking. These results suggest that ingredient proportions need further adjustment. The Montina™-based bread exhibited an even texture and minimal cell compaction at the base of the bread. In comparison, the commercial product had the largest and most consistent cell size throughout the slice.

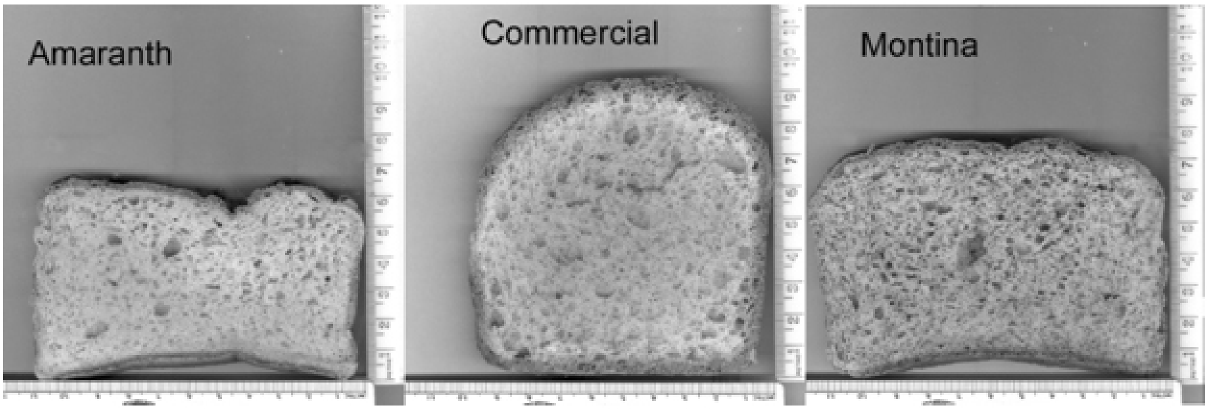

Figure 2. Cell size and density measurements of developed gluten-free breads compared to a commercially-marketed gluten-free bread

Not only was the texture of each bread visually different, but the color of the products also varied. The amaranth-based bread appeared most like the commercial bread despite a slight yellow tint which was also noticeable in the raw amaranth flour. The Montina™-based bread contained black particles throughout which was similar to the flour itself, exhibiting a multi-grain appearance. The commercial GFB product was similar in color to commercial multi-grain breads which contain bran particles due to the inclusion of the whole grain including the bran and germ; however, the dark particles were not as pronounced in color as the bread developed with Montina™. If a consumer preferred white bread, then the amaranth-based bread may be more appealing, whereas if consumer preference was for whole grain-type breads, then the Montina™-based bread may be more visually appealing.

Based on results of the study, considerations for future research include adjusting starch level and/or water content along with adjusting the sugar and fat content. Overall, the GFB developed with Montina™ exhibited a good rise, acceptable cell size, and acceptable texture. However, modifications such as those mentioned above should be made to improve sensory acceptability.

Amaranth which is known for its nutty flavor and unique texture has a low level of amylase starch, and as such, it often requires significant modifications of the base recipe when used in GFB. Current literature suggests replacing the original starch with up to 10% amaranth so as not to affect sensory qualities in a baked product (Arendt et al., 2009; Arendt & Moore, 2006). In this study, amaranth was substituted at 20% of the total flour based on acceptability results of pilot test; however, this could explain the lower volume observed. According to Schoenlechner et al. (2010), adjusting the water content in amaranth-based breads has a significant influence on bread quality. Thus, increasing the water content would be expected to enhance starch gelatinization and hydration of the proteins resulting in a softer, less gummy bread with improved cell size and bread volume. For this reason, additional research is needed to determine if decreasing the amount of amaranth flour or increasing the recipe's water content may produce a GFB with sensory qualities more comparable to commercial GFB.

4. Conclusion

4.1 Limitations

Although a commercial GFB product was used as the comparison sample in establishing consumer expectations for sensory and quality attributes of GFB, it also may have been a limitation of the study. The commercial GFB was not a lean bread, and thus it contained added fat and sugar which may have influenced consumer preference. In future studies, it may be more advantageous to compare the Montina™- and amaranth-based breads to the original lean bread recipe which contained only rice, potato, tapioca, and soy starches instead of to a non-lean commercial product. Another potential limitation of the study is the higher proportion of college-age participants which may limit the ability to generalize to all age groups within the CD and GI populations.

4.2 Recommendations

Developing an acceptable nutrient-dense GFB is possible, yet challenging as reported in this and other research studies. Additional research is needed to assist in the development of consumer-friendly GF products and in the development of GFB recipes for persons with CD that are interested in in-home preparation of GFB with similar sensory qualities to commercial GFB. The later type of research and development is critical in promoting dietary compliance among CD and GI individuals especially among those who commonly serve GF products and meals in the home to family members with non-restricted diets. For example, it has been shown that when the entire family eats GF, it is less difficult to follow a GF diet (Garcia-Manzanares & Lucendo, 2011). Recommendations for future testing and development of amaranth-and Montina™-based breads include modifying the flour ratios and/or adding sugar, fat, and water into the recipe for improving the palatability of the bread and making it more comparable to commercial GFB. Registered dietitians can use this information to counsel clients with CD or GI by assisting the client with advice on preparing GFB in the home especially in regions of the country where the variety of GF products is limited.

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